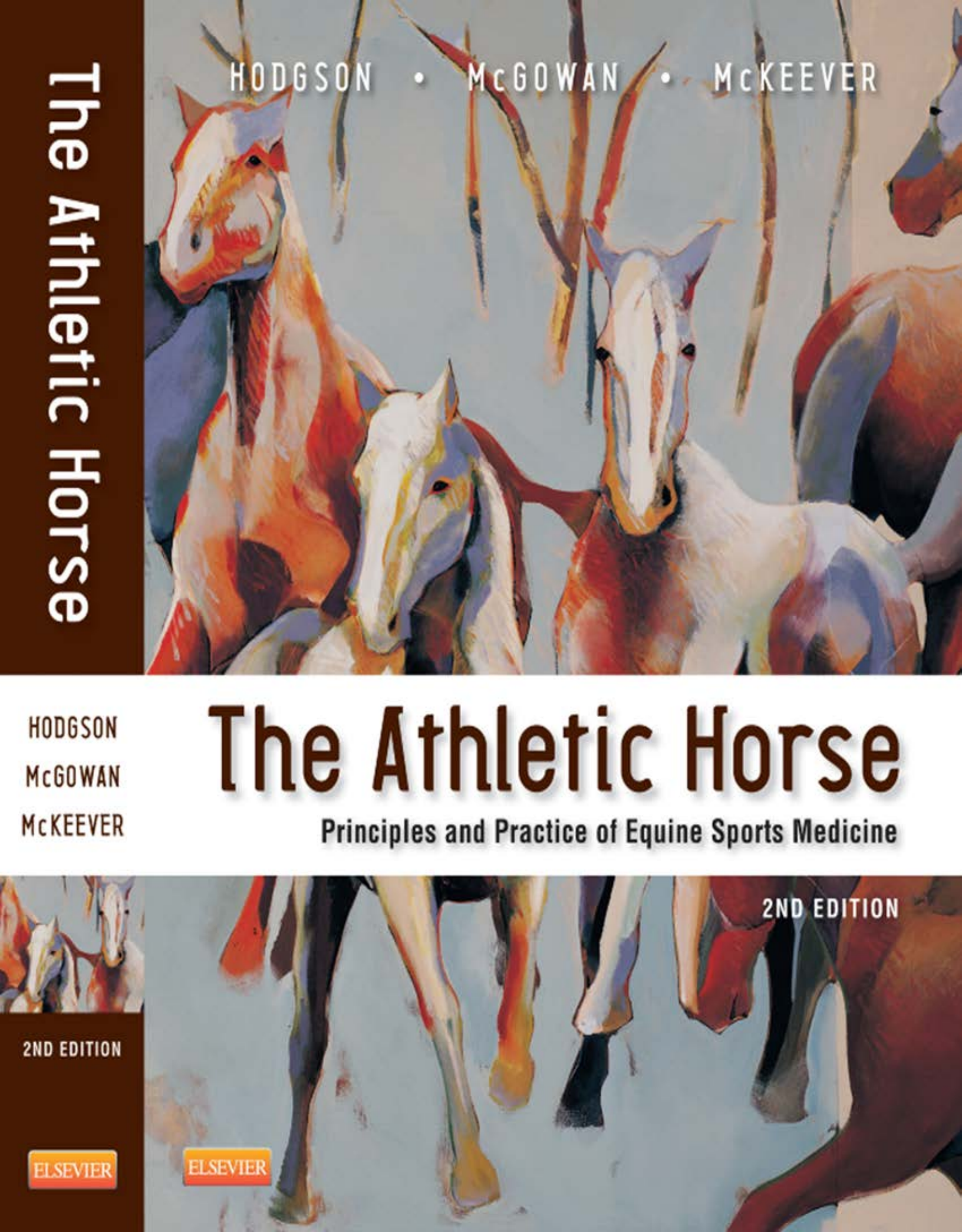
HODGSON • McGOWAN • McKEEVER
The Athletic Horse
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The Athletic Horse
Principles and Practice of Equine Sports Medicine
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The Athletic Horse

Principles and Practice of Equine Sports Medicine

SECOND EDITION

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Dedicated to Jennie and all the horses that made the myriad studies reported in this book possible.
David Hodgson

To my wife Jennifer and daughters Nicole, and Natalie for their love, patience, and support.
Kenneth McKeever

To Tom, Lizzie and Dylan who are my constant support.
Cathy McGowan

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Preface

We are delighted to welcome the publication of the second edition of *The Athletic Horse*. This edition has been a long time in development. The first edition was published in 1994. Much has changed since that first edition. One being that the first edition was one of the few books on veterinary lists dealing with the physiology of exercise in horses. As we move well into the second decade of the twenty first century, veterinary lists have expanded dramatically as our knowledge has grown and more and more specialties have been formed. Publishing and methods for dissemination of knowledge have changed inexorably since the mid 1990's. Thus although this edition is being produced in hardback, similar to the first edition, color has been widely utilized as have many sophisticated production tools by the publishers, Elsevier. With the advent of cloud computing, extraordinary search engines, and on-line publishing it is inevitable that in the foreseeable future texts such as these will undergo substantial change as to how they are produced and delivered to you the end user. That stated, we as authors owe a great debt of gratitude to the highly experienced, capable and endlessly patient team at Elsevier. Those who have nurtured us through this process and deserve special mention are: Penny Rudolph, Shelly Stringer, Sara Alsup, and Lauren Harms. Their team approach, gentle coaxing, and guidance is appreciated by the authors more than they will likely appreciate.

The original concept for *The Athletic Horse* came as a result of Reuben Rose and one of the editors (DRH) working together at the Equine Research Laboratory at the University of Sydney in the 1990's. One of the results of that collaboration was the first edition of *The Athletic Horse*. Dr. Rose has now retired from life at the University of Sydney taking up residence at his 140-year-old family farm in south eastern Australia. Given the rigors of maintaining a flock of about 5,000 sheep he thought it wise not to commit to working on this edition. However, one of the individuals Drs. Rose and Hodgson admired during the halcyon days of horse research in the 1980's and 90's was Dr. Ken McKeever. Thus it was only logical that Dr. Rose recommended Dr. McKeever as his replacement in this project. Further, in the early 1990's Drs. Hodgson and Rose identified that a young star was emerging onto the scene in exercise physiology in horses: Cathy McGowan. Given Cathy's work ethic, intellect and organizational skills Drs. Rose and Hodgson agreed that it was essential that Cathy join the editorial team. Two of us (DRH and KHM) attest that this proved to be a masterstroke as Cathy has been the one constant in this project and we, DRH and KHM, are fully cognizant of the fact that the book would never have reached fruition without Cathy's assiduous nature and regular (and necessary) counseling and cajoling of her co-editors.

No book of this nature is possible without the hard work and little rewarded effort of the many authors who have contributed to this project. We asked experts in their discipline areas and they did not disappoint. I hope you believe as we do that these invited authors have made and extraordinary effort in the writing of this text.

We trust you will find this edition useful no matter what your area of interest: veterinarian, exercise scientist, physiologist, student, to name just some groups we have tried to appeal to with this edition.

DRH, KHM, CTM

Contents

SECTION I STRUCTURE CONSIDERATIONS IN EQUINE SPORTS MEDICINE

CHAPTER 1

An Overview of Performance and Sports Medicine

DAVID R. HODGSON, CATHERINE McGOWAN

The study of equine sports medicine, although now out of its infancy, can only be said to be in its adolescence compared with investigations into human exercise and sports science, which appear to be scaling new heights with ever more sophisticated technologies. Early studies of equine exercise physiology at the end of the nineteenth century (Zuntz, 1898) through until the mid-1930s (Procter, 1934) focused on energy metabolism, with particular relevance to the work horse. The working equid is still used widely in many parts of the world (there are an estimated 100 million across the planet) (Food and Agriculture Organization [FAO], 2009), but increasing mechanization in the Western countries has resulted in the horse being used mainly for recreational purposes, with approximately 16 million used for this purpose (FAO, 2009). Revenue from gambling has been an important driving force in the development of the racing industries in many developed countries. As an extreme example, the Hong Kong Jockey Club holds about 700 races per year with a betting turnover of approximately $US9 billion, that is, approximately $12 million per race.

In the 1950s and 1960s, there was an upsurge in interest in the physiology of the athletic horse (Holmes, 1966; Irvine, 1958; Karlsen, 1964; Persson, 1967; Steel, 1960; Steel, 1963); and over the last 20 years, there has been a dramatic acceleration in information that is available from a range of research studies performed around the world. The real pioneer of equine exercise science is Professor Sune Persson, who commenced his studies of the Swedish trotter in the early 1960s. Persson was the first person to use the treadmill to study the physiology of exercise, and the treadmill has gone through a period in which it was widely used for research and on occasion commercial training. Persson's work has stimulated a range of studies throughout the world examining the science of equine exercise. The widespread interest in the physiology of the athletic horse can be gauged by the response to the regular International Conferences on Equine Exercise Physiology (ICEEP) held every 4 years since the first meeting in 1982 in Oxford, England. Clearly, there is both scientific and commercial interest in factors that contribute to the successful athletic performance of the horse.

EQUINE SPORTS MEDICINE AND THE ATHLETIC HORSE

In contrast to earlier investigations into exercise, which were stimulated by the horse's role in agriculture, many more horses are used for recreation today, with the range of equestrian activities becoming increasingly diversified. A variety of breeds are involved in an assortment of athletic endeavors, including Thoroughbred, Standardbred, and Quarterhorse racing; endurance riding; dressage; show jumping; eventing; driving events; vaulting; and the rapidly expanding western riding activities: rodeo, polo, polocrosse, and bull fighting. Riders, drivers, trainers, and veterinarians are better informed, and there is acknowledgment that traditional training and feeding methods require investigation. Appropriate changes can be made, and have been made, to such areas as training strategies in light of new information from research studies. However, it must be acknowledged that there is some disillusionment with equine sports medicine with regard to expectations that the principles used in human exercise science could be transposed easily to training the athletic horse. Several popular publications proposed simple recipes for success in training horses, but these regimens had not received scientific investigation, and many methods proposed were time consuming and ultimately produced no improvement in athletic performance or, indeed, increased injury rates (Ivers, 1983; Swan, 1984). There appear to be no easy methods for producing athletic success in horses. However, the extensive information that is now available from various research studies does provide the opportunity for some guidelines for horse owners, trainers, and veterinarians. Superior athletic performance is multifaceted and is the result of integration of the major body systems involved in delivering energy, as well as critical biomechanical factors (Figure 1-1). Although it is clear that physiologic capacity is closely related to athletic performance, it appears almost impossible to define what contributes to that elusive "will to win" that distinguishes the champion horse within an elite group.

ENERGY DEMANDS OF EXERCISE AND IMPLICATIONS FOR TRAINING

If one compares events as diverse as endurance riding (160 kilometers [km]) and racing (up to 8500 meters [m]),

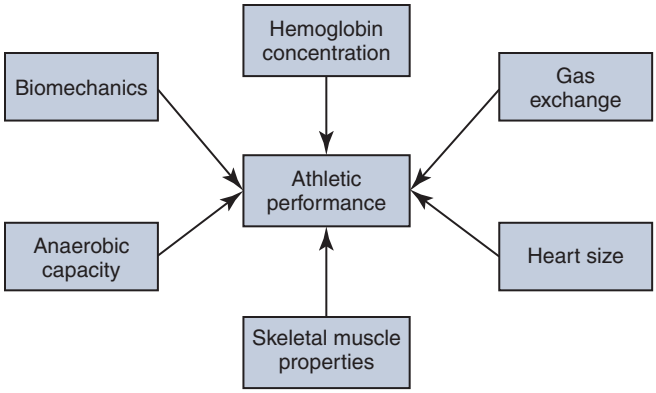

FIGURE 1-1 Some of the major physiological factors contributing to superior athletic performance in horses.

it is clear that there are great differences in energy demands, biomechanical function, thermoregulation, and training strategies. Other athletic activities such as dressage and show jumping focus on biomechanical skills and muscle memory rather than on energy availability. However, in all these activities, an important consideration is the provision of energy from the available reserves, which are chiefly glycogen in the liver and skeletal muscle and fat in various fat depots. Aerobic energy delivery—a function of heart rate, stroke volume, and oxygen extraction by muscle—is the result of a complex chain of events involving the oxygen transport chain (Figure 1-2). In contrast, anaerobic energy delivery is more direct and predominates in the rapid delivery of energy for brief periods of intense exercise (Figure 1-3). A detailed consideration of energy utilization during exercise is provided in Chapter 3.

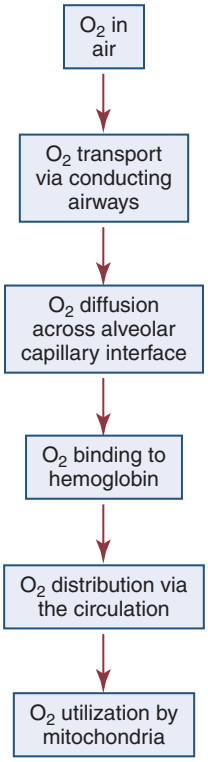

FIGURE 1-2 The oxygen transport chain showing the various steps in transport from the air breathed in to final utilization by the mitochondria.

Knowledge of the patterns of energy use in different competitive events allows specific training strategies to be adopted to maximize the adaptations in various body systems. Although aerobic and anaerobic energy supplies coexist in all events, aerobic energy production predominates in the majority of equine competitive activities. This has important implications for training strategies because an emphasis on aerobic training appears to be an important foundation for all events. However, much more information is required for assessing different training schemes and experimental methods to determine such factors as:

- The selection of horses for specific events prior to the onset of training
- Specificity of training for speed versus stamina versus muscle memory
- The rate of decrease in fitness following cessation of training
- The optimal age to commence training for the discipline the horse will participate in

Many of these questions can be answered by specific experiments, but it is doubtful whether sufficient resources are available to determine whether physiologic measurements may be used to forecast future athletic performance accurately.

TRAINING AND MUSCULOSKELETAL INJURIES

The major problem in training horses for athletic activities is to keep them free of injury. Studies examining racehorses in training have shown that by far the most common reason for wastage is musculoskeletal injury (Evans, 2002; Jeffcott, 1982; Perkins, 2005; Rossdale, 1985). A variety of factors are involved, including the conformation, training surface, age of the horse, and stage of training. However, as yet, few studies have examined the role of training in maximizing the strength of soft tissue and bone. Those studies which have been performed are reviewed in Chapter 13.

LIMITATIONS TO PERFORMANCE

Fatigue is a complex chain of events, with central as well as peripheral contributions. Short-duration, high-intensity exercise such as is performed in Thoroughbred, Quarterhorse,

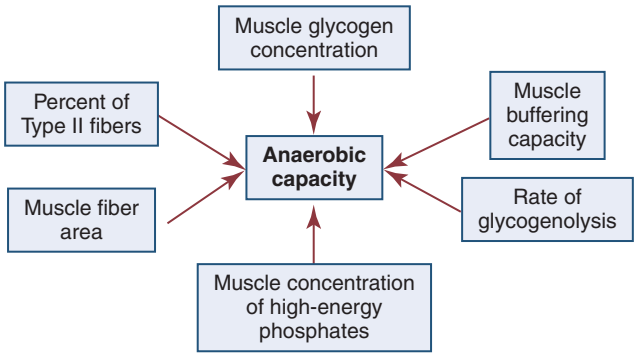

FIGURE 1-3 A summary of important factors contributing to anaerobic capacity.

and Standardbred racing is not limited by availability of substrates but, more likely, by failure of energy production associated with an increase in protons and a decrease in adenosine triphosphate (ATP). In contrast, longer-duration exercise such as endurance riding results in substantial muscle glycogen depletion, which eventually may limit the horse's capacity to continue to exercise. (See also Chapter 3). In addition, long-distance exercise also imposes substantial thermoregulatory demands, with evaporative cooling from sweat production as the major mechanism for heat dissipation (see Chapter 8).

OVERVIEW OF THE APPLICATION OF SPORTS MEDICINE AND EXERCISE SCIENCE IN THE ATHLETIC HORSE

The clever application of science to horse racing will achieve much more than faster racing times. The benefits range from fewer working hours spent in training with identification of the most effective training methods to fewer injuries and improved equine welfare. The long-term financial benefits for owners and trainers are likely to be substantial. Indeed, the involvement of science in the horse racing industry should be encouraged as a matter of priority if the existence of the industry is to be secured for the future.

AIM OF SCIENTIFIC INVESTIGATION

The aim of science is to test hypotheses through objective measurement. Much that is written or spoken about horses represents little more than qualitative opinions. For example, the horse's appearance often is assessed by observation. Muscles are described as "looking firm," or the jockey "feels" that the horse is "running better." In contrast, science deals with measurable numbers: How many grams of carbohydrate were utilized, and how much oxygen was consumed in a 2000 m race? What was the horse's blood lactate concentration after a prescribed bout of exercise? What were the actual speed of training, cumulative distance at different speed ranges, altitude, and heart rate, and how do these factors correlate with race performance or injury?

The scientist tests the validity of any hypothesis by applying statistics to the numbers that have been collected. This allows an assessment of whether the intervention under study produced an effect and whether that effect could be ascribed to chance alone or resulted directly from the specific intervention.

Questions that are open to scientific investigation and worthy of analysis include:

- What key physiologic factors determine athletic success in racing horses?
- What is the nature of the fatigue experienced by racing horses?
- How can race horses be better prepared to resist the onset of fatigue? That is, what are the optimal training methods?
- How can we ensure fewer injuries during training and racing?

Some of these issues have, of course, been addressed over the past decade, particularly those relating to "wastage," that is, days or horses lost to training and racing. However, much of the available information on the other points listed above has changed relatively little.

TECHNIQUES AND INSTRUMENTATION NEEDED FOR LABORATORY RESEARCH OF THE ATHLETIC SPECIES

Horses are considerably harder to study than are humans. Apart from the difficulty in obtaining their complete cooperation, the added complication is that precautions need to be taken to ensure their safety and that of the researchers. Despite this, various tools have been developed to enable the evaluation of various aspects of exercise physiology.

TREADMILL

The treadmill allows the investigator to study the athlete during exercise of any intensity as well as at rest before or after exercise. Tests more specific to horses that can be performed on a treadmill include gait analysis, endoscopic evaluation of upper airway function, and tests of exercise performance. It is the last test that has been used most widely for, among others, determining superior athletic ability; explaining poor racing performance in individual horses; identification of possible physiologic factors that may alter with training; and studying the biochemical and physiologic nature of fatigue during exercise (Figure 1-4).

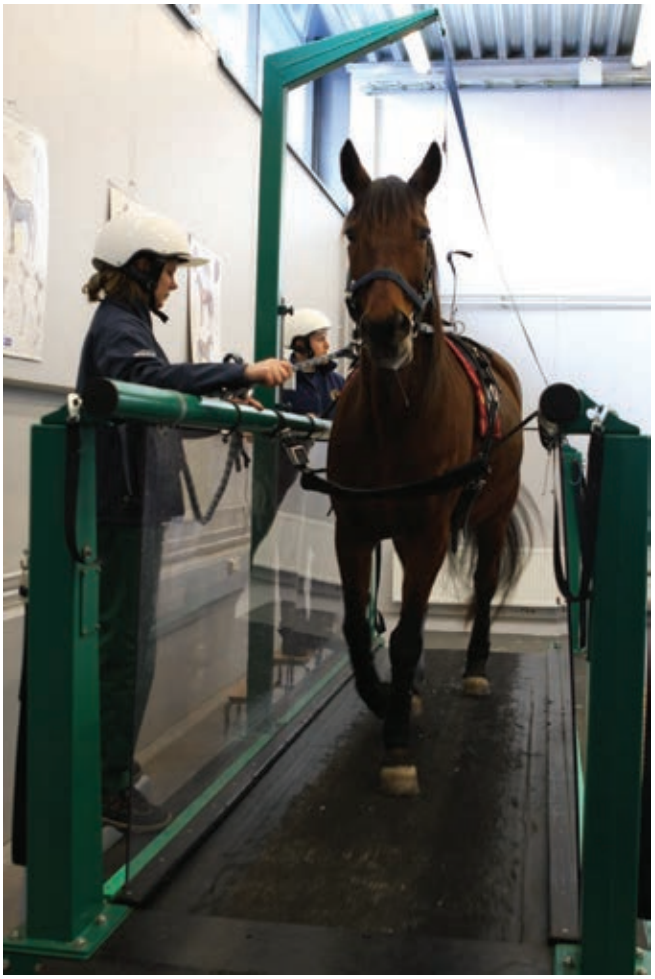

FIGURE 1-4 A Säto (SÄTO AB) used for performance evaluation at the University of Helsinki Equine Hospital. *(Photo courtesy Päivi Heino.)*

Treadmill exercise is not equivalent to track exercise. The effects of air movement, track surface, and rider impact are not duplicated on the treadmill, and horses have no forward momentum on the treadmill because the moving belt provides the driving force. Therefore, the amount of work performed by a horse on the treadmill is quantitatively different from work on the track. For a track exercise test, horses require only a short habituation period and can be worked in their standard manner, often with the usual rider or driver (Sloet van Oldruitenborgh-Oosterbaan and Clayton, 1999). Nevertheless, there are clear advantages to studying responses to exercise on the treadmill. A consistent exercise surface, controlled environmental conditions, precise control over intensity of exercise, and ease of measuring physiologic variables to monitor fitness are all strong indications to pursue treadmill-based studies (Sloet van Oldruitenborgh-Oosterbaan and Clayton, 1999). By positively inclining the treadmill, a horse can be exercised at its maximum power output at a relatively slower speed than if it were on a flat plane (Sexton and Erickson, 1990). This potentially reduces the risk of musculoskeletal injury because speeds above 12 to 13 meters per second (m/s) are unnecessary, but the steeper the slope, the more likely is an adverse impact on normal biomechanical function. It is suggested that muscles may be recruited differently when the horse is exercised on a slope versus a flat plane (Sloet van Oldruitenborgh-Oosterbaan and Barneveld, 1995). A slope of 10% (5.71 degrees) is recommended for treadmill testing, as most horses will reach their maximum oxygen uptake at speeds of 10 to 12 m/s compared with 14 to 15 m/s on a flat plane. It is desirable to standardize the incline that exercise tests are performed on, to allow better comparison between studies from different institutions (Sloet van Oldruitenborgh-Oosterbaan and Clayton, 1999).

Significant differences in locomotor and metabolic variables have been reported in studies comparing track exercise with treadmill exercise, and future research may continue to elucidate the etiology of these differences. Currently, treadmill tests are preferable for most research purposes, but track tests may be of greater importance when examining the locomotor variables and fitness of sport horses (Sloet van Oldruitenborgh-Oosterbaan and Clayton, 1999).

RESPIRATORY GAS ANALYSIS AND DETERMINATION OF $\dot{V}O_{2MAX}$

Air expired during exercise can be measured for its oxygen and carbon dioxide content, thereby allowing the calculation of the rate of oxygen consumption during exercise of different intensities. Measurement of oxygen consumption allows calculation of energy expenditure at any specific workload or running speed and gives an idea of an individual horse's efficiency or "economy of movement." The respiratory exchange ratio (RER), which is calculated as the ratio of carbon dioxide (CO_2) production to oxygen (O_2) consumption, provides an indication of the relative proportions of the metabolic fuels that are used at a specific exercise intensity.

Maximal Oxygen Consumption

Maximal oxygen consumption ($\dot{V}O_{2max}$) is the maximal amount of oxygen used by the athlete during maximal exercise to fatigue. It is determined by increasing the workload or speed of the treadmill in a stepwise manner with continuous monitoring of the rate of oxygen consumption. $\dot{V}O_{2max}$ is sometimes termed the *peak aerobic power*.

Thoroughbred racehorses have $\dot{V}O_{2max}$ values of 160 to 200 mL O_2/kg/min (Noakes, 1992; Rose et al., 1988), more than twice that of elite human athletes on a per kilogram bodyweight basis. The higher $\dot{V}O_{2max}$ values of the racehorse are best understood in terms of the physiologic factors that determine how rapidly oxygen can be transferred from the air to the active muscles where it is used (see Figure 1-2).

The rate of oxygen consumption can be calculated as the product of the cardiac output (CO) multiplied by the difference in the oxygen content between arterial and venous blood. The difference in the (high) oxygen content of arterial blood traveling to the muscles and the (much lower) oxygen content of venous blood returning to the heart, having delivered much of its oxygen to the active muscles, is known as the *arteriovenous* O_2 *difference* [(a-v)DO_2].

Therefore,

$$\dot{V}O_2 = CO \times [(a\text{-}v)DO_2]$$

CO is the volume of blood pumped by the heart each minute. It is the product of heart rate (HR) and stroke volume ($S\dot{V}$), which is the amount of blood ejected from the heart with each contraction.

$$CO = HR \times SV$$

Cardiac output increases during exercise as a result of an increase in both HR and SV. During exercise, both these responses occur to a greater extent in horses than in humans. For more details see Chapters 3, 9 and 11.

Blood Oxygen Content

The amount of oxygen carried in arterial blood is dependent on the concentration of red blood cells (RBCs) in the circulation and their hemoglobin content. Respiratory disorders that may interfere with the transport of oxygen from the atmosphere to the pulmonary vasculature can be assessed for their significance by measuring arterial blood gas tensions. Arterial blood samples are normally collected from a catheterized transverse facial artery during a treadmill exercise test. Values should be corrected for central venous blood temperature. Hypoxemia and hypercapnea are recognized responses to high-intensity exercise (Bayly et al., 1983; 1987) and the severity of hypoxemia increases with training. There is a strong negative correlation between minimum arterial oxygen content and $\dot{V}O_{2max}$ in trained horses, indicating the importance of assessing both variables before interpreting blood gas data (Christley et al., 1997).

Another measure of the number of circulating RBCs is the hematocrit, which is the percentage of the total blood volume occupied by RBCs. The horse has the unique ability during exercise, to release a large number of RBCs from their storage site in an intra-abdominal organ, the spleen. As a result, the hematocrit of the horse can increase from around 32% to 46% to 60% to 70% during maximal exercise (Snow and Vogel, 1987). This ability dramatically increases the oxygen-carrying capacity of the horse's blood during exercise.

HEART RATE

Specific heart rates are seldom used as a predictor of athletic performance. However, equine physiologists use a measure termed V_{200}, which is the velocity a horse achieves at a heart

rate of 200 beats per minute. V_{200}, which is said to approximate the maximal aerobic power achieved by the horse, is calculated from heart rates measured during a series of runs at different speeds on a treadmill or on the racetrack. V_{200} can be used as a simple, yet effective, measurement to monitor training adaptations during and at the completion of a training program. Increases in V_{200} would be interpreted to indicate a favorable training adaptation, whereas the reverse would apply if V_{200} fell.

In addition, measurement of heart rate can be a most valuable training aid because it allows for accurate control of the intensity of any exercise training session (Foreman et al., 1990). In general, it is now believed that intensity is the most important variable in the training program, since the extent of the training adaptation is determined by the intensity, rather than the volume, of training (Noakes, 1992). However, if training intensity is excessive, injury and overtraining are the likely results. For more detail of the use of heart rate in training the reader is referred to Chapter 11.

BLOOD TESTS

Blood samples are taken routinely during exercise tests as specific indicators of exercise intensity and fitness and to monitor the health of athletes prior to racing. For more information see Chapter 5.

Blood Lactate Concentration

In humans as well as horses, the most common blood biochemical measurement is the *blood lactate concentration*. During exercise of progressively increasing intensity, the lactate concentration rises progressively from the resting concentration of about 1 millimole per liter (mmol/L) (Figure 1-5). The exercise intensity at which lactate concentrations rise more steeply is often termed the *lactate threshold*, which occurs at a lactate concentration of 4 mmol/L, the V_{LA4}. Traditionally, the V_{LA4} has been considered to approximate the anaerobic threshold, mirroring the metabolic transition from predominantly aerobic to anaerobic energy sources, and this calculated value increases with improved fitness. This threshold has been used as a measure of both fitness and athletic ability in humans and possibly horses. In general, less fit horses show a rise in blood lactate concentration at lower exercise intensities or running speeds, and hence they exhibit an earlier lactate threshold compared with fit horses. The same relationship is seen in better-performing human athletes (Noakes, 1992) (Figure 1-6). Other studies have shown that blood lactate concentrations after maximal exercise are higher in better-performing horses (Persson and Ullberg, 1974; Räsänen et al., 1995).

It is believed that increasing acidity, shown as a fall of the pH in active muscles, is a contributor to fatigue during exercise of very high intensity and short duration, as typified by horse races of 1000 to 3000 m. Muscles with an increased buffering capacity are more resistant to changes in pH and thus have a greater capacity to continue contracting during high-intensity exercise.

Blood Glucose and Insulin Concentrations

Premature fatigue during prolonged exercise lasting more than 1 to 2 hours can be caused by hypoglycemia (low blood glucose concentration) (Coggan and Coyle, 1991). Monitoring of the blood concentrations of glucose and insulin, the hormone that regulates the blood glucose concentration, can determine if hypoglycemia is the cause of fatigue during this type of exercise.

Hormone Profiles

Standardized exercise tests are suggested to provide a way to detect subtle changes in hormonal responses in the individual, which may make an important contribution to the detection of early overtraining (McGowan and Whitworth, 2008).

De Graaf-Roelfsema et al. (2009) successfully used the resting pulsatile growth hormone (GH) secretion pattern to detect the (over-)training status of Standardbreds in an experimental setting, although in the field setting measurements of the serum concentrations of insulin-like growth factor 1 (IGF-1) and IGF-2 are more practical and good indices of GH status. Furthermore, the serum concentrations of IGF-1 and IGF-2 are relatively constant during the day, so stimulation testing or multiple sampling is not necessary (de Graaf-Roelfsema et al., 2009).

Blood Enzyme Assays

The condition of rhabdomyolysis, or "tying-up syndrome," can be diagnosed by measuring the activity of certain enzymes

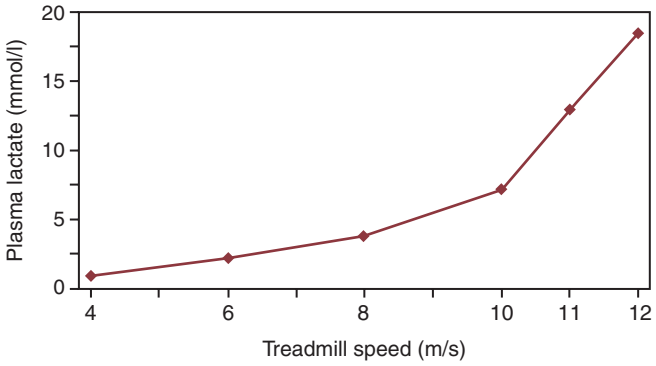

FIGURE 1-5 Normal plasma lactate response to exercise in a 3-year-old thoroughbred horse during an incremental exercise test on a treadmill inclined at a slope of 6 degrees (10%).

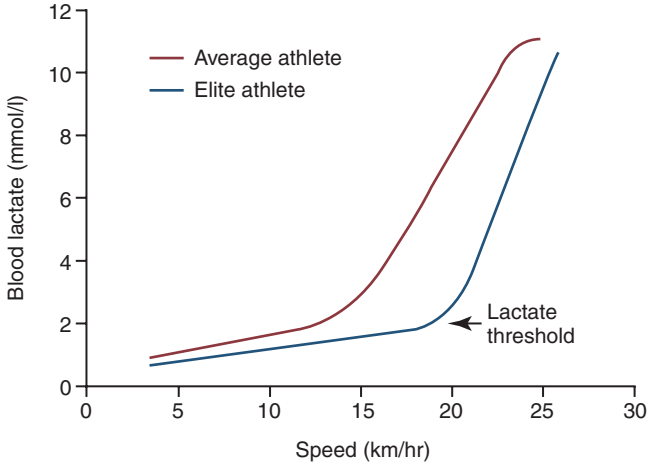

FIGURE 1-6 The lactate curve with increasing speed for an average and elite human athlete.

released from damaged muscles into blood. The condition is brought on by exercise and can present variably from stiffness to an inability to move. As a result of the muscle damage that causes the condition, the activities of the muscle enzymes creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) increase in the bloodstream. Muscle enzymes can also increase due to cumulative muscle damage associated with intense, unaccustomed, or eccentric exercise and may be associated with overtraining (McGowan, 2008; see Chapters 5 and 12).

MUSCLE BIOPSY

In this procedure, a small sample of muscle is removed via insertion of a hollow cutting needle through a small cut in the skin (Snow and Guy, 1976). Muscle samples are commonly tested for carbohydrate (glycogen) content, muscle enzyme activities, and muscle fiber typing. Muscle fiber composition has been correlated with locomotor patterns in horses (stride frequency and stride length) (Rivero et al., 2006) and, therefore, may indirectly influence the economy of locomotion. For more detail of muscle adaptations to training the reader is referred to Chapter 12.

VALIDATION OF NEWER TECHNICAL SYSTEMS FOR USE IN HORSES

All scientists are tempted to quickly adopt the most recent and most innovative of equipment as it becomes available on the commercial market. Most of this newer equipment is routinely developed for use in the human medical market. The veterinary marketplace is much smaller and secondary to the size, volume, and financing available in the human medical equipment market. Especially with equipment developed primarily for use in human medicine, it is critical to remember that unlike the famous movie line "If you build it, they will come," the adoption of newer technology should not be a matter of "If you can measure it, it must be correct." It is incumbent on all equine exercise physiologists, as for all scientists, to validate the use of that equipment in the equine patient or equine athlete.

Heart Rate Monitors

An early example cited above is the validation of the early on-board HR monitors for use in equine exercise physiology research (Evans and Rose, 1986). Conversely, one recent paper using HR monitors cited validation data in humans but not in horses and then went on to use the monitor as if it were validated for use in horses (Cottin et al., 2006). Again, ideally, it would have been best if this equipment had been validated for use in the species of interest in the paper reporting the data. Newer HR monitors such as the Kruuse Televet system are validated for use in horses and have overcome many of the early difficulties with contact, interference, and movement (Figure 1-7).

FIGURE 1-7 Televet electrocardiography (ECG) system (Kruuse, UK) being used on a horse, Philip Leverhulme Equine Hospital, University of Liverpool.

Blood or Plasma Lactate Analyzers

The studies by Evans and Golland (1996) and Butudom et al. (2010) have both documented the accuracy of the YSI lactate analyzers for use in equine exercise physiology. Other portable on-site lactate analyzers still used daily in equine research have been shown to be less than accurate when compared with benchtop standards or to the validated YSI analyzers or have been shown to be accurate but with limited ranges of accuracy (Butudom et al., 2010; Evans and Golland, 1996). Some nonlactate dedicated point-of-care analyzers have also been shown to be accurate for use in equine research (Silverman and Birks, 2002).

Plasma lactate values are 30% to 50% higher than whole blood lactate values. However, because of great interindividual variation in lactate distribution between plasma and RBCs after exercise and in the rate of lactate influx into RBCs, there is no consistent relationship between the two lactate reservoirs (Pösö et al., 1995). Recent evidence suggests that when estimating the accumulation of lactate from exercising muscle, whole blood lactate concentrations should be measured to minimize any variations caused by factors that influence transport of lactate from plasma into RBCs (Väihkönen et al., 1999). If whole blood is to be used, the sample should be immediately deproteinized to halt postcollection production of lactate within the RBC; however, storage at 0°C (32°F) for up to an hour before deproteinization does not affect the lactate concentration (Ferrante and Kronfeld, 1994). Whether plasma or whole blood lactate is assessed, one method should be adhered to by the laboratory or the investigator to reduce variability in measurements.

Respiratory Gas Analyzers

Many original studies were published using large nonportable oxygen collection systems for determination of ventilation and oxygen consumption (Bayly et al., 1987; Evans and Rose, 1988). The problem with these systems is that they are cumbersome and restricted to use in the laboratory setting. Initial attempts at a portable respiratory measurement system were troubled by insufficient reliability. Newer technology has allowed the development of portable respiratory measurement systems. These small self-contained systems include a plastic airtight face mask and miniaturized portable analyzers that allow use of the system in field settings such as galloping training for Thoroughbred racehorses (Art et al., 2006). Twin low-resistance turbines are placed, one over each nostril, to measure air flow from the nostril using an electronic optic reader, which determines the number of turbine rotations per second. The manufacturer specifies

that the system has a linear response for ventilation from 0 to 5000 liters per minute (L/min), with resistance of each nasal turbine of 0.7 cm H_2O/L at a flow rate of 120 liters per second (L/s). Expired air is sampled breath by breath from a sampling port located at one of the nasal turbines to a 210-cm sampling line feeding into miniaturized oxygen and carbon dioxide analyzers. Data can be relayed by telemetry or stored for later downloading after the exercise is completed. This new portable system was shown to yield reproducible results in one trial (Art et al., 2006). In a second trial, results were comparable with an accepted reference method of $\dot{V}O_2$ determination at the walk, trot, canter, and gallop in five healthy saddle horses (Art et al., 2006), but there were differences in VCO_2, V_E, and $FETCO_2$, which became "more marked with increasing exercise intensity and more significant the higher the workload."

Global Positioning System

The global positioning system (GPS) is used routinely for obtaining locations and directions while driving a vehicle. Several groups have recently published their works that examine the use of GPS technology to measure the speed and distances over which horses train in everyday settings (Gramkow and Evans, 2006; Hebenbrock et al., 2005; Kingston et al., 2006; Vermeulen and Evans, 2006).

Kingston and coworkers (2006) reported on the combined use of GPS and HR monitors in assessing training load in young Thoroughbred racehorses. Their intent was to determine the validity of using the GPS to measure work speed as opposed to the traditional use of trainers' stopwatches. They reported that the GPS had a sample rate of once every 5 seconds with a variation in measured speed of up to 0.6%. "The average speeds determined with the GPS system were in agreement with average speeds timed by the trainer. However, peak speeds reached during training were significantly greater than those estimated with stopwatch timing." The authors stated, "The results from this study show that a GPS/heart rate monitor system provides a reliable measure of daily workload in horses during training." The investigators concluded that this technology provides "an indication of relative work intensity together with the means to download and store such data. This information should be useful in assessing responses to training . . . ."

In a similar study on Thoroughbred racehorses in training, Vermeulen and Evans (2006) showed that combining GPS technology and heart rate monitors allowed determination of traditional measures such as HR_{max}, VHR_{max}, and V_{200}. They showed that VHR_{max} was reliable regardless of the velocity, and that VHR_{max} and V_{200} increased with training. They concluded that "velocity and HR measurements during field gallop exercise provided reliable measures of fitness which enabled a measurement of the response to training . . . . This approach offers a simple, noninvasive method for monitoring adaptations to training in the field." In a second study from the same laboratory using the same technologies (Gramkow and Evans, 2006), Thoroughbred racehorses with higher VHR_{max} earned more money per start ($r = 0.41$, $p < 0.05$) and horses with VHR_{max} <14.5 m/s had mean per race earnings <$2500. It was concluded that "field studies of the relationship between HR and velocity with a GPS enable identification of horses with limited earnings" and that "the technique has potential application in commercial training environments assisting with decisions concerning racing careers of individual racehorses."

The applicability of GPS technology is not unlimited. The system works only outdoors and only when true signals are obtained from and to the satellite; cloudy weather may impede the signal. Although detailed records of a horse's workout are obtainable, someone must still expend the time and effort to examine and make conclusions from the data obtained and processed through the commercial software.

FUTURE OF EQUINE SPORTS MEDICINE

For generations, the care and training of athletic horses have been based on tradition. This accent on horse husbandry continues to be a key to successful athletic performance, but some of the principles of exercise physiology have found their way into commercial training programs, as well as being available for use by specialized veterinary practices. An increase in the training of research students in the exercise sciences has provided, and will continue to provide, the knowledge base and expertise for wide areas of the equine industry.

Much of the basic knowledge of the physiology and biochemistry of equine exercise is now known. However, a great deal of the available information is purely descriptive, and therefore, considerable further research is required before specific recommendations can be made about optimal training methods or selection for performance potential. It is open to debate whether the time and funds are available to reach these goals. The high expectations for improved performance in athletic horses as a result of the use of human athletic training principles have given way to more modest hopes. Applications of the principles of exercise physiology have enabled, and should enable, improved fitness of athletic horses and a reduction in the incidence of limb injuries, which is currently quite high.

Identifying the horse with outstanding physiologic potential for a particular competitive event prior to commencement of training is one of the unrealized dreams of those involved in equine exercise physiology. Although the various factors associated with superior performance are well known, the weighting of these and the potential for forecasting athletic success from physiologic indices remain unknown. To undertake a project to examine the predictors of performance, a huge range of resources is required so that a large number of weanlings and yearlings could be assessed and evaluated against some objective indices of performance. This has such obvious commercial ramifications that it seems likely that the project will eventually be undertaken, although the results may never be published.

CONCLUSION

The athletic horse is a remarkable animal, with both grace and stamina. A high ratio of heart weight to body weight, large mass-specific cardiac output, and substantial capacity for oxygen carriage resulting from splenic erythrocyte release during exercise all contribute to the potential of the athletic horse to run at speeds up to 10 to 12 m/s for long distances and reach peak speeds of 17.5 to 18 m/s. We need to better understand the limitations to equine performance as well as the adaptations that are possible for the wide range of equine competitive activities.

A more scientific approach to the application of training methods shown to be effective in humans might both improve the athletic ability of Thoroughbred horses and also reduce their high incidence of injury. Indeed, human athletes benefit from the input of a team of advisers, including coaches, trainers, exercise physiologists, physiotherapists, and medical doctors. In the sport of horse racing, in which communication with the athlete is vital but virtually impossible, it would seem that the assistance of a qualified team would be even more important. The probable result of this team approach would likely be improved racing times, fewer injuries, stronger and healthier racehorses, and a more prosperous horse racing industry.

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CHAPTER

Comparative Aspects of Exercise Physiology

2

DAVID R. HODGSON, AND J.H. FOREMAN*

Besides appreciating their own athletic ability, humans, uniquely, have also trained other animals to compete in athletic events, stimulating scientific investigation of the athletic capabilities of many other species, including the racehorse. This chapter examines some different animal species involved in athletic sports; it briefly considers historical aspects of that involvement, the physiologic factors that might explain differences in athletic ability among different species, and the nature of the improvements in the athletic achievements of these different species over the last century. This information provides insight into those possible factors whose modification might enhance the efficiency and success of training Thoroughbred racehorses.

MAIN ATHLETIC SPECIES

Of all the athletic species in the world, four have dominated exercise physiology studies. These are (1) the athletic human, (2) the racehorse, (3) the greyhound, and (4) the racing camel (Figure 2-1). Of these species, the human athlete has received the most attention and the greyhound and the camel the least. The racehorse occupies an intermediate position.

Interest in the physiologic changes that occur during exercise in humans began at the end of the nineteenth century. By the early 1920s, scientists began to address practical questions, including the possible biochemical and physiologic causes of the fatigue that develops during exercise (Gordon, 1925; Hill, 1923). However, it was only from the late 1960s that research in sports medicine and the exercise sciences began to develop as a reputable academic discipline. Perhaps two key reasons stimulating this development were (1) the trend toward an increased interest in health internationally, and (2) the growing dominance of international sport by athletes from eastern European countries, especially the former German Democratic Republic (East Germany). The success of athletes from socialist countries posed a challenge to the Western nations, especially those from the United States, who were anxious that the perceived superiority of the capitalist system in all spheres of human endeavor should not be undermined. Thus, financial and political support for sports-related research increased in most Western countries, stimulating the rebirth of these disciplines on a global scale.

In contrast, the exercise sciences have received little financial and intellectual support in the horse racing community. In his book, *The Fit Racehorse*, Tom Ivers (1983) has written that "[the racehorse] industry honors the past more ferociously than it defends its own existence." This ethos likely remains true in the twenty-first century. It is very apparent that the principal focus of the horse racing industry remains in its past, that is, in examining the breeding history of the species, especially of its champions. Limited attention is paid to its future, which should involve the application of scientific knowledge and techniques to the study of horses and horse racing. As a direct result, progress in equine exercise science research lags behind human exercise research science by decades.

Perhaps there are two major reasons for the general lack of interest that the horse racing community has for the application of science to its industry. First, any new development, be it a training technique, nutritional advance, apparatus, or drug treatment, that may aid performance is seldom, if ever, adequately evaluated in a scientifically valid manner. In part, this may be because of financial pressures that induce artificial time constraints. In their perpetual search for a "quick fix," owners and trainers eschew the protracted process necessary for adequate scientific evaluation of these interventions. As a result, no distinction can be made between quackery and interventions that may be of real long-term value to the industry. This is clearly to the ultimate detriment of the horse racing industry.

A second possible explanation is that in the majority of races, the winning time is unimportant because there are no additional financial rewards for record performances. Patrick Cunningham (1991) wrote that "nobody is much interested in improving the average racing times . . . what does it matter if all horses race 10 percent faster?" Hence there is no incentive to improve the performances of all racehorses progressively and in a systematic manner.

THE HUMAN ATHLETE

Competitive racing for humans and horses shares a common origin—the use of these species for transport. The Greeks and the Romans used runners to deliver messages by foot, often over long distances. This tradition continued in Britain in the tenth century A.D. However, only from the seventeenth century onward was running established as a competitive sport, originating mainly in Britain (Noakes, 1992).

Distances

The very earliest human footraces were usually over extremely long distances. It is recorded that a race of 237 kilometers (km) (147 miles) took place in Rome during the Roman Empire (Noakes, 1992). Recognized distances for modern footraces range from sprints of 100 meters (m) to ultramarathon races of 1000 km or more, lasting many days. The top speeds achieved by human sprinters exceed 36 kilometers per hour

*The authors acknowledge the work of T.D. Noakes on this chapter in the previous edition.

FIGURE 2-1 The four main athletic species showing the relative maximum speeds during exercise. Maximum speeds in the different species are 19 meters per second (m/s) (Thoroughbred horse), 16.6 m/s (greyhound), and 10 to 11 m/s (human athlete and racing camel).

(km/h; 22 miles/h), whereas speeds of around 16 km/h (10 miles/h) are more common in marathon races of up to 100 km and of 6 to 8 km/h (4 to 5 miles/h) in races of 1000 km or more.

THE EQUINE ATHLETE

Horse racing originated with the Bedouins of the Middle East, who dehydrated their horses and trained them to race to the nearest water hole. It was an event in the Greek Olympic Games as early as 664 B.C. The first recorded race meeting in Britain was in the twelfth century in London. However, it was only in the late seventeenth century (circa 1665) that organized Thoroughbred horse racing, as we now know it, first took place in New York and in Newmarket, England (Kidd, 1976).

Distances

The earliest horse races were run over distances of 6 miles. However, the distances of modern track races for Thoroughbreds vary from 1000 m for "sprinters" to the longer 3000- to 7000-m races for "stayers." Endurance races of 80 to 160 km (50 to 100 miles) and longer are also held over all types of terrain. Quarterhorses race over 400-m tracks, attaining top speeds of up to 70 km/h (44 miles/h).

THE RACING CAMEL

The camel is known for its endurance ability in hot, dry environments. In the Middle East, specially bred camels are raced over distances of 4 to 10 km and achieve speeds of approximately 36 km/h (22 miles/h) (Evans et al., 1992).

RACING DOGS: THE GREYHOUND

From as early as 1835, greyhounds were raced against each other, sometimes in races involving as many as 64 participants (Genders, 1990). In 1858, the sport was officially organized with the formation of a governing body, the National Coursing Club, in Britain.

DISTANCES

The length of greyhound races varies from 250 m sprints to "long-distance races" of 600 to 1000 m. Dogs can reach speeds of up to 60 km/h (37 miles/h) during races of up to 500 m.

RACING DOGS: THE HUSKY

The endurance ability and resistance to cold temperatures of the husky and related species have long been recognized. These dogs are the principal means of transport for polar travel. The first races were held in Alaska in 1907 over 600 km (400 miles) (Sayer, 1989). The most famous modern race is the Iditarod, in which dogs race from Fairbanks to Nome in Alaska over 1050 to 1150 km. This race takes 8 to 14 days to complete (www.iditarod.com/about/, 2011).

COMPARATIVE PHYSIOLOGY OF THE ATHLETIC SPECIES

AEROBIC POWER OR $\dot{V}O_{2MAX}$

Oxygen Consumption

In both humans and horses, measurement of oxygen consumption allows calculation of energy expenditure at any specific workload or running speed and gives an idea of an individual horse's efficiency or "economy of movement." Maximal oxygen consumption ($\dot{V}O_{2max}$) is the maximal amount of oxygen used by the athlete during maximal exercise to exhaustion. $\dot{V}O_{2max}$ is sometimes referred to as the *peak aerobic power.*

In humans, there is a trend for the best athletes to have the highest $\dot{V}O_{2max}$ values. Elite human athletes have $\dot{V}O_{2max}$ values ranging between 69 and 85 milliliters oxygen per kilogram per minute (mL O_2/kg/min), whereas Thoroughbred racehorses have $\dot{V}O_{2max}$ values twice as high, about 160 to 200 mL O_2/kg/min (Noakes, 1992; Rose et al., 1988).

Cardiac Output

Cardiac output (CO) is the volume of blood pumped by the heart each minute and increases during exercise as a result of an increase in both heart rate and stroke volume. During exercise, both these responses occur to a greater extent in horses than in humans.

Resting heart rate (HR) values are in the low twenties in fit horses, whereas values of 40 to 60 are more usual in athletic humans under the same conditions. During exercise, maximal HR values of between 240 and 250 beats per minute (beats/min) have been recorded in racehorses, whereas maximal values in the range of 180 to 200 beats/min are more common in athletic humans (Evans and Rose, 1988; Noakes, 1992).

Thus, the Thoroughbred racehorse has the ability to increase its HR almost 10-fold from rest to maximal exercise, whereas in humans this range is of the order of three- to fourfold. This difference contributes in large measure to the greater $\dot{V}O_{2max}$ of the Thoroughbred racehorse compared with the elite human athlete.

The greyhound has a maximal heart rate of about 300 beats/min, only a threefold increase from resting values (Snow, 1985). $\dot{V}O_{2max}$ in this species is in excess of 100 mL O_2/kg/min, although this has proven difficult to measure.

The camel, in contrast, has the lowest $\dot{V}O_{2max}$ value (51 mL/kg/min) of the four common athletic species, the horse, the greyhound, the camel, and the human (Evans et al., 1992) (Table 2-1). The racing camel can increase its HR fourfold from a resting rate of about 33 beats/min to about 150 beats/min during maximal exercise.

Stroke Index

The resting stroke index (SI, divided by body weight) for horses is between 1.3 and 2.3 mL/kg, increasing to 2.5 to 2.7 mL/kg during maximal exercise (Physick-Sheard, 1985), similar to the human resting value of 1.1 to 1.4 mL/kg, which increases to around 1.5 mL/kg during maximal exercise (Ganong, 1985). Although the stroke index increases in both humans and horses, the increase is, at most, of the order of one- to twofold. Hence it is the much larger (fourfold to

TABLE 2–1

Comparative Table for $\dot{V}O_{2max}$, Heart Rate, Peak Blood Lactate, Hematocrit, Stroke Index, and Muscle Fiber Composition

	Human Athlete[a]	Thoroughbred Racehorse	Greyhound Dog[b]	Racing Camel[c]
$\dot{V}O_{2max}$ (mL O_2/kg/min)	69–85	160[d]	100	51
Resting HR (beats/min)	40–60	20-30[e]	100	33
Max exercise HR (beats/min)	190	240[f]	300	147
Resting stroke index (mL/kg)	1.1–1.4[g]	1.3–2.3[f]	–	–
Max stroke index (mL/kg)	1.5[g]	2.5–2/7[f]	–	–
Resting hematocrit (%)	40–50	32–46	54[h]	33[i]
Max hematocrit (%)	40–50	60–70	64[h]	36[i]
Peak lactate (mmol/L)	15	30[f]	20	12
Muscle fiber composition	Sprinters type II >75%	Sprinters type II[e]>80%	Type II >75%	Type I >70 %
	Endurance type I >75%	Endurance type I[k]~30%	–	–

Data from [a]Noakes; [b]Snow; [c]Rose and colleagues; [d]Rose and colleagues; [e]Snow and Vogel; [f]Physick-Sheard; [g]Ganong; [h]Snow and colleagues; [i]Evans and colleagues; [j]McMiken; [k]Rose.

10-fold) increase in HR that is the main contributor to the increase in CO during exercise in both racehorses and human athletes.

Blood Oxygen Content

The hematocrit is the percentage of the total blood volume occupied by red blood cells (RBCs). The human athlete maintains this hematocrit value at between 40% and 50% (Wilmore and Costill, 1988). During exercise, in humans, the hematocrit tends to rise slightly as a result of a fall in the amount of fluid, the plasma volume, in which the RBCs circulate. The total number of RBCs contained in that volume may increase only slightly during exercise in humans.

In contrast, the horse has the unique ability, specifically during exercise, to release a large number of RBCs from the spleen. As a result, the hematocrit of the horse can increase from around 32% to 46% to 60% to 70% during maximal exercise (Snow and Vogel, 1987). This ability dramatically increases the oxygen-carrying capacity of the horse's blood during exercise. Greyhounds have high resting hematocrit levels of about 54%; these increase to around 64% during maximal exercise. It is not known whether this is caused by the release of RBCs from the splenic reserve or results from a decrease in plasma volume causing an increase in the concentration of red blood cells (Snow et al., 1988). The hematocrit of the racing camel increases from 33% at rest to 36% at maximal exercise (Evans et al., 1992). Of the four athletic species, the camel, therefore, has the lowest concentration of RBCs. This, together with a relatively low maximum HR, might explain the relatively low $\dot{V}O_{2max}$ of this species.

Some human athletes have attempted to mimic this physiologic response by using an illegal technique known as "blood doping." In this procedure, RBCs are withdrawn from the athlete and frozen. At some point, usually hours before competition, the stored RBCs are reinjected, thereby increasing the oxygen-carrying capacity of the blood and potentially aiding performance (Buick, 1980). This procedure appears to have limited effects in the horse, possibly because of the innate capacity of the horse to increase the packed cell volume (PCV) in response to exercise. Another way to increase this effect is by using high altitude in training programs with the current recommendation of live high, train low for altitude training in people. However, in both humans as well as horses, the effects are relatively small and not consistent (de Paula and Niebauer, 2010; Wickler and Anderson, 2000).

The maximum arteriovenous oxygen difference, or (a-v) DO_2, measured in the horse during maximal exercise is only very slightly greater than values measured in elite human athletes under similar conditions. Thus, a larger (a-v)DO_2 accounts for only about 23% of the greater $\dot{V}O_{2max}$ of the racehorse compared with the elite human athlete; the much greater CO and oxygen-carrying capacity of the blood accounts for the other 77% (Physick-Sheard, 1985).

$\dot{V}O_{2max}$ as a Predictor of Athletic Ability

Although $\dot{V}O_{2max}$ is generally considered the best predictor of athletic potential, there is evidence to dispute this belief (Noakes, 1988). For example, although elite human athletes do have high $\dot{V}O_{2max}$ values, so, too, do many less conditioned athletes. Athletes with similar athletic abilities may have quite different $\dot{V}O_{2max}$ values (Noakes, 1992).

The same relationship is found in racehorses, in which there is no significant difference between the $\dot{V}O_{2max}$ values of Standardbred (165–180 mL/kg/min) and Thoroughbred, (164–200 mL/kg/min) horses. Clearly, if $\dot{V}O_{2max}$ was the sole predictor of athletic ability, the value should be much higher in Thoroughbred than in Standardbred horses. Hence factors other than $\dot{V}O_{2max}$ must be important in determining the superior athletic ability of the Thoroughbred racehorse.

Two important factors are locomotive efficiency, which is the oxygen cost per kilogram per kilometer traveled, and the percentage of $\dot{V}O_{2max}$ that can be sustained during prolonged exercise (Coetzer et al., 1993; Hammond et al., 1984; Noakes, 1992). Subjects able to run at a higher percentage of their $\dot{V}O_{2max}$ for longer periods exhibit superior fatigue resistance. For example, the $\dot{V}O_{2max}$ of the Thoroughbred racehorse is about three times

greater than that of the racing camel, which is of similar mass (Rose et al., 1992) (see Table 2-1). Yet the camel can exercise at a very high intensity (100% $\dot{V}O_{2max}$) for a much longer period than can the horse (18 min versus 3–5 min). Thus, the performance of the camel in races lasting more than 10 to 15 minutes is likely to be more similar to that of the racehorse because of the former's ability to exercise at a much higher percentage of $\dot{V}O_{2max}$ for much longer despite a substantially lower $\dot{V}O_{2max}$. Hence, in the assessment of an athlete's potential, it is necessary to consider not only the $\dot{V}O_{2max}$ but also the percentage of $\dot{V}O_{2max}$ that can be sustained during prolonged exercise.

An important factor in determining success in human runners and cyclists is *economy of locomotion*, or a lower than average oxygen cost at any running or cycling speed (Coyle et al., 1991; Noakes, 1992). This has yet to be evaluated in racehorses and the other athletic species. However, when comparing the oxygen cost of exercise in camels and horses, it is clear that the oxygen cost per kilometer traveled in camels is much less than in the horse, indicating superior economy of locomotion.

Thus, the greater capacity for oxygen transport in the racehorse is the result of a larger capacity to increase CO, with heart rate being the main contributor; the greater oxygen-carrying capacity of the blood during exercise; and finally, a small increase in the capacity to extract oxygen in the active muscles, measured as a greater (a-v)DO_2 (Evans and Rose, 1988).

BLOOD LACTATE RESPONSE TO EXERCISE

Both the horse and the dog are able to produce higher peak blood lactate concentrations after maximal exercise compared with humans (Rose et al., 1988; Snow et al., 1985) (see Table 2-1). However, the correlation between muscle lactate and pyruvate concentrations and the muscle pH is similar in humans and horses, indicating a similar buffering capacity (Harris et al., 1984). Also, respiratory compensation for this metabolic acidosis increases 16-fold in both the horse (100–2000 L/ min) and the human athlete (6–100 L/min). Thus, the higher peak lactate concentrations after maximal exercise in the horse are associated with lower muscle pH levels than in human athletes (6.2 versus 6.6) (Costill et al., 1983; Snow et al., 1985; Snow and Harris, 1987).

MUSCLE FIBER TYPES

There are two main muscle fiber types, classified as *type I* (slow twitch, or ST) and *type II* (fast twitch, or FT). Type II fibers are further subdivided into type IIa and type IIb fibers. The ratio of type I to type II fibers is genetically determined. Moreover, the muscle fiber type present in any individual may predispose that individual to success in specific athletic activities. Human sprinters have a majority (>75%) of type II fibers, whereas endurance athletes have a predominance (>75%) of type I fibers (Noakes, 1992).

The muscle fiber composition of horses also varies according to their athletic abilities. Endurance horses have a larger percentage of type I fibers than do sprint horses. However, as a species, the horse has a low percentage of type I fibers, with a maximum of around 40% in endurance horses, compared with human endurance athletes, who usually have more than 75% type I fibers (Noakes, 1992; Rose, 1986).

Although the proportion of muscle fibers is strongly genetically controlled, training can alter the relative proportions, at least to a limited extent. Especially, a transition of type IIB to type IIA fibers, and vice versa, might occur, depending on the training regimen (Essen-Gustavsson and Lindholm, 1985; Tyler et al., 1998).

The racing camel has a large percentage of type I fibers (70%), with the remainder being type IIa, with few or no type IIb muscle fibers (Rose et al., 1992). Thus, the camel is well suited to low-intensity endurance activities. In contrast to the camel and the racehorse, the greyhound has an almost complete dominance of type II muscle fibers (Snow, 1985)

RELEVANCE OF PHYSIOLOGIC TESTING

The main reasons for the physiologic testing of racehorses are to predict their athletic capacity, to monitor improvements with training, and to determine the likely causes for impaired exercise performance. It is, therefore, important to ensure that the variables being tested in the laboratory will give information that is of relevance to the clinical setting. For example, the human runner has minimal external interference to his or her performance. The same equipment is used on the track and on the laboratory treadmill. In contrast, during competition, but not during laboratory testing, the horse needs a jockey to control positioning and speed. In addition, there is the racing tack, including the bridle and the saddle, necessary for competition, and this introduces variables that need to be considered in the laboratory testing. Care must be taken when predictions of track racing performance are made on the basis of laboratory measurements of physiologic function during treadmill exercise in racehorses.

For example, studies have determined that oxygen consumption measured in humans during treadmill running in the laboratory is not significantly different from values measured in the field (Basset et al., 1985). Because of the weight of the jockey, the equipment, and the track conditions, it is not known whether the same is true for horses. Thus, studies have been performed in an attempt to identify all the possible variables that might influence the interpretation of laboratory test results in racehorses. As in humans, it appears that maximal oxygen consumption in the horse in the field is higher than in the laboratory setting (Franklin et al., 2012).

HISTORY OF IMPROVEMENT IN RACING TIMES IN THE DIFFERENT SPECIES

IMPROVEMENT IN RACING TIMES IN HORSES, DOGS, AND HUMANS

Records in all athletic activities improve with time. However, the athlete has to ultimately reach some limiting speed or distance beyond which no further improvement is possible. This stage has yet to be reached in any human athletic event, although experts agree upper limits are close to being achieved in several athletic disciplines. Rather, world records for all events continue to improve. However, the rate of improvement of these records differs in different sports and among the different athletic species. To illustrate this difference, we analyzed the records of three famous athletic events contested by three different species. All these races are run at roughly the same exercise intensity (90–100% $\dot{V}O_{2max}$). These races are the 1 mile (1609 m) foot race for humans, the Kentucky Derby (1.25 miles, 2018 m) for Thoroughbreds, and the Puppy Derby (460 m) for greyhounds. The slope of the regression lines in Figure 2-2 represents the rate of improvement in

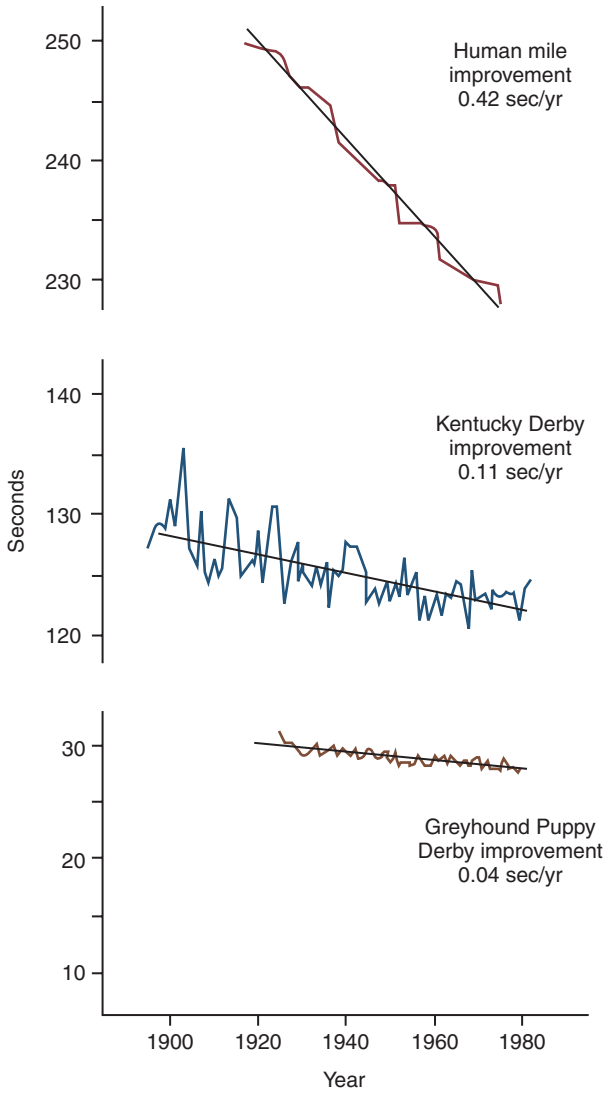

FIGURE 2-2 The winning times over the years for the human mile, Kentucky Derby, and the greyhound Puppy Derby.

the winning times in the different events. The rate of progression of the winning time in the human foot race (0.42 seconds per yard [s/yd) is four times greater than in the Kentucky Derby (0.11 s/yr) and 11 times greater than in the Puppy Derby (0.038 s/yd). Expressed as a percentage of the record times in 1935, the rates of improvement per year for the different species in the different events are 0.17%, 0.09%, and 0.13% for the three species, respectively.

This comparison invites the following question: Why should the rate of improvement of human athletic records be superior to that of Thoroughbreds and somewhat better than that of greyhounds? To answer this question, we need first to consider the factors that determine racing performance and how these may be influenced by different factors in the different species.

FACTORS DETERMINING ATHLETIC ABILITY IN ANY SPECIES

Athletic ability, regardless of sport or species, is determined by three main factors: (1) genetics, (2) environment, and (3) training.

Genetics

It has been said that the most effective way to become a champion athlete is to be selective when choosing one's parents. A measure of this genetic contribution to athletic ability is provided by studies of groups of identical twins, whose $\dot{V}O_{2max}$ values are almost identical ($R = 0.92$) (Bouchard et al., 1986; 1992). Further, the endurance capacity of identical twins during prolonged exercise is quite similar and is more similar than is the endurance capacity of nonidentical twins, whose performances are also more similar than those of brothers (Bouchard, 1986). This indicates a strong genetic component for athletic performance, estimated to be between 40% and 60% for $\dot{V}O_{2max}$ and endurance capacity, respectively.

Although genetic factors determine the ultimate limit of each athlete's performance, environmental and training factors determine how closely each athlete approaches that limit. Paradoxically, unlike the human athlete, the Thoroughbred racehorse has been bred with one objective—to run faster than any other horse. The evidence provided in Figure 2-3, however, suggests that careful breeding has failed to produce the desired result.

Thoroughbred breeding records have been kept since 1791, when James Weatherby established the *Stud Book*. Since then, the breeding of horses has been recorded in elaborate detail. Weatherby's book indicates that just over 50% of all the genes in the present Thoroughbred population come from only 10 horses and 80% from 31 horses (Gaffney and Cunningham, 1988). Thus, a very small genetic pool exists in the racing breed.

The first book including the breeding records of greyhounds was published in 1882. Controlled breeding of greyhounds, therefore, began much later than that of Thoroughbred horses. Again, this question must be asked: If genetic factors are such important determinants of athletic performance, especially in Thoroughbred racehorses, why then has the breed with the longest history of controlled breeding not made the greatest improvements in racing performances? One explanation for the slow improvement in the winning times of Thoroughbred racehorses contends that inbreeding has led to a limited gene pool, leaving little room for further improvement. Support for this contention is the finding that between 1804 and 1910, the winning times for certain Thoroughbred races improved more rapidly than in subsequent years. The assumption is that as the Thoroughbred breed has become progressively more inbred, the potential for further improvement has decreased (Cunningham, 1991).

To examine the effect of controlled breeding, Cunningham (1991) used Timeform ratings as a measure of racing performance in 31,263 3-year-old Thoroughbred racehorses. He compared the Timeform ratings of half-brothers and half-sisters with those of randomly selected groups and of parents and their offspring with random pairings from two consecutive generations. He concluded that only 35% of the variance in ability is explained by hereditary factors; the remaining 65% is attributed to environmental factors such as training and nutrition.

If genetic factors explain only 35% of the variance in athletic performance in Thoroughbreds, is it therefore appropriate to expend so much time, money, and effort on selective breeding? The answer is both yes and no. According to Gaffney and Cunningham (1991), selective breeding contributes to an increase of about 1% per annum in Timeform ratings. Other research in the Quarterhorse also suggests that selective breeding will continue to improve racing performances in future

generations (Willham and Wilson, 1991). But perhaps the most important practical point is that since genetic factors explain less than 40% of a Thoroughbred's racing potential, and since that contribution is fixed at birth in any individual horse, it follows that more attention should be paid to those environmental and training factors that determine performance and that can be actively and successfully modified.

Environment

Environmental factors that influence athletic performance include all the equipment necessary to participate in the sport, the surface on which the sport is performed, and the nutrition of the athlete. The jockey adds an additional environmental component that must be considered in the racehorse.

Athletic Nutrition

The aim of athletic nutrition is to meet the athlete's energy and nutrient requirements. Athletes consume 50% to 75% more energy than nonathletes (Grandjean, 1989). Carbohydrate, fat, and protein form the three primary nutritional fuels. All ingested foods are either used directly by the body or converted and stored for later use. As a completely herbivorous animal, the horse has developed a digestive system to break down vegetable cellulose. Thus, the equine athlete's nutritional processes and demands are far more complex than those of the human athlete. The horse's digestive system, like that of all herbivores, operates with an intake limitation, functioning optimally only at a certain fullness (Frape, 1988).

Therefore, to meet the energy demands of the racehorse undergoing athletic training, the trainer must increase the frequency of feeds instead of increasing only the amount of food. Also, the energy density of the diet can be increased (Lawrence, 1990). Both humans and horses use nonesterified fatty acids (NEFAs) as the main fuel for muscles during rest or when walking at a slow pace. This is shown by a resting energy requirement (RER) of less than 0.8, indicating fat utilization. As the exercise intensity increases, fats can no longer supply the high rate of energy demands, and carbohydrates become progressively more important as the energy source (RER = 0.8–1.0). Carbohydrates are derived from muscle glycogen or glucose transported in the bloodstream.

In the early 1920s, scientists realized that low blood glucose concentrations were associated with fatigue in human athletes (Levine et al., 1924) and that carbohydrate ingestion could rapidly reverse this fatigue. These findings suggested that human athletes should eat a high-carbohydrate diet in the 24 hours prior to a race and consume carbohydrates during long-distance races (Gordon et al., 1925). Modern research has confirmed these findings in human athletes (Coggan and Coyle, 1991), but caution needs to be exercised in direct extrapolation of these findings to horses because both diet and digestion are different in horses. For more information, see Chapter 4.

The timing of precompetition meals may be of importance to both humans and horses. Studies performed in human athletes who ingested carbohydrate 45 minutes prior to exercise showed that their blood glucose concentrations fell as a result of increased serum insulin concentrations (Costill et al., 1977). A similar study in horses that exercised 2 hours after a feed also showed that blood glucose concentrations fell during subsequent exercise (Arana et al., 1988). Whether or not this fall in blood glucose concentration impairs exercise performance remains to be clearly established.

The National Research Council (NRC) of North America recommends that the dietary intake of horses in heavy athletic training should contain 33% roughage and 67% concentrate. The main source of carbohydrate is obtained from cereals and hay, which also provide roughage. Horses in heavy training are sometimes fed fats, in the form of soya bean or maize oils, as a potential high source of energy. Concentrations of fat of up to 20% (in terms of calories consumed) of the diet have been found to be tolerated by horses, even though the regular training diet contains only 1% to 2% fat. Human athletes are advised to eat diets composed of 50% to 65% carbohydrate, 12% to 15% protein, and 20% to 30% fat (Leaf and Friska, 1989).

Track Surfaces

Running surfaces often can contribute to injuries in both equine and human athletes. Advances in the design of track surfaces for racehorses have trailed behind advances made in human athletics. For example, a "sprung" track tuned to the specific biomechanical characteristics of the human body has been developed and may reduce injury risk in human athletes (McMahon and Green, 1979).

Equine racetracks traditionally have been either turf or dirt. Each has its own drawbacks. Turf can withstand only limited use, whereas dirt tracks rely on the soil type and moisture level for optimal performance (Pratt, 1989). However, new advances in hydrophobic (water-repelling) polymers have enabled coating of the sand particles. This innovation is preparing the way for new equine racetracks, which may combine the benefits of both turf and dirt tracks. Clearly, more needs to be done to develop surfaces that reduce injury risk. This is critically important given the large number of training injuries in Thoroughbred racehorses.

Shoes

Advances in human sporting achievements have always relied on equivalent technological advances in sporting equipment. From lighter and more aerodynamic bicycle designs, exemplified most recently by the Lotus bicycle raced to victory by Chris Boardman in the 1992 Barcelona Olympic Games, to the aerodynamically designed javelin that could be thrown so far that the safety of the spectators was threatened, humans have always searched for unique ways to improve their sporting achievements. Perhaps the most visible modern development has been the design of running shoes for humans. Running shoes have become so sophisticated that there are now over 150 different shoe types to choose from. Since no man or woman is anatomically identical, running shoe companies have manufactured shoes to deal with this wide variety of anatomic imperfections.

In contrast, the horse shoe has changed little during the last century, yet lameness accounts for 70% of lost training days in racehorses, with 80% of injuries occurring in the front legs. This is caused by the nearly 5000-kg of force that is transferred through the horse's front legs when racing. Thus, some horses may require novel innovations to soften impact loading during training and racing. However, this possibility has not been addressed until recently, with greater attention being paid to the optimal training surfaces and with introduction of a range of "shock-absorbing" horse shoes, which have had some success in rehabilitating lame horses (Grant et al., 1989). These shoes are made either from an acetal resin or from polyurethane in place of the

usual hard metal and are lighter than metal shoes. This is of importance, since weight added to the hoof has been found to affect the stride length of the horse. Aluminum horse shoes were developed to decrease the weight of the shoe; however, the durability of the shoe is inferior because of the light-weight material now used.

Thus, some progress has been made in the development of more advanced shoes for horses. However, there is a need to increase this research effort so that horses may be at a lesser risk of disabling injuries during both training and racing.

The Jockey

Unlike humans, the equine athlete's positioning, speed, and racing strategy are determined by the jockey. The jockey's weight, positioning, and experience play an integral part in the sport. To succeed, the jockey must be an expert judge of the horse's pace and its capabilities.

The jockey represents not only added weight for the horse but also additional surface area, which increases the overall aerodynamic drag. It is in this area that the jockey can learn from competitors in other high-speed sports, including cycling and downhill ski racing. Competitors in these events have discovered the value of wearing clothing and equipment designed to reduce the aerodynamic drag. It is estimated that 25% of the horse's energy is expended to overcome air resistance at high speeds. Improvements in the aerodynamic profiling of the jockey by paying attention to the jockey's riding position, equipment, and clothing would likely reduce this percentage and could conceivably influence the outcome of closely contested races. We should, therefore, expect more attention to be paid to this area in the future.

Training

After genetics, training is the single most important variable determining athletic success. An essential advantage enjoyed by coaches of human athletes is that these athletes can communicate ideas and especially sensations to their trainers. Unfortunately, the equine trainer lacks the benefit of direct feedback from the horse, so it is less likely that training programs are ever absolutely specific to the unique needs of individual horses. Since the elite athlete, whether human or horse, always treads a fine line between peak condition and overtraining, the absence of this feedback complicates immeasurably the task of training. Nevertheless, exercise scientists have developed techniques to monitor the effects of training, including monitoring of HR and performance during specific workouts. It is vital that trainers and exercise physiologists work together so that this knowledge from the human experience can be transferred and applied, with benefit, to the training of horses. With the wise application of scientific principles established in human athletes, the trainer and exercise physiologist working together can bridge the "communication gap" and develop specific training programs for individual horses that will ensure that each achieves its optimal performance relative to genetic abilities.

Training Methodology

With the advances that have been made in the human exercise sciences, an ever-increasing interest has centered on improving training methods by scientifically evaluating the real (as opposed to surmised, but untested) effects of different training methods. Unfortunately, many trainers of either horses or humans continue to use training methods that have been handed down from generation to generation without ever undergoing scientific validation. Worse, many trainers are reluctant to innovate.

A traditional training method for Thoroughbred racehorses consists of a single exercise session with one single bout of exercise, for example, a run of 800 m. The intensity and duration may vary from day to day, with an occasional hard gallop or "breeze" at about racing speed. The distance covered is usually shorter than the racing distance.

Interval training is a training method in which a single exercise bout is divided into segments that vary according to intensity, duration, and frequency. Interval training was developed by the German athletics coach Woldemar Gerschler in the 1930s. It is claimed that training of this type will delay the onset of fatigue and strengthen the weight-bearing structures, thereby reducing the risk of injury. A few horse trainers have introduced some of these techniques but, due to an inappropriately sudden transition to a high-intensity training program in horses unprepared for the change, the horses have experienced problems, particularly lameness. This invites the question: Can horses really be trained on the basis of the principles used by human athletes?

Trainers of both humans and horses use a variety of different training methods. However, most use a variation on a basic model that incorporates three phases, including some form of interval training in the final, pre-competition phase. The three phases are (1) a foundation phase, (2) a cardiovascular or aerobic interval phase, and (3) an anaerobic interval phase (Costill, 1986).

The foundation phase is probably the most important, since it is in this phase that bones, tendons, and the muscular system are strengthened; in equine terminology, this is referred to as *continuous training* or *endurance training*. During this phase, a great deal of patience is required. Should the phase not be completed properly for any reasons, injuries are likely to occur subsequently. It is important that the training load be increased gradually to prevent any sudden unusually large demands on the horse. No competitive racing should be undertaken during the foundation phase.

In phase II, the aerobic interval phase, the training volume is maintained, but sessions of higher-quality, more-intensive training are included. In these sessions, repeated runs at a faster pace with brief rest intervals introduce the horse to more intensive training (Figure 2-3). This allows the heart rate to recover fully during the rest phase. There is debate about the optimal length of the recovery phase. Costill (1986) suggests that brief rest periods of as little as 5 to 15 seconds should be used in human athletes. Phase II takes between 10 to 12 weeks in the horse.

Phase III, the anaerobic interval stage, is used to develop the strength required for racing at top speed and to optimize the development of coordinated neuromuscular functioning. A high-intensity workout over short distances is performed. The rest periods between exercise bouts must be of sufficient length to facilitate HR recovery to about 60% to 70% of the maximum HR (Figures 2-4). This phase is suggested to last for about 6 weeks for horses (Harkins et al., 1990). However, it should be used as a continual method for maintaining fitness and racing condition throughout the competitive racing season.

FIGURE 2-3 Aerobic or cardiovascular interval training and heart rate response in the Thoroughbred horse.

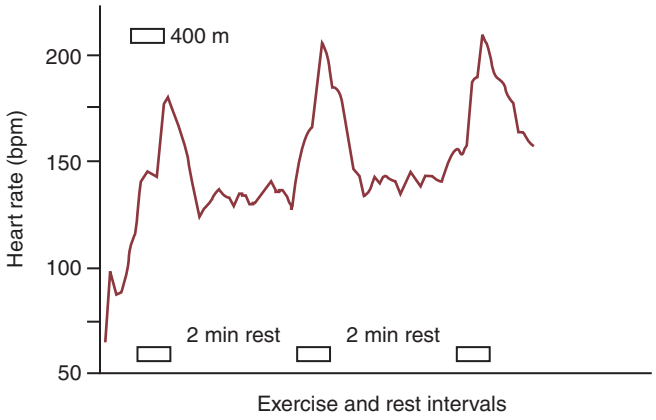

FIGURE 2-4 Anaerobic interval training and the heart rate response in the Thoroughbred horse.

Interval training would seem to be of benefit to human and equine athletes. However, more studies of the specific design of training programs need to be completed so as to establish methods that achieve the maximum training benefits with minimal injuries. Studies also must be done to examine the effect of interval training and a more gradual and delayed introduction to racing at an older age on the competitive longevity of the horse. It must be stressed that there is no substitute for a solid training foundation, especially when initiating an interval training program.

Swimming training or running in water have long been used by horse trainers as additional methods of training. Human athletes also have used these methods, although mainly for injury rehabilitation. A specially designed buoyant vest has been developed to enable human athletes to run in water. To achieve the same effect, horses either swim in a circular pool or run on a submersed treadmill or horse walker. HR in both humans and horses is lower when exercising in water compared with when running at a similar intensity on land. Despite limited training effects, horses undergoing swimming exercise, especially where they must swim and not train through water on a treadmill or walker, are often perceived to be exercising at high intensity. Perceived exertion also was higher in humans running in water than over land (Svedenhag et al., 1992). The effect in horses may be attributed to the increased external pressure on the thoracic cavity limiting respiratory capacity, which is not an ideal situation.

It would seem that the main use of swimming and running in water is for the purposes of rehabilitation from injury, for training young horses, since the buoyancy properties of water decrease the concussive forces on the bones and joints, and for adding variety to training programs. As Snow and Vogel (1987) stated, the use of large amounts of swimming training for horses would only be beneficial if horses also competed in swimming races.

CONCLUSION

Apart from being the fastest competitive athletic species, the Thoroughbred racehorse shows some rather unique physiologic adaptations to exercise. This makes it difficult to apply findings from human physiologic studies directly to the horse. Hence, there remains a real need to develop the body of scientific knowledge specific to the racing horse. The need for this information is perhaps best shown by the finding that despite the longest history of selective breeding for athletic success, the improvement in winning race times is much less in Thoroughbreds than in humans. It is unlikely that this is caused by a small genetic pool that limits further improvement, and therefore, other possible factors should be considered.

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CHAPTER 3

Energetic Considerations of Exercise

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The horse is a superb athlete, the result of the evolutionary adaptations required to live as a species on open landscapes. These have resulted in speed to escape predators and endurance necessary in the search for nutrition. Selective breeding by humans has further modified these evolutionary traits, and domesticated horses have been selected for certain characteristics dependent on intended use. Heavy breeds were selected for draft or military work, whereas lighter horses were chosen because of their speed and endurance. This has resulted in a myriad of breeds and capacities of each. For example, Thoroughbreds can achieve speeds of >65 kilometers per hour (kph) when racing over 800 to 7000 meters (m), Standardbreds trot or pace at up to 55 kph for up to 4000 m, Quarterhorses sprint over 400 m (¼ mile) at speeds approaching 90 kph, yet endurance horses (Arabian) can cover 160 km at average speeds of approximately 25 kph. In contrast, draft horses pull sleds weighing >4000 kg over 6 m and such breeds as Warmbloods may participate in eventing, showjumping, and dressage competitions.

The athletic capacity of horses results from physiologic properties (see also Chapters 1 and 2), attributed in particular to:

1. High maximal aerobic capacity ($\dot{V}O_{2max}$)
2. Large intramuscular stores of energy, particularly glycogen
3. High respiratory capacity of skeletal muscle
4. Splenic contraction, which results in the oxygen-carrying capacity of blood increasing by up to 50% soon after the onset of exercise
5. Highly efficient and adaptable gait(s)
6. Well-developed capacity for effective thermoregulation

Vertebrate locomotion requires the controlled integration of numerous physiologic and metabolic pathways, which then impact on the musculoskeletal system, providing the organism with mobility. Perhaps the most important pathways are those concerned with the production of energy, for without energy muscles cannot contract and mobility is not achieved. Muscular movement requires the transformation of chemical energy stored in metabolic *substrates* or *fuels* to the kinetic energy of muscular contraction. All pathways integral to energy supply are concerned with the ultimate production of adenosine triphosphate (ATP), the final carrier of energy "packages" utilized by muscles for contraction. For muscles to contract, there is a coupling of thin actin and thick myosin filaments to form cross-bridges, and then these filaments slide relative to each other by a change in the orientation of the cross-bridges (Guyton, 1986; see Chapter 12). This process is repeated millions of times during muscular contraction. Energy is necessary for the change in orientation of the cross-bridges to occur, and the energy comes from the cleavage or hydrolysis of ATP at the head of each myosin filament. The cleavage of a high-energy phosphate bond from ATP is catalyzed by the enzyme myosin adenosine triphosphatase (ATPase) and produces adenosine diphosphate (ADP), free phosphate, a proton, and the release of energy (Figure 3-1). The energy released is utilized by the working muscle for contraction (Cain and Davies, 1962). In addition, ATP is the source of energy required to restore the contracted muscle to a relaxed or resting state via the distribution of calcium ions (Åstrand and Rodahl, 1986).

Under normal conditions, there is a finite store of ATP within muscle, sufficient to maintain muscular activity for only a few seconds (Åstrand and Rodahl, 1986; Lindholm, 1979). Therefore, for continuous muscular exertion, it is necessary to resynthesize ATP, and this is done by the pathways of aerobic (oxidative) and anaerobic phosphorylation (Figure 3-2).

PRODUCTION OF ENERGY

OXIDATIVE PHOSPHORYLATION

Oxidative or aerobic phosphorylation production of ATP via aerobic pathways occurs within the inner membrane of mitochondria in a series of single oxidation reactions known as the *electron transport* or *respiratory chain*. *Oxidation* is the donation or loss of electrons (often in the form of hydrogen) from an atom or molecule, whereas *reduction* is the acceptance of electrons (hydrogen) by an atom or molecule. When electrons are donated, considerable chemical energy is liberated, and a portion of this energy is captured for the rephosphorylation of ADP to ATP, with the remainder being lost as heat energy (Guyton, 1986). Nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) act as hydrogen carriers (acceptors) during glycolysis, beta (β)–oxidation, and the tricarboxylic acid (TCA) cycle and, therefore, are reduced to NADH and $FADH_2$. These coenzymes are essential for aerobic and

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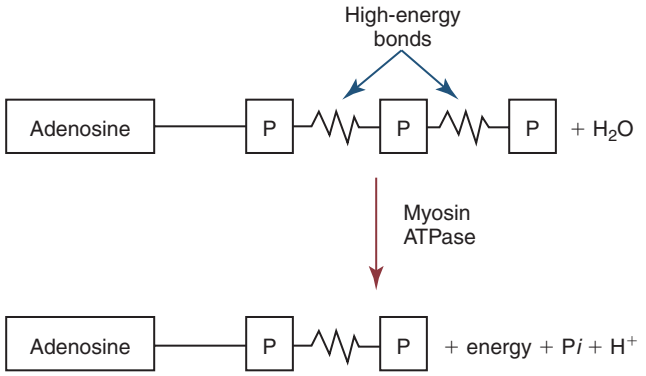

FIGURE 3-1 Hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) by the enzyme myosin adenosine triphosphatase (ATPase), with the release of energy for use by working muscle. *P*, Phosphate; *Pi*, orthophosphate.

anaerobic phosphorylation, but their concentrations within the muscle are low. Therefore, NADH and $FADH_2$ must be reoxidized to NAD^+ and FAD via the electron transport chain. Specific mitochondrial enzymes incorporated in the electron transport chain catalyze the oxidation through a process of dehydrogenation. The donated hydrogen atoms provide the electrons that are transported from one enzyme complex to another by electron carriers, for example, *cytochrome c*. The importance of oxygen (O_2) in this whole process is that it acts as the final hydrogen acceptor to form water. The energy released by the step-by-step transfer of electrons from NADH or $FADH_2$ to O_2 via the electron carriers is used to pump protons from the inner matrix of the mitochondrion into the outer chamber between the inner and outer mitochondrial membranes. This creates a strong transmembrane electric potential. Energy for the phosphorylation of ADP to ATP is obtained as the protons flow back through an inner membrane enzyme complex called *ATP synthetase* (Guyton, 1986; Stryer, 1988).

The availability of O_2 to the exercising muscle is the rate-limiting step for oxidative phosphorylation. Oxygen immediately available to the muscle at the onset of exercise, from myoglobin within the muscle (MbO_2), hemoglobin within the circulatory system (HbO_2) or O_2 dissolved in the body fluids, is in sufficient quantities for only a few seconds of exercise. Therefore, the delivery of O_2 to the exercising muscles via the cardiorespiratory system is crucial for the capacity to continuously produce energy via aerobic means.

The two major electron donor substrates for aerobic phosphorylation are carbohydrates (CHO) and fatty acids. Glucose is the main CHO, and if it is not used for immediate energy production, it is stored as glycogen, mostly in skeletal muscle and to a lesser extent in the liver (Hodgson et al., 1983, 1984; Lindholm, 1979). Adipose tissue constitutes the largest store of fatty acids (FA) (Robb et al., 1972; Stryer, 1988). Adipocytes store fat within their cytoplasm as triglycerides. Triglyceride storage also occurs to a much lesser extent in muscle (Lindholm, 1979).

AEROBIC GLYCOLYSIS

The importance of CHO as a substrate for energy production increases as exercise intensity increases (Hodgson et al., 1985; Lawrence, 1990). Glucose diffuses into the muscle cell cytoplasm from the circulation and is phosphorylated to glucose-6-phosphate (G-6-P) in a reaction catalyzed by the enzyme *hexokinase* (HK) and requiring one ATP molecule. G-6-P is then transferred into the glycolytic pathway for immediate energy production or reversibly converted to glucose-1-phosphate (G-1-P) and then glycogen for storage. Glycogen stores provide most of the glucose required for energy production during exercise. In the glycolytic pathway, G-6-P is phosphorylated to fructose-6-phosphate (F-6-P), which is then phosphorylated to fructose-1,6-bisphosphate (F-1,6-BP) in a reaction catalyzed by *phosphofructokinase* (PFK) and at the expense of one ATP molecule. F-1,6-BP is subsequently split into the triose phosphate isomers, glyceraldehyde-3-phosphate (Gl-3-P) and dihydroxyacetone-phosphate (DiH-P). DiH-P is readily converted into Gl-3-P by *triose phosphate isomerase* (TPI). Therefore, one molecule of glucose or glycogen gives two molecules of Gl-3-P. Gl-3-P then proceeds through a series of reactions, the end result being the production of pyruvate, ATP, NADH, water, and hydrogen ions. The net reaction for the glycolytic pathway utilizing glucose is:

$$\text{Glucose} + 2\text{Pi} + 2\text{ADP} + 2\ \text{NAD}^+ \longrightarrow 2\ \text{Pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\text{H}^+ + 2\text{H}_2\text{O}$$

Under aerobic conditions, the hydrogen atoms are transferred to the electron transport chain, and pyruvate is transported into the mitochondrial matrix as a substrate for acetyl coenzyme A (acetyl CoA). Acetyl CoA then enters the TCA cycle by combining with oxaloacetate to form citrate. The normal function of the TCA cycle requires three NAD^+ and one FAD to accept hydrogen atoms during the oxidative conversion of citrate back to oxaloacetate. When O_2 is available, the NADH and $FADH_2$ are then reoxidized back to NAD^+ and FAD in the electron transport chain, thus replenishing the adenine dinucleotide stores and producing ATP (see Figure 3-2). The complete aerobic utilization of one mole of glucose generates 36 to 38 ATP. When O_2 is not available, pyruvate is converted to lactate as described later.

FATTY ACID UTILIZATION

Following lipolysis, nonesterified fatty acids (NEFAs) are released into the circulation and are subsequently available as hydrogen donors for energy production in skeletal muscle. NEFAs likely diffuse into muscle cells down a concentration gradient as well as being actively transported across the cell membrane. At the cytoplasmic surface of the outer mitochondrial membrane, the NEFAs are esterified (activated) enzymatically forming long-chain acyl CoA molecules. The acyl CoA molecules are then linked to carnitine and shuttled across to the matrix side of the inner mitochondrial membrane. In the mitochondria, the acyl CoA molecules undergo a series of four reactions known as *β-oxidation*. With each cycle of β-oxidation two-carbon (C_2) units are sequentially removed from the acyl CoA molecule and acetyl CoA, NADH, and $FADH_2$ are produced (see Figure 3-2). NADH and $FADH_2$ subsequently donate their electrons in the electron transport chain, generating ATP and being reoxidized to NAD^+ and FAD in the process. Acetyl CoA is utilized in the TCA cycle as previously described. The splitting of C_2 units from the parent acyl CoA molecule is repeated until the whole chain has been cleaved into the acetyl CoA molecules. The number of carbon atoms in the parent FA chain will determine the net energy yield from β-oxidation. Complete oxidation of one palmitic acid molecule produces 129 molecules of ATP.

ANAEROBIC PHOSPHORYLATION

The pathways of anaerobic phosphorylation occur solely in the muscle cell cytoplasm, with no reactions in the mitochondria as there are for aerobic phosphorylation. With the initiation of exercise, there is a lag period before oxidative energy production becomes an important source of ATP.

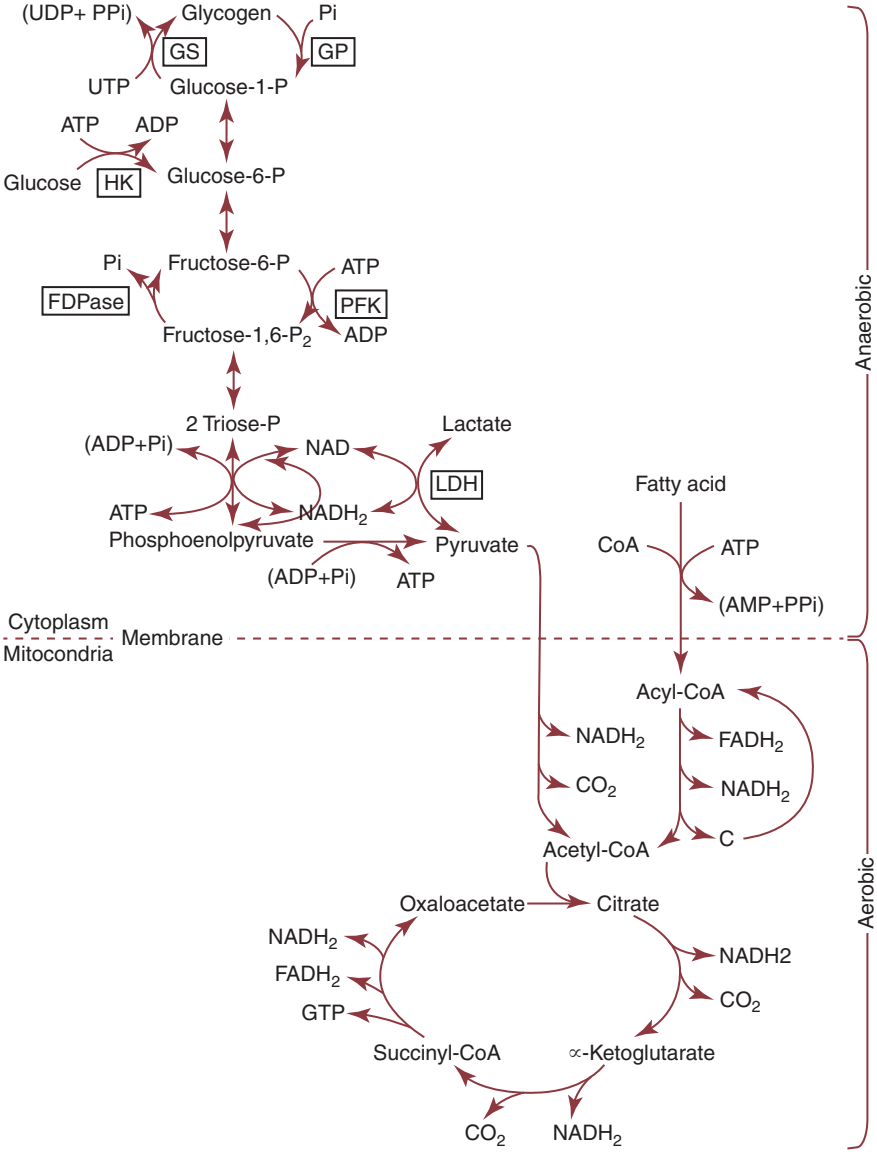

FIGURE 3-2 Schematic representation of the principal components of glycolysis, fatty acid oxidation, and the tricarboxylic acid cycle in a muscle cell. Adenosine triphosphate (ATP), guanosine triphosphate (GTP), uridine triphosphate (UTP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), coenzyme A (eoA), orthophosphate (P), pyrophosphate (PP), glucose-l-phosphate (G-l-P), glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), fructose-l,6-diphosphate (F-l,6P), nicotinamide-adenine-dinucleotide (NAD), flavin-adenine-dinucleotide (FAD), glycogen synthetase (GS), glycogen phosphorylase (GP), hexokinase (HK), phosphofructokinase (PFK), fructose diphosphatase (FDPase), and lactate dehydrogenase (LDH) are shown.

During this time, rapid supplies of ATP must still be available if muscular contraction is to continue. Stores of ATP in skeletal muscle are limited (4–6 millimoles per kilogram [mmol/kg] wet muscle) and contribute little to the total energy supply (Lindholm and Piehl, 1974; McMiken, 1983). Until aerobic phosphorylation makes a substantial contribution to energy supply, rapid regeneration of ATP must occur in the absence of O_2. The anaerobic phosphorylation of ADP is achieved by three pathways: (1) the phosphocreatine reaction, (2) the myokinase reaction, and (3) anaerobic glycolysis. The former two pathways may be described as anaerobic alactic reactions because no lactate is produced as it is in the latter process (Clayton, 1991).

Phosphocreatine Reaction

In this pathway, the enzyme *creatine kinase* catalyzes a reversible reaction where creatine phosphate (CP or phosphocreatine) donates its high-energy phosphate to ADP producing ATP:

$$CP + ADP \rightarrow Creatine + ATP$$

In the gluteus medius muscle of Standardbreds, the size of the CP pool is estimated to be 15 to 20 mmol/kg wet muscle (Lindholm and Piehl, 1974; Lindholm, 1979). This source of ATP replenishment would support maximum intensity exercise for no more than a few seconds (Åstrand and Rodahl, 1986; Clayton, 1991).

Myokinase Reaction

The *myokinase* enzyme catalyzes the synthesis of ATP and adenosine monophosphate (AMP) from two ADP molecules:

$$ADP + ADP \rightarrow ATP + AMP$$

At rest, this reaction proceeds at an approximately equal rate in both directions with little net ATP being produced. In working muscle, *AMP-deaminase* reduces AMP concentration by converting it to ionosine monophosphate (IMP) and ammonia. This provides the driving force for the myokinase reaction toward the production of ATP (McMiken, 1983). Again, this pathway only has the capabilities of providing small amounts of ATP.

Anaerobic Glycolysis

The anaerobic production of two molecules of pyruvate from one molecule of glucose or glycogen is identical to that described for aerobic glycolysis. In the absence of available O_2, pyruvate accepts hydrogen atoms from NADH and is converted to lactate, rather than being converted to acetyl CoA and entering the TCA cycle. The reaction is catalyzed by *lactate dehydrogenase*, and the regeneration of NAD^+ during the reduction of pyruvate to lactate sustains glycolysis under anaerobic conditions (see Figure 3-2). The net result of anaerobic glycolysis is the production of three molecules of ATP from one molecule of glycogen or two molecules of ATP from one molecule of glucose. This form of energy production is relatively rapid compared with aerobic glycolysis but yields a significantly lower amount of ATP, and substrates are limited.

REGULATION OF AEROBIC AND ANAEROBIC PATHWAYS

At all exercise levels, both systems of energy supply are active; however, one will predominate, depending, in particular, on the intensity and duration of the activity. A complex method of metabolic regulation controls the input of each pathway. Substrate and enzyme availability, end product concentrations, and various feedback mechanisms contribute to pathway dynamics.

Oxygen supply to muscles and the ratio of ATP to ADP are the most significant regulators of the energy producing pathways. Although there is adequate O_2 available, aerobic phosphorylation persists, providing a driving force for substrate to enter the TCA cycle and produce high concentrations of citrate. Citrate retards the activity of PFK, the enzyme responsible for the irreversible conversion of F-6-P to F-1,6-BP in the glycolytic pathway. This has the effect of inhibiting the metabolism of glucose and glycogen. Accumulation of glycolytic intermediates before the PFK step, including G-6-P, inhibits the activities of HK and phosphorylase, thus also dampening the glycolytic pathway. High cellular concentrations of ATP (i.e., a high ATP:ADP ratio) also inhibits PFK activity. *Pyruvate kinase*, the enzyme which converts phosphoenolpyruvate to pyruvate, is the third control site in glycolysis. Pyruvate kinase is inhibited by ATP and activated by F-1,6-BP. When the relatively slow production of energy by oxidative phosphorylation is unable to meet the demands for ATP, the ATP:ADP ratio swings in favor of ADP, and this, in turn, stimulates PFK and enhances glycolysis and the utilization of glucose and glycogen stores. The pyruvate produced is metabolized to lactate, and this allows the reoxidization of NADH to NAD^+ for continued electron acceptance in the glycolytic pathway.

Control of the TCA cycle starts with regulation of the irreversible oxidative decarboxylation of pyruvate to acetyl CoA by *pyruvate dehydrogenase* (PDH). Acetyl CoA, NADH, and ATP inhibit PDH activity. Once acetyl CoA is formed, its combination with oxaloacetate to produce citrate is inhibited by high concentrations of ATP. At least two other sites in the TCA cycle have regulatory mechanisms that respond to the cellular concentrations of ATP. Overall, the rate of the TCA cycle is reduced when abundant ATP is present, but it should also be remembered that the TCA cycle operates only under aerobic conditions because of the constant need for NAD^+ and FAD, regenerated or reoxidized from NADH and $FADH_2$ by the transfer of their electrons to O_2 during oxidative phosphorylation.

The rate of oxidative phosphorylation is regulated by the cellular ADP concentration. This is known as *respiratory control*. For electrons to flow through the electron transport chain to O_2, the simultaneous phosphorylation of ADP to ATP is usually required. When ATP is utilized, ADP concentrations increase; therefore the rate of oxidative phosphorylation increases, that is, if an adequate O_2 supply is available. So, oxidative phosphorylation is coupled to ATP utilization via the relative ADP concentration.

Enzymes in the β-oxidation pathway are inhibited by NADH and acetyl CoA; however, the rate of FA oxidation is determined greatly by substrate availability.

ENERGY PATHWAY CONTRIBUTIONS IN THE EXERCISING HORSE

The duration and intensity of exercise determine the metabolic requirements of muscle. At any given time, the most effective combination of the various energy-producing pathways will occur; again, it should be emphasized that no form of exercise by the horse is entirely aerobic or anaerobic in nature.

At the initiation of exercise, the immediate source of energy is locally available ATP. As previously noted, this supply of ATP is limited and rapidly depleted; therefore, for exercise to continue, ATP must be replenished by other processes. Creatine phosphate and the phosphocreatine pathway provide the next rapidly available ATP source; however, this energy supply is also of limited capacity (McMiken, 1983). The myokinase reaction provides a further means of regenerating ATP, but it is restricted to certain muscle fiber types and is used in anaerobic exercise only when these fibers are recruited (Meyer et al., 1980). The myokinase reaction is believed to have a minor role in energy production overall.

The glycolytic pathway with the production of pyruvate and lactate provides the main ongoing ATP source and reaches peak metabolism within about 30 seconds of the onset of exercise. The delay in maximal glycolytic output is possibly caused by the multiple and complex reactions required (McMiken, 1983). The large stores of glycogen in equine muscle (Hodgson et al., 1984; Lindholm, 1979; Lindholm and Piehl, 1974; Lindholm et al., 1974; Nimmo and Snow, 1983) allow this pathway to provide an early consistent source of energy, but still there is a finite limit to this substrate.

Aerobic mechanisms for ATP replenishment represent the most efficient means of substrate production. However, it is the slowest pathway to respond to exercise demands, owing to the cardiovascular lag in supplying O_2 to the cells and the intricacy of the reactions. Oxidative processes are in full production within 1 minute after the onset of exercise, and then muscular energy is more likely to be dependent on the rate of O_2 transport to the cells rather than substrate availability (McMiken, 1983). In fit racehorses, the time to peak energy production via oxidative processes can be as short as 20 seconds, especially following warm-up (Tyler et al., 1996).

At rest and during low-intensity exercise (walking and trotting), aerobic pathways provide most energy requirements

after the initial lag period. At this exercise intensity, the ratio of ATP to ADP will be high; PFK will, therefore, be inhibited and β-oxidation of fatty acids will provide the main method for ATP regeneration (Hodgson et al., 1985; Lawrence, 1990). Such is the case during endurance rides, in which case, it is well recorded that blood concentrations of nonesterified fatty acids (NEFA) increase and the glycogen utilization rate is low (Hodgson et al., 1983; Hodgson et al., 1985; Lucke and Hall, 1980; Snow et al., 1982) (Table 3-1 and Table 3-2).

As exercise intensity increases, ADP accumulates, and this stimulates anaerobic glycolytic energy production and a dramatic increase in the use of CHO substrates (see Table 3-1). Galloping and bursts of intense exercise such as during polo games and jumping rely heavily on anaerobic energy supply. The self-limiting nature of anaerobic power output (substrate exhaustion) means the horse can only maintain maximal speed for about 600 to 800 m. After this distance, energy supply falls back to the slower aerobic pathways, necessitating a reduction in speed of exercise (Hodgson et al., 1985; McMiken, 1983).

ENERGY SUBSTRATES

Scientific data on the relationship between nutrition and equine performance has occupied the attention of many researchers in recent years; however, it is difficult to design controlled experiments that only isolate nutritional influences on performance. Subtle, yet important effects of nutritional alterations may go undetected, partly because the power of statistical studies is limited by the small numbers of horses often used in experiments (Hintz, 1994). The relationship between energy and exercise is complex and inseparable. The amount of energy required depends on the type and duration of activity and the horse's body weight. Maintenance digestible energy (DE) requirements are linearly related to body weight (Pagan and Hintz, 1986a). During submaximal exercise energy expenditure is exponentially related to speed and proportional to the body weight of the riderless horse or the combined weight of the horse plus rider (Pagan and Hintz, 1986b). The method used by Pagan and Hintz (1986b) for calculating energy requirements was based only on the amount of work performed and may not account for any follow-on demands for energy in recovery that the work bout stimulates (Lawrence, 1990).

The stores of major fuels in the horse for muscular contraction are outlined in Table 3-3 as calculated by McMiken (1983). It is clear that "fast" energy stores (i.e., ATP, creatine phosphate, and glycogen) are limited despite the high capacity for glycogen storage in equine muscle. The primary dietary sources of energy stores for the horse are soluble and fiber derived CHOs and fats. Protein is considered to play a minor role as an energy source.

TABLE 3–1

Rates of Glycogen Utilization in Horses Performing Various Types of Athletic Activities *(Adapted from Hodgson, 1985.)*

Type of Exercise	Average Speed (m/min)	Glycogen*
ENDURANCE RIDE		
40 km	187	1.47
110 km	173	0.41
160 km	180–135	0.69
THREE-DAY EVENT		
Cross-country phase (23.1 km)	293 (range 220–690)	4.08
TROTTING		
4 hr	300	0.50†(2.0)
1 hr	500	0.175†(7.0)
5 min	750	15.0†(60)
RACING		
506 m	870	149.4
800 m	920	191.9
1025 m	846	129.3
1200 m	960	126.5
1600 m	756	66.5
3620 m	684	18.8

Note: At speeds greater than 500 to 600 m/min, intensity of exercise is such that aerobic phosphorylation is unable to meet all the energy needs of the working muscle; therefore, anaerobic glycolysis plays a major role in the supply of energy. As a result, glycogen utilization at these speeds and greater increases dramatically.

*Millimoles glucose units per kilogram muscle per minute (dry weight).

†Millimoles glucose units per kg muscle per min (wet weight). Approximate figures for dry weight values are presented in parentheses

TABLE 3–2

Mean Plasma Nonesterified Fatty Acid Concentration in Horses Performing Various Athletic Activities *(Adapted from Hodgson, 1985.)*

Type of Exercise	Mean Plasma NEFA Concentration (μmol/L)
80 KM ENDURANCE RIDE	
Pre-exercise	47
Postexercise	1254
THREE-DAY EVENT *(CROSS-COUNTRY PHASE)*	
Pre-exercise	156
After steeplechase and	
roads and tracks section	586
After cross-country section	324
GALLOPING (1.2 KM)	
Pre-exercise	246
Postexercise	279

TABLE 3–3

Energy Stores *(Adapted from McMiken, 1983.)*

Energy		
FUEL	KILOJOULES (KJ)	KILOCALORIES (KCAL)
Adenosine triphosphate (ATP)	38	9
Creatine phosphate	188	45
Glycogen	75,300	17,988
Fat	640,000	152,889

Note: These values are estimations for a 500 kg horse with a muscle mass of 206 kg (approximately 55 kg being locomotor muscles), adipose tissue of 25 kg, and a liver of 6.5 kg.

Carbohydrates

Absorbed CHO is immediately available as an energy source in the form of blood glucose. Muscle and liver are the reservoirs where excess CHO is stored as glycogen. Numerous studies have documented the depletion of muscle glycogen stores that occurs with exercise in the horse. The rate and percentage of depletion that results is a function of the intensity and duration of exercise. Muscle glycogen utilization per minute is greatest at faster speeds over shorter distances (Hodgson et al., 1984; Nimmo and Snow, 1983) (see Table 3-1), but the total percentage of glycogen depletion increases with increasing duration of exercise (Hodgson et al., 1983; Snow et al., 1981; 1982). Liver glycogen stores are also depleted significantly during exercise (Lindholm et al., 1974; Lindholm, 1979).

Fat

Assimilated fats are stored as triglycerides (uncharged esters of glycerol) in adipose tissue and muscle. Quantitatively, adipose tissue constitutes the largest energy store in the body (see Table 3-3). Triglyceride concentrations in muscle are considerably less than in fat (Lindholm, 1979). The triglycerides are highly concentrated stores of energy because they are reduced and anhydrous (Stryer, 1988). The initial event in the utilization of triglycerides as an energy substrate is their hydrolysis by lipases to glycerol and free fatty acids (FFA). Lipolysis is stimulated by epinephrine, norepinephrine, glucagon, and adrenocorticotrophic hormone and inhibited by insulin. Glycerol is converted in a number of steps to Gl-3-P, which is an intermediate in both the glycolytic and gluconeogenic pathways. The FFAs undergo β-oxidation and enter the TCA cycle as previously described. Oleic, palmitic, and linoleic acids represent the major FAs in the horse (Robb et al., 1972).

Fat has been shown to be the major energy substrate during low-intensity exercise. This is best evidenced by a decrease in the respiratory exchange ratio (R) (McMiken, 1983; Pagan et al., 1987; Rose et al., 1991) and an increase in plasma NEFA concentrations (Essen-Gustavsson et al., 1991; Lindholm, 1979; Rose et al., 1980) that occurs with prolonged submaximal exercise. R is calculated by dividing the volume of carbon dioxide (CO_2) expired by the volume of O_2 consumed during exercise. R values around 0.7 indicate fat utilization, whereas for CHO utilization, the value is 1.0. Values within this range reflect various mixtures of FA and CHO metabolism. When anaerobic metabolism predominates, R values will exceed 1.0 because lactate production is high, thereby adding to the CO_2 load to be eliminated.

Protein

Digested protein is absorbed from the small intestine as amino acids and small peptides. When amino acids are available in excess of the animal's requirements, they may be broken down to provide energy. Degradation by deamination or transamination reactions occurs mostly in the liver, with the final product being acetyl CoA for utilization in the TCA cycle. Leucine, a branched-chain amino acid, may undergo oxidation directly in muscle (Lawrence, 1990). The contribution of amino acids to energy production during exercise is minor compared with that of CHO and FA (Åstrand and Rodahl, 1986), perhaps in the range of 1% to 15% (Lawrence, 1990). High-protein diets (up to 16%) were once thought necessary to sustain the performance of mature equine athletes, but now it is considered that approximately 10% protein in the diet is adequate (see Chapter 4).

EFFECTS OF DIETARY ALTERATIONS ON ENERGY SUBSTRATE UTILIZATION

Many published reports have described the effect that altering components of the normal diet has on substrate utilization and performance in the horse. The consumption of large amounts of digestible CHO within a few hours of strenuous activity may depress the performance of that exercise (Åstrand and Rodahl, 1986). This is possible because insulin-stimulated uptake of blood glucose results in hypoglycemia, a greater dependence on muscle glycogen, and, therefore, earlier onset of fatigue. Free FA mobilization is also inhibited by insulin (Frape, 1988). The effects of consumption of CHO or fat before or during exercise on metabolism are summarized in Table 3-4.

However, a lack of available CHO during submaximal exercise can also limit performance; there is strong evidence supporting the use of high CHO diets in humans for endurance exercise. In humans, muscle glycogen loading was achieved by performing intense exercise and then consuming a CHO-rich diet (Lindholm, 1979). Current practice is to combine a program of decreased activity with increased CHO consumption a few days before competition to achieve a glycogen load. Glycogen loading in horses has been accomplished, but no obvious improvement in work performance has been demonstrated (Frape, 1988; Lawrence, 1990; Snow, 1994; Topliff et al., 1983; 1985). Intravenous, but not oral, glucose supplementation has increased glycogen repletion rates after exercise (Davie et al., 1994; 1995). Although glycogen loading is not recommended in the horse, adequate CHO intake must still be ensured (Hintz, 1994). A low CHO diet and regular exercise

TABLE 3–4

Effects of Dietary Alterations on Energy Substrate Utilization

Food Type	Effects on Carbohydrate Metabolism	Effects on Fat Metabolism	Other Effects
Starch/glucose	Increased insulin, decreased blood glucose, increased glycogenolysis	Decreased lipolysis	Decreased endurance and speed?
Fats	Increased citrate, decreased phosphofructokinase, decreased glycogenolysis		Glycogen sparing?
Glucose during exercise	Increased glycemia		Extends endurance time?
Glucose (intravenously) after exercise	Increased glycemia		Increased rate of glycogen resynthesis

lead to glycogen depletion and decreased performance in horses (Topliff et al., 1983; 1985). In a study in which fit Standardbreds were exercised strenuously for 3 consecutive days to achieve a 55% depletion of the muscle glycogen store, anaerobic, but not aerobic, capacity was seen to be impaired (Lacombe et al., 1999). However, the association between glycogen depletion and impaired anaerobic metabolism is not conclusive as confounding effects of other exercise-induced changes on performance could not be eliminated (Lacombe et al., 1999). When muscle glycogen was depleted by 22%, there was no significant effect on the performance of Thoroughbreds exercising at high intensities (Davie et al., 1996). A CHO supplement taken an hour or two before exercise does not seem to benefit endurance performance, but intake of glucose during exercise may supplement waning plasma concentrations and delay the onset of fatigue (Lawrence, 1990).

The beneficial effects of feeding high-fat diets to horses on substrate utilization and performance remain controversial, although benefits for horses with problems such as recurrent tying up and gastric ulceration are unequivocal. Differences in the condition of the horses, type of exercise, the length of the adaptation period to the diets, the type of fat used as the supplement, and the level of fat supplemented, particularly in relation to CHO, make comparing the published results difficult. Many variations in study designs influence the results. Feeding an increased level of fat is suggested to cause metabolic adaptations that permit horses to preferentially utilize fat and spare glycogen during exercise, but the evidence to support such a proposal is inconclusive (Hintz, 1994; Lawrence, 1990; Snow, 1994).

Interest in amino acid requirements of the performance horse is growing, and there is a suggestion that improvements in oxidative capacity can be brought about by certain amino acid supplements (Hintz, 1994; Lawrence, 1990). Higher-than-necessary protein diets are often fed to performance horses, but studies to demonstrate that this practice enhances exercise capabilities are lacking (Lawrence, 1990). In the case of CHO-rich and fat-rich diets, plasma concentrations of glucose, ammonia, lactate, alanine, and the muscle concentrations of G-6-P and lactate were higher at the end of exercise, compared with normal diets (Essén-Gustavsson et al., 1991). Higher pre-exercise muscle glycogen concentrations and FFA concentrations were present when horses were fed a CHO-rich diet compared with the fat-rich and normal-diet fed periods. No significant difference in performance during trotting at submaximal intensity on a horizontal treadmill was detected between the three diets (Essén-Gustavsson et al., 1991) with the average time to fatigue being 51 to 56 minutes. Whether or not the diets would alter performance in shorter or longer exercise periods remains unanswered. The effects on protein metabolism need to be further investigated as both the CHO-rich and the fat-rich diets, compared with normal diet, were associated with significant increases in branched-chain amino acids in plasma during and at the end of exercise (Essén-Gustavsson et al., 1991). The resting plasma concentration of the branched-chain amino acids was increased by 26% with the fat-rich diet but only by 8% with the CHO-rich diet. A 9% (control) or 18.5% (high) crude-protein diet had no effect on hepatic or muscular glycogen utilization and did not affect exercise performance in Quarterhorses exercising at submaximal intensities (Miller-Graber et al., 1991). Performance of Arabian endurance horses was not augmented by excessive protein in the diet (Hintz, 1983). In contrast, Standardbreds fed a high-protein diet (20%) or high-fat diet (15% soybean oil) showed greater muscle and liver glycogen utilization during prolonged exercise than when fed a control diet of 12% crude protein (Pagan et al., 1987). During higher-intensity, shorter-duration exercise, glycogen utilization was less when horses were fed high-protein or high-fat diets.

The timing of feeding and what to feed before exercise have considerable influence on the metabolic and physiologic responses to exercise (Harris and Graham-Thiers, 1999; Lawrence et al., 1995). In one study, it was concluded that feeding only hay shortly before exercise would not adversely affect performance but feeding grain would and that grain therefore should be withheld (Pagan and Harris, 1999).

Of course, many other nutritional components not discussed in this chapter may play roles in equine performance. These include water, electrolytes, acid–base balance, minerals, and vitamins. For more information see Chapter 4.

ENERGY EXPENDITURE

AEROBIC POWER OR OXYGEN UPTAKE

The oxygen consumed by the body at a given time is a measure of the body's total aerobic metabolic rate and is termed the *oxygen uptake* ($\dot{V}O_2$). Units of measurement are usually milliliters of oxygen per kilogram of body weight per minute (mL/kg/min) or liters per minute (L/min), therefore representing a rate of consumption and not a finite capacity. The maximum rate of oxygen uptake is called the $\dot{V}O_{2max}$. Oxygen consumption by the body is principally a function of the cardiorespiratory system to supply O_2 and the capacity of end organs to utilize O_2. The sequence of events is described as the *oxygen transport chain* (see Chapter 1). It is influenced by the O_2 concentration in the air, ventilation of the lungs, diffusion of O_2 through the alveolar wall, circulatory perfusion of the lungs and affinity of hemoglobin (Hb) for O_2, distribution of O_2 to the periphery by the circulation, extraction by the end organ (muscle) and, finally, O_2 utilization by the mitochondria. A large number of physiologic variables contribute to the capacity of the O_2 transport chain.

Oxygen Uptake at Rest and during Submaximal Exercise

At rest, $\dot{V}O_2$ is in the order of 3 to 5 mL/kg/min or 1.5 to 2.5 L/min for a 500 kg horse (Thornton et al., 1983). It can be difficult to accurately obtain a basal $\dot{V}O$ prior to exercise as often horses are excited in anticipation of impending activity. Therefore, a resting $\dot{V}O_2$ level of 2 mL/kg/min may be more realistic. During submaximal exercise, a number of factors will influence the level of $\dot{V}O_2$, including speed of exercise, load being carried, degree of incline on which exercise is being performed, duration of exercise, thermoregulation, and track surface.

Speed

There is a well-established linear relationship between $\dot{V}O_2$ and the speed of exercise at submaximal intensities in horses (Evans and Rose, 1987; 1988; Hörnicke et al., 1983; Hoyt and Taylor, 1981; Rose et al., 1990) and humans (Åstrand and Rodahl, 1986). When speed increases such that $\dot{V}O_{2max}$ is approached, this linear relationship is lost as $\dot{V}O_2$ plateaus and anaerobic sources of energy production become significant. In addition, if horses exercise at unnatural (extended or

restricted) gaits, the linear relationship will be lost due to a loss in economy of locomotion (Griffin et al., 2004; Hoyt and Taylor, 1981; Preedy and Colborne 2001; Wickler et al., 2001) (Figure 3-3 and Figure 3-4).

Load

Few equine sports are performed without the horse carrying an extra load in the form of a rider or driver. Oxygen consumption (or energy expenditure) increases in proportion to the load carried (Gottlieb-Vedi et al., 1991; 1997; Pagan and Hintz, 1986b; Taylor et al., 1980; Thornton et al., 1987). Taylor and colleagues (1980) reported that when a 10% load was added to the horse when trotting, $\dot{V}O_2$ increased approximately by 10%, and this direct proportionality was consistent for loads between 7% and 27% of the horse's body mass. A direct proportionality between load and $\dot{V}O_2$ was also demonstrated in trotting rats, trotting and galloping dogs, and running humans (Taylor et al., 1980). As a consequence, small animals use more O_2 and expend more energy to carry each gram of a load a given distance than do large animals, be it their own body mass or an additional load carried. Pagan and Hintz (1986b) demonstrated that a 450-kg horse with a 50-kg rider would expend the same amount of energy as would a 500-kg horse carrying the same weight. Thornton and colleagues (1987) found no significant difference between loaded and unloaded horses in the O_2 cost per kilogram per meter traveled. The increase in $\dot{V}O_2$ due to load is achieved largely by an increase in ventilation until maximum tidal volume is approached (Thornton et al., 1987), and this is readily explained by the close and linear relationship between $\dot{V}O_2$ and pulmonary ventilation (Gottlieb-Vedi et al., 1991; Hörnicke et al., 1983). The implications for racing performance should be considered. Compared with a heavier horse, a horse of less mass will expend proportionally less total energy to move the same distance.

Slope and Terrain

The degree of incline on which exercise is being performed has a significant impact on $\dot{V}O_2$. For Standardbreds, trotting on a 6.25% inclined treadmill at an average speed of 5.2 meters per second (m/s), $\dot{V}O_2$ increased from a mean of 17.7 L/min on the flat surface to 31.1 L/min on the slope (mean change of 13.4 L/min (76%, $p < 0.001$) (Thornton et al., 1987). The addition of a load when doing the inclined exercise did not significantly add to the O_2 cost of the exercise. Thoroughbreds exercising on a treadmill at speeds of 1 to 13 m/s also showed a substantial increase in$\dot{V}O_2$ when the treadmill slope was elevated from 0% to 5% and 10% (Eaton et al., 1995a) (Figure 3-5). Exercising on a 10% slope can double the energy expenditure at some speeds. When trotting on a treadmill at an incline over a range of speeds, $\dot{V}O_2$ max is higher than with level running (McDonough et al., 2002), and a greater volume of muscle would have to be recruited to generate an equivalent force for body support, which is reflected in significant increases in the electromyography (EMG) intensity (IEMG) of muscles (Wickler et al., 2005).

Wickler et al. (2004) found that in the horse, the costs of swinging the limbs are considerable and that the addition of weights to the distal limb can have a profound effect on not only the energetics of locomotion but also on the kinematics, at least in the hindlimb. Thus, they proved that the use of weighted shoes, intended to increase animation of the gait, increases the metabolic effort of performance horses to a disproportionate

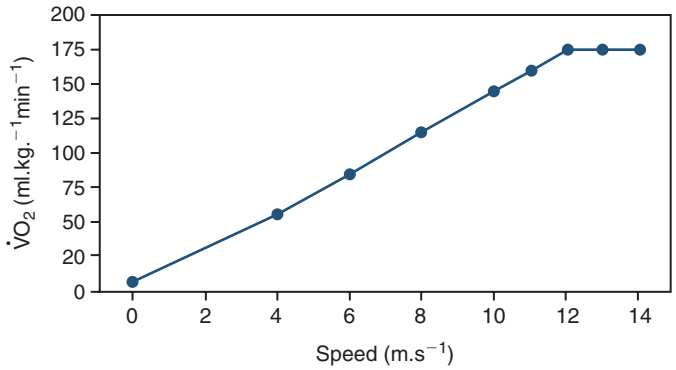

FIGURE 3-3 The relationship between aerobic capacity ($\dot{V}O_2$) and speed and maximal oxygen uptake. Horses exercising on a treadmill set at a 10% incline demonstrate the linear relationship between speed and $\dot{V}O_2$ up to the speed where maximal oxygen uptake is reached (in this case at 175 mL/kg/min at 12 m/s). Once maximal oxygen uptake is attained, further increases in speed will not elicit any further increases in $\dot{V}O_2$ and the extra energy required to run at these speeds must be provided by anaerobic pathways.

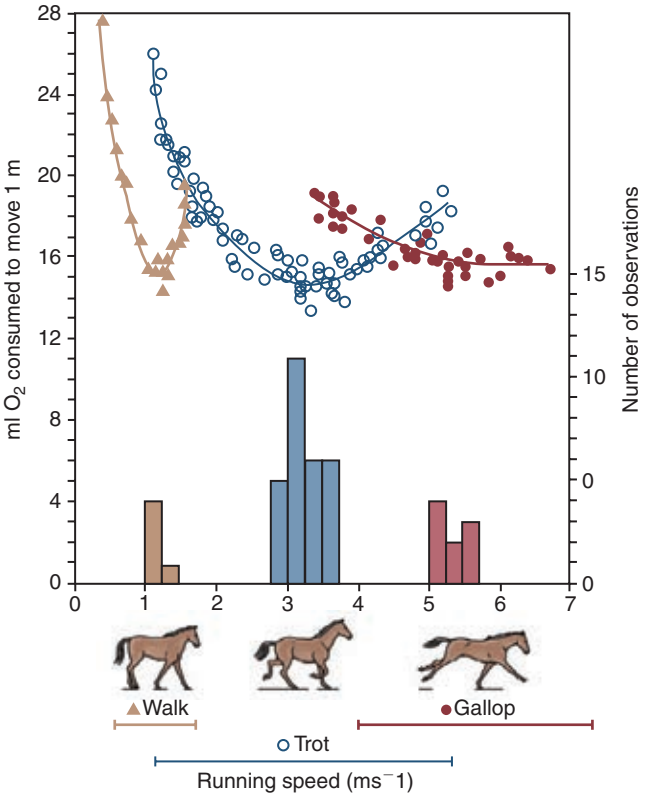

FIGURE 3-4 Economy of locomotion. The oxygen cost to move a unit distance (rate of oxygen consumption divided by speed) declined to a minimum and then increased with increasing speed in a walk and trot. It also declined to a minimum in a gallop, but the treadmill did not go fast enough to make it possible to observe any increase at higher galloping speeds. The minimum oxygen cost to move a unit distance was almost the same in all three gaits. The histogram shows gaits when a horse was allowed to select its own speed while running on the ground. The three speeds chosen coincided with the energetically optimal speed for each gait. (From Hoyt DF and Taylor CR: *Gaitand the energetic of locomotion in horses,* Nature 292:239-240, 1981)

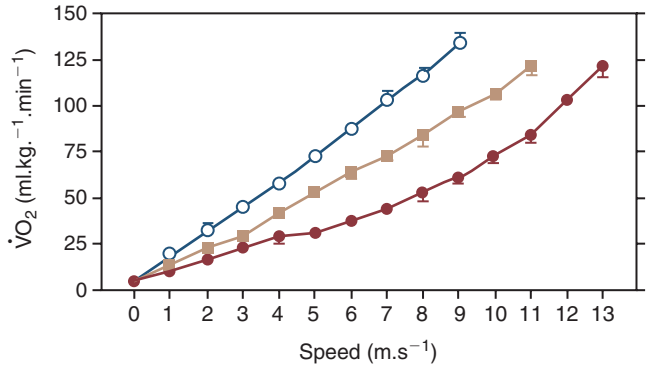

FIGURE 3-5 The effect of slope on aerobic capacity ($\dot{V}O_2$). Horses exercising on a treadmill increase $\dot{V}O_2$ as the incline of the treadmill increases. At some speeds, exercising on a 10% incline will double the energy expenditure compared with exercise on the flat (• = 0% incline; ■ = 5% incline, and 0 = 10% incline).

amount. The additional mass also increases the joint range of motion and, potentially, the likelihood of injury.

The terrain of endurance rides and cross-country tracks in 3-day events ensure that much work up and down gradients will be performed, and this will play a large role in determining energy expenditure. Little investigation has been done with regard to the effect of a downhill gradient on energy expenditure in horses; in humans, however, the energy cost of moving down a slope decreases up to a certain steepness and then becomes more expensive compared with level exercise (Åstrand and Rodahl, 1986). A similar response is likely for the horse.

Track surfaces may affect the economy of locomotion due to altered stride patterns (change in frequency and length of stride) in slippery, uneven or "heavy" conditions. Quantifying track effects on energy expenditure is difficult, but Thoroughbreds and endurance horses have longer race times in heavy conditions. Different treadmills also influence the energy cost of locomotion: It costs less energy for horses to walk, trot, or canter on a stiffer treadmill than on a more compliant treadmill (Jones et al., 2006).

Duration

The effect that duration of exercise has on $\dot{V}O_2$ has not been frequently investigated. Rose and Evans (1986) monitored cardiorespiratory and metabolic alterations during 90 minutes of submaximal exercise in Standardbreds. The horses trotted on a slope of 2% at 3 m/s. Many respiratory variables were measured, including $\dot{V}O_2$, which reached a steady state within 5 minutes of the start of exercise and remained stable for the duration of the exercise period. Oxygen consumption from 5 minutes onward did not alter significantly until a slight decrease was identified at 90 minutes. It was proposed that fluid and electrolyte losses in the sweat, contributing to thermoregulatory and circulatory problems, would be key factors in the horse's ability to perform endurance activity (Rose and Evans, 1986). Naturally, the intensity of exercise will be a determinant of the duration of any activity.

Temperature

The effect of temperature on $\dot{V}O_2$ will be a consequence of any impedence that thermoregulation may have on energy demands. Redistribution of cardiac output to skin for heat dissipation, fluid shifts, and metabolic disturbances may all contribute to a less-efficient O_2 transport chain and, therefore, diminished performance (see also Chapter 8). The optimal temperature range for O_2 utilization has yet to be established.

Rider

It has been reported that major horse race times and records improved by 5% to 7% around 1900 when jockeys adopted a crouched posture. When animals carry loads, there is a proportionate increase in metabolic cost, and in humans, this increase in cost is reduced when the load is elastically coupled to the load bearer. Pfau et al. (2009) showed that jockeys move to isolate themselves from the movement of their mounts, which would be difficult or impossible with a seated or upright, straight-legged posture. This isolation means that the horse supports the jockey's body weight but does not have to move the jockey through each cyclical stride path. This posture requires substantial work by jockeys, who have near-maximum heart rates during racing.

Maximum Aerobic Power

When $\dot{V}O_2$ no longer increases despite an increase in workload, the horse is defined as having reached $\dot{V}O_{2max}$. This value represents the maximum or peak aerobic power (see Figure 3-3). In humans, $\dot{V}O_{2max}$ has been considered the "gold standard" by which prolonged exercise capacity can be judged. Thoroughbred horses have mean $\dot{V}O_{2max}$ values around 160 mL/kg/min (Evans and Rose, 1987; Rose et al., 1990).

The horse's tremendous ability to achieve a higher $\dot{V}O_{2max}$ compared with other athletic species is related to its massive heart rate (HR) response and ability to substantially augment its circulating red blood cell mass, and therefore oxygen-carrying capacity, during exercise (Thomas and Fregin, 1981; see Chapter 2). In horses, a positive correlation between running speed and $\dot{V}O_{2max}$ has been described, and this correlation became stronger as the distance ran increased (Harkins, 1993). It has been suggested that faster horses utilize more oxygen during maximal exercise intensity. In addition, there is anecdotal evidence of a relationship between $\dot{V}O_{2max}$ and racing performance, although this has yet to be proven.

ANAEROBIC POWER

Anaerobic energy supply becomes significant when exercise intensity is at a level beyond that which aerobic pathways alone can accommodate. The faster glycolytic pathways may be recruited under one of two conditions: (1) when energy demand increases so rapidly that the slower aerobic systems cannot match the supply rate required and (2) when the total energy demand exceeds what the aerobic pathways are capable of supplying at peak capacity. Workloads at intensities beyond that provided for by $\dot{V}O_{2max}$ have been referred to as *supramaximal intensities*. This level of energy utilization is experienced by racing Thoroughbreds, Standardbreds, and Quarterhorses during competition. Shorter duration races, for example, Quarterhorse 400 m sprints, rely predominately on the rapid supply of energy by anaerobic means. Anaerobic power is considered a finite capacity and not a rate because the supply of substrates for anaerobic phosphorylation is limited. Factors that influence anaerobic capacity include muscle concentration of high-energy phosphates, muscle glycogen concentration and rate of glycogenlysis, percentage of type II

(especially type IIB or type IIX) muscle fibers, muscle fiber area (which is larger for type II muscle fibers), and muscle buffering capacity.

Theorizing that anaerobic capacity is a function of the area of type II fibers in the locomotor muscles, McMiken (1983) stated that to measure maximal anaerobic capacity, one should calculate the type II fiber area and the activities of anaerobic pathway enzymes in the muscle. Maximum accumulated oxygen deficit (MAOD) has been investigated as a measure of anaerobic capacity in horses (Eaton et al., 1992; Eaton et al., 1995b) following preliminary studies indicating its usefulness in humans (Mebø et al., 1988; Scott et al., 1991). Oxygen deficit refers to the deficiency in $\dot{V}O_2$ that occurs at the commencement of exercise until the responding cardiorespiratory system meets the O_2 demand of the tissues. The total O_2 deficit that accumulates during exercise at supramaximal intensities is the MAOD, and this is the difference between the O_2 demand and the actual $\dot{V}O_2$ achieved. The O_2 demand is calculated by extrapolating from the linear relationship between $\dot{V}O_2$ and speed at submaximal intensities (Figure 3-6). To determine MAOD, horses on a treadmill are rapidly accelerated to speeds equivalent to supramaximal intensities (defined as a percentage of $\dot{V}O_{2max}$ measured in a previous exercise test and extrapolated from the $\dot{V}O_2$ versus speed plot). The $\dot{V}O_2$ is measured at frequent intervals until the horse becomes fatigued. The area between the O_2 demand and the $\dot{V}O_2$ curve is the MAOD (Eaton et al., 1995b) (see Figure 3-6).

For exercise intensities requiring 105% to 125% $\dot{V}O_{2max}$, the MAOD was similar at 31 mL O_2 equivalents per kilogram of bodyweight, but the proportion of energy supplied by anaerobic processes increased from 14% to 30% (Eaton et al., 1995b). $\dot{V}O_{2max}$ was not correlated to MAOD, which suggested that anaerobic capacity is unlikely to be dependent on the rate of O_2 uptake. Eaton and colleagues (1995b) proposed from their results that anaerobic energy supply would contribute less than 30% of the total energy input in Thoroughbred and Standardbred races, which is considerably lower than previously suggested (Bayly, 1985). The use of peak blood or plasma lactate concentrations as an indicator of anaerobic capacity appears limited because of the many variables that affect lactate concentrations, including rates of flux between fluid compartments (Evans et al., 1993).

In humans, the power versus time-to-fatigue (P:TTF) relationship has been used as an accepted method for assessing anaerobic work capacity, and this relationship has now been investigated in horses (Lauderdale and Hinchcliff, 1999). In humans, the relationship is best described by the hyperbolic equation:

$$t = W9/(P\ 2Ø_{PA})$$

where t is the time to fatigue (s); P is power (watts); $Ø_{PA}$ is power asymptote, or critical power, which represents the maximum sustainable power output or anaerobic threshold, and W9 is a constant representing anaerobic capacity or the finite amount of work that can be performed above $Ø_{PA}$ (Figure 3-7). Similar to that in humans, the P:TTF relationship in Standardbreds is best represented by a hyperbolic

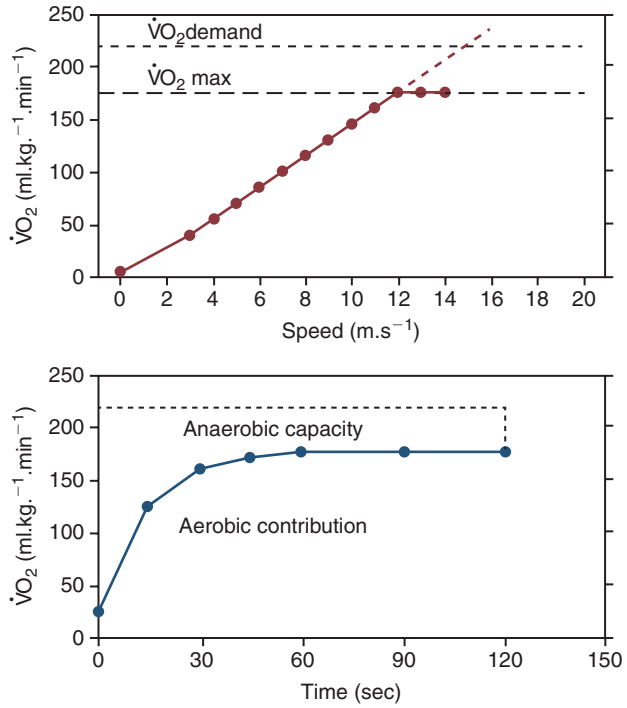

FIGURE 3-6 Determination of maximum accumulated oxygen deficit (MAOD). **A,** Initially, the aerobic capacity ($\dot{V}O_2$) versus speed plot is generated by performing a standardized incremental exercise test on an inclined treadmill. The high maximal aerobic capacity ($\dot{V}O_{2max}$) is 175 mL/kg/min, and for this horse to exercise at an intensity of 125% of $\dot{V}O_{2max}$, by extrapolation, it can be seen that the $\dot{V}O_2$ demand would be 219 mL/kg/min. To exercise at this supramaximal intensity, the horse would need to run at 15 meters per second (m/s). **B,** This figure demonstrates the relationship between $\dot{V}O_2$ and time for the horse exercising at 125% of $\dot{V}O_{2max}$. The previously calculated oxygen (O_2) demand is drawn in as the dotted line at 219 mL/kg/min. At the onset of the exercise, there is a lag in $\dot{V}O_2$, but it quickly reaches $\dot{V}O_{2max}$. The exercise ceases when the horse can no longer keep pace with the treadmill. The difference in the O_2 demand and the actual O_2 uptake is defined as the MAOD and is a measure of the anaerobic capacity.

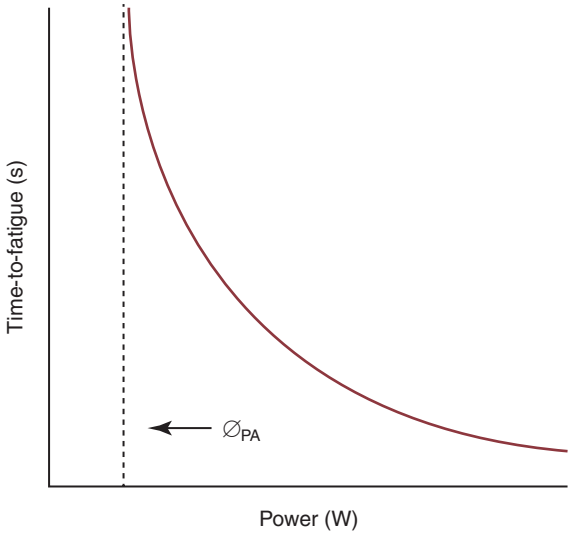

FIGURE 3-7 Schematic of the hyperbolic relationship between power (P) and time-to-fatigue (t) represented by the equation $t = W'/(P2ØPA)$. W', anaerobic capacity; $Ø_{PA}$, critical power; power units = watts (W).

function; however, the technique needs to be validated against the more traditional MAOD measure of anaerobic capacity before its usefulness in horses as a predictor of fitness and anaerobic capacity can be investigated (Lauderdale and Hinchcliff, 1999). The P:TTF does not require collection of respiratory gases or blood for its calculation, and this may be an advantage over the more intensive effort required to measure blood lactate concentrations or determine MAOD. However, currently, a high-speed treadmill test and multiple high-intensity exercise tests are still prerequisites, as the ability to calculate the P:TTF relationship in field trials has yet to be determined. For any measure of anaerobic capacity in the horse, the relationship that exists between performance and anaerobic capacity remains to be examined.

Anaerobic Threshold

Anaerobic threshold is defined as the level of work or $\dot{V}O_2$ consumption just below that at which metabolic acidosis and the associated changes in pulmonary gas exchange occur (Wasserman et al., 1973). It represents the transition during which anaerobic means of energy supply becomes important during exercise. In humans, this level of work has been correlated with a blood lactate concentration of 4 mmol/L (Åstrand and Rodahl, 1986). As exercise intensity increases, lactate accumulation in the circulation rises in an exponential manner. Hence, the anaerobic threshold has also been described as the *onset of blood lactate accumulation (OBLA)*. The velocity or intensity of work at which a blood lactate concentration of 4 mmol/L (V_{LA4}) is reached has been used to assess the relative fitness of horses and humans and their response to training (Auvinet, 1996; Persson, 1983; Thornton et al., 1983). The V_{LA4} increases with training (Eaton et al., 1999; Thornton et al., 1983); in general, the higher the V_{LA4}, the fitter is the horse (Rose and Hodgson, 1994).

Anaerobic threshold and OBLA are determined during an incremental exercise test. Anaerobic threshold is identified by the point of nonlinear increase in respiratory variables such as minute ventilation and CO_2 production (Wasserman et al., 1973). It is assumed that this point is highly correlated with the OBLA, but this may not be the case (Åstrand and Rodahl, 1986).

POSTEXERCISE OXYGEN CONSUMPTION

At the cessation of exercise, O_2 continues to be consumed above basal rates as it declines in an exponential manner to resting levels. This is referred to as *excess postexercise oxygen consumption* (EPOC) or *oxygen debt* (Figure 3-8). The EPOC may only account for a small fraction of the net total O_2 cost (NTOC) of exercise. In humans, during exercising at 30% to 70% of $\dot{V}O_{2max}$ for up to 80 minutes, the EPOC was only 1% to 8.9% of the NTOC of the exercise (Gore and Withers, 1990). In the only comprehensive study to date in horses, Rose and colleagues (1988) measured O_2 debt as the area under the O_2 recovery curve following a bout of exercise to fatigue at an intensity equivalent to 120% of $\dot{V}O_{2max}$. Oxygen debt represented nearly 52% of the NTOC, which is dramatically higher than that in the previously quoted study of humans. This can be attributed, in part, to the very different exercise intensities performed in the two studies and the relative fitness of the subjects.

EPOC is considered to have an initial fast phase and then a slower phase. In the horse, these phases were shown to be

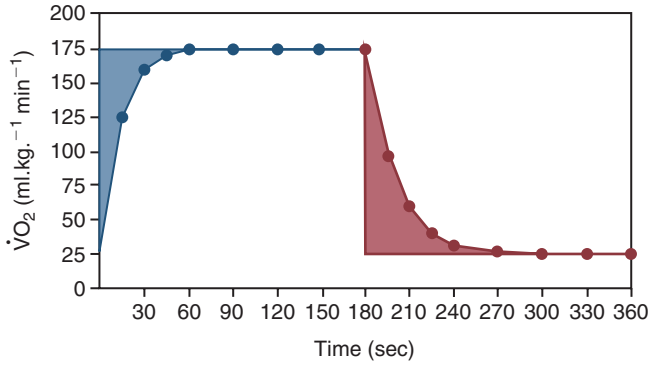

FIGURE 3-8 Oxygen deficit and oxygen debt. At the start of exercise, there is a lag in oxygen uptake relative to the energy demand; this "lag" is termed the *oxygen deficit*. Thus, in the early stages of exercise, the energy demand is met by O_2 stores within the body and anaerobic energy supply. At the cessation of exercise, metabolism does not immediately return to resting levels; this postexercise period is characterized by excessive postexercise oxygen consumption (EPOC), also termed the *oxygen debt* (*blue shading* = oxygen deficit; *red shading* = excessive postexercise oxygen consumption).

completed in 1.4 and 18.3 minutes, respectively, after supramaximal exercise (Rose et al., 1988). The fast phase is associated with the resaturation of myoglobin and hemoglobin and the replenishment of the high-energy phosphagen pool (CP and ATP). Perhaps less than 1.5% of the EPOC was required to restore the muscle CP pool in the horse, and this occurred at a slower pace than may have been expected (Rose et al., 1988). Postexercise tachycardia and tachypnea would also contribute small components to the EPOC because of increased consumption of O_2 by the myocardium and respiratory muscles until resting levels are reached. The slow phase is associated with the oxidation of lactate that accumulates during exercise. However, not all the lactate that is metabolized is accounted for by the EPOC, and some of it is utilized in gluconeogenesis and amino acid synthesis. A poor relationship existed between the restoration of muscle metabolites to pre-exercise levels and the recovery of $\dot{V}O_2$. Muscle and plasma lactate concentrations remained elevated after 60 minutes of recovery whereas $\dot{V}O_2$ had returned to near pre-exercise levels (Rose et al., 1988).

A number of other factors considered associated with EPOC, including exercise-induced hyperthermia, have not been quantified in the horse.

ENERGY PARTITIONING

As mentioned above, energy supply is not derived from one source because of the integration of aerobic and anaerobic pathways. During low-intensity exercise, virtually all the energy requirements will be met by aerobic mechanisms, and this would be the case in endurance rides. Sports requiring sudden bursts of activity (e.g., jumping and polo) or sustained high-intensity performance (e.g., racing) will require an increased anaerobic energy component.

At the start of supramaximal exercise, the reserves of oxygen (in the lungs, hemoglobin, and myoglobin), as well as stores of ATP and phosphocreatine in muscle, will provide the immediate supply of energy for exercise lasting for a few seconds. As the exercise continues, the anaerobic processes that utilize glycogen will provide energy, and as the duration of exercise increases, the aerobic pathways will become more

important. The intensity of exercise at which aerobic ATP production fails to meet energy demands and anaerobic processes commence will vary, depending on a range of factors. The most important is the rate of O_2 delivery to the exercising muscle. Other factors include mitochondrial density, catecholamine levels, intracellular enzyme concentrations, prior training, nutritional status, warmup, and the rate of increase in the workload.

The proportion of energy derived from aerobic versus anaerobic sources further highlights the importance that $\dot{V}O_{2max}$ may have on equine athletic performance, since in most competitive events aerobic energy delivery predominates. During a run at a speed eliciting an intensity of 125% $\dot{V}O_{2max}$ and lasting for nearly a minute, the majority of the energy (70%) was supplied aerobically (Eaton et al., 1995b). This intensity can be compared with a sprint race of 1000 m. In humans, the anaerobic capacity assumes a more substantial role, contributing 50% of the energy supply in supramaximal exercise of 1-minute duration, corresponding to an intensity of 175% $\dot{V}O_{2max}$ (Hermansen and Medbø, 1984). From these data, it can be suggested that horses that are considered *sprinters* still rely on aerobic energy delivery. Apart from the initial acceleration period, horses in longer races (e.g., 3200 m) provide a substantial proportion of their energy requirement via aerobic pathways. It is only in races of very short duration (i.e., 400 m lasting around 20 to 22 seconds) that anaerobic sources predominate (Figure 3-9).

ECONOMY OF LOCOMOTION

The *economy of locomotion* refers to the net energy cost in milliliters of O_2 per kilogram of body weight per meter traveled (mL O_2/kg/m). It is independent of speed and load (or body weight). There are conflicting reports on the values for economies of locomotion, but the importance of gait in these studies needs to be recognized. Horses on treadmills may be forced to work at defined speeds and, in doing so, utilize extended or restricted gaits that might alter the true cost of locomotion they would otherwise naturally incur if allowed to control their own pace. Thornton and colleagues (1987) have reported values of 0.122 mL O_2/kg/m for speeds of 4.5 to 6.25 m/s and 0.124 mL O_2/kg/m for speeds of 6.5 to 8.14 m/s obtained on a horizontal plane. These results are similar to the 0.133 mL O_2/kg/m that can be derived from the results of Taylor and coworkers (1980) for a 119 kg pony working at 3.11 m/s. Eaton and colleagues (1995a) recorded a range of 0.10 to 0.16 mL O_2/kg/m at speeds of 5 to 13 m/s on a horizontal treadmill. Exercising on a positively inclined treadmill will increase the O_2 cost of work by about 2 to 2.5 times that measured on a flat plane, depending on the steepness of the slope (Eaton et al., 1995a; Thornton et al., 1987). When averaging a range of speeds for each gait, Hörnicke and colleagues (1983) reported higher values for the walk (0.21 mL O_2/kg/m) and for the trot and gallop (0.19 mL O_2/kg/m) for horses exercising on the track. By averaging the economies of a number of speeds, a higher O_2 cost can be expected as values will include less efficient gait velocities than that at which the horses may be required to exercise (Hörnicke et al., 1983).

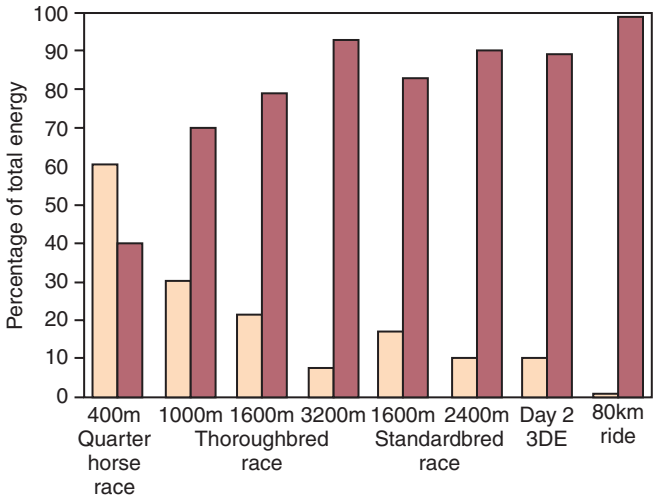

FIGURE 3-9 Energy partitioning. Estimates of the proportion of energy that is derived from aerobic and anaerobic pathways during competitive events (*light shading* = anaerobic contribution; *dark shading* = aerobic contribution).

It is well documented that horses will choose a gait at any speed that results in the least possible expenditure of energy (Griffin et al., 2004; Hoyt and Taylor, 1981; Preedy and Colborne, 2001; Wickler et al., 2001). Ponies (110–170 kg) were trained to walk, trot, and gallop and to extend their gait on command on a treadmill. Rates of O_2 consumption increased curvilinearly with speed for walking and trotting. The maximum speed of the treadmill prevented sufficient data from being obtained for galloping velocities. Gait transition occurred at speeds when the O_2 consumption was similar for the two gaits, but when the ponies were forced to exercise at an extended gait beyond the normal range of speeds, O_2 consumption was higher (Hoyt and Taylor, 1981). Thus, there was a speed for each gait where the energy cost of locomotion was minimal, and this cost was similar for the walk, trot, and gallop (Hoyt and Taylor, 1981) (see Figure 3-4). So, at the optimal speed of each gait, the amount of energy consumed to move a given distance is much the same. When a horse was allowed to move at its natural pace freely, it did so by selecting speeds within each gait around the most energy efficient speed (Hoyt and Taylor, 1981). The optimal value for economy was similar to the 0.122 to 0.133 mL O_2/kg/m values derived elsewhere for flat treadmill exercise (Taylor et al., 1980; Thornton et al., 1987).

Stride frequency is a logarithmic function of walking speed in all species, a linear function of trotting or running speed, and nearly independent of speed in galloping. Griffin et al. (2004) found that the absolute walk–trot transition speed increased with size, but it occurred at nearly the same speed-to-length ratio. In addition, horses spontaneously switched between gaits in a narrow range of speeds that corresponded to the metabolically optimal transition speed. These results support the hypotheses that the walk–trot transition is triggered by inverted-pendulum dynamics and occurs at the speed that maximizes metabolic economy. At the trot–gallop transition, horses are hypothesized to change from spring mechanics to some combination of spring and pendulum mechanics, changing the slope of metabolic rate versus speed; this differs from that of other species where a linear relationship is maintained (Hoyt et al., 2006).

The effects of training and athletic activities on horses are subject to the needs to be considered with regard to the economy of locomotion. Endurance horses forced to use extended gaits for prolonged periods may become fatigued more

rapidly than if they were to move at their natural speed for each gait. The development of unnatural gaits such as pacing also has consequences related to energy expenditure. In an experiment where Standardbred horses had been trained to pace wearing hobbles or exercise using natural gaits, while the horses were working on a treadmill between 4 and 12 m/s, there was a trend for $\dot{V}O_2$ to be higher when pacing compared with trotting. Clearly, it becomes a matter of achieving the right balance of speed and energy consumed to complete the distances and be successful in such events.

FATIGUE

Fatigue is a complex and intricate physiologic response to exercise, leading to the inability to sustain further activity at the current intensity. Fatigue can be categorized as structural, acute, or chronic. *Structural fatigue* refers to biomechanical failure of tissues, for example, tendons, ligaments, and bone, that inadequately adapt to the stresses placed upon them. *Chronic fatigue* is a function of prolonged conditions such as chronic anemia and starvation. *Acute fatigue* is directly related to energy production in muscles and occurs in events requiring maximal work effort for short periods, for example, in Thoroughbred or Standardbred racing. It has been labeled *anaerobic fatigue* (McMiken, 1983) and has different causal factors to those that limit aerobic performance in endurance-type events. Fatigue appears to involve central (psychological or neurologic) and peripheral (muscular) contributions. Overtrained horses could become listless and "sour," and their performance may decline; this may be partly a manifestation of psychological fatigue. McGowan and Whitworth (2008) differentiated overtraining syndrome from *overreaching*, a term used in horses which, after suffering a loss of performance without an obvious clinical reason, recover their performance within 1 or 2 weeks. When excessive training stress is applied and recovery time is insufficient, performance reduction, and chronic maladaptation occurs. Overtraining syndrome is recognized as a complex condition that afflicts top-level horses in training. Peripheral causes of fatigue have been studied more widely as they are easier to define. Recently, De Graaf-Roelfsema et al. (2009) induced overtraining (performance decreased by nearly 20% compared with controls) by intensified training and found that in overtrained horses their resting pulsatile growth hormone (GH) secretion had become altered. The increased irregularity of nocturnal GH pulsatility pattern is indicative of a loss of coordinated control of GH regulation. Longer phases of somatostatin withdrawal were hypothesized to be the underlying mechanism for the observed changes in GH pulsatility pattern.

Fatigue in response to high-intensity exercise is likely caused by a combination of factors, including depletion of the phosphagen pool (ATP and CP), decreased intracellular pH, and possibly accumulation of lactate (Essén-Gustavsson 1999; Hodgson et al., 1985; McMiken, 1983). The main event appears to be a reduction in the concentration of ATP. Acidity can impair the respiratory capacity of muscle and have a direct effect on the contractile apparatus. In addition, acidosis and increased muscle temperature could be associated with impaired functioning of the sarcoplasmic reticulum. Finally, altered electrolyte gradients (potassium and calcium) will add to overall deleterious effects on muscle metabolism.

Hyperthermia, altered fluid and electrolyte balance, and fuel depletion have all been considered contributors to fatigue during prolonged, submaximal exercise (Hodgson et al., 1985; Lucke and Hall, 1980; Snow et al., 1982). Performance capacity or onset of fatigue in horses and humans has been correlated with depletion in glycogen stores in working muscle (Åstrand and Rodahl, 1986; McMiken, 1983; Snow et al., 1981; 1982). A decline in extracellular glucose concentrations may also be important. However, fatigue or reduced muscle power occurs before the complete depletion of any substrate, and it is the rate of ATP production that appears crucial.

Neural fatigue in short events is considered very unlikely but may be important in endurance events (McMiken, 1983). However, in supramaximal exercise lasting only a few seconds, fatigue may be related to an inability of the neuromuscular junction to maintain the propagation of excitatory action potentials into muscle fibers, possibly leading to early musculoskeletal damage (Leach and Sprigings, 1979).

CONCLUSION

It is imperative that anyone with an interest in the equine athlete should understand energy production and utilization in the horse. Both aerobic and anaerobic pathways of energy supply are necessary for all forms of exercise. In recent years, evidence has suggested that the aerobic system has a much greater role to play than previously thought, in short-duration, supramaximal exercise bouts. The effect of nutrition on performance has been heavily investigated but continues to be an area of considerable controversy. Comparison between studies is difficult, and at this time, meaningful conclusions cannot be drawn.

The various energy supply pathways have far-reaching implications in terms of specific training programs for horses competing in different athletic events. An understanding of the patterns of energy substrate supply and utilization in different athletic events allows tailored training strategies to be adopted to maximize the adaptations in various body systems. Although no activity is exclusively aerobic or anaerobic in nature, aerobic energy production is vital in all cases. Hence, an emphasis on establishing an effective "aerobic foundation" is considered paramount in any training program (Rivero, 2007). A basic model of training incorporates three phases: (1) a foundation phase, (2) a cardiovascular or aerobic phase, and (3) an anaerobic interval phase. The foundation phase is considered continuous or endurance-type training and is vital for the cardiovascular and musculoskeletal systems to make early adaptations to the stresses placed on them. Shorter runs, with every subsequent phase increased in intensity, are included in the program.

The economy of locomotion refers to the optimal gait at a given speed of exercise at which the energy cost is least, and this gait is naturally chosen by freely moving horses.

The horse is unique in its capacity to utilize O_2, as seen by the high values for $\dot{V}O_{2max}$ and the rapid kinetics of O_2 uptake. In all but the most intense exercise, aerobic pathways contribute the majority of the energy supply. It is this phenomenal ability to utilize O_2 that allows the athletic horse to work at extremely high intensities such as those during Thoroughbred and Standardbred races and also for prolonged durations such as during endurance rides. The measurement of $\dot{V}O_{2max}$ remains the single most important assessor of a horse's relative fitness.

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CHAPTER

4 Nutrition of the Performance Horse

SHANNON E. PRATT-PHILLIPS AND LAURIE M. LAWRENCE

EQUINE DIGESTION, ABSORPTION, AND METABOLISM

DIGESTION AND ABSORPTION

Being nonruminant herbivores, horses utilize both enzymatic digestion and microbial fermentation to break feedstuffs down into smaller particles for absorption into the bloodstream and for metabolism. Enzymatic digestion takes place primarily in the small intestine with enzymes from the pancreas and along the brush border of the epithelial surface. Microbial fermentation of food takes place through the symbiotic relationship horses have with bacteria, fungi, and protozoa present in the large intestine. These microbes ferment fibrous particles resistant to mammalian enzymes into usable products.

Starches are broken down in the small intestine by pancreatic amylase to maltose. Disaccharides such as maltose, sucrose, and lactose are further digested by the brush-border enzymes maltase, sucrase, and lactase, respectively, into simple sugars. Maltase activity is high in the horse and is found along all regions of the small intestine. Sucrase activity has been shown to be highest in the proximal small intestine, whereas lactase activity is similar throughout the small intestine (Dyer et al., 2002). The monosaccharides glucose, fructose, and galactose are absorbed and transported in blood to tissues for energy metabolism or storage. The glucose and galactose transporter, SLGT-1 (sodium/glucose cotransporter 1), is expressed principally in the proximal small intestine and has a limited capacity for these sugars (Dyer et al., 2002). Therefore, at high levels of grain intake, sugar digestion and absorption may exceed the capacity of the small intestine, and these carbohydrates may pass to the large intestine (Dyer et al., 2002; Potter et al., 1992). This occurrence may contribute to digestive disturbances associated with high grain intake (Dyer et al., 2002).

Dietary triglycerides are emulsified by bile salts from the liver and then broken down by lipases from the pancreas. The resulting fatty acids and glycerol are absorbed and transported to sites of storage and metabolism. Although equine diets are relatively low in fat, horses appear to be able to digest most fats efficiently. Digestibility of fat included in the diet is close to 100% at ranges between 15% to 23% dry matter (Kronfeld et al., 2004). This is likely supported by relatively high concentrations of lipase within the pancreas (Lorenzo-Figueras et al., 2007).

Dietary proteins are initially hydrolyzed by hydrochloric acid and pepsin within the stomach; specifically, pepsin acts on peptide bonds involving tryptophan and phenylalanine. Proteins are further broken down into their constituent amino acids in the small intestine by pancreatic enzymes such as trypsin and chymotrypsin. At the brush border, peptidases digest the remaining peptides and individual amino acids are transported in blood to tissues for use in protein synthesis or energy metabolism.

Dietary fiber is fermented by microbial populations within the large intestine. Cellulose and hemicellulose are the primary fibers digested in the large intestine, but pectin, fructan, and other carbohydrates that are not susceptible to mammalian enzymes are fermented in the large intestine also. The main products of fermentation are the volatile fatty acids (VFAs, also called short chained fatty acids or SCFAs), namely, acetate, propionate, and butyrate. These are produced in varying quantities and ratios, depending on the diet, with acetate being the principal VFA produced. The VFAs may be used by the horse for immediate energy production or for the synthesis of glucose or fat. Any soluble carbohydrate (starch or glucose) that reaches the large intestine also will be subjected to microbial fermentation, resulting in VFA production.

Some dietary protein may also escape digestion within the small intestine, particularly when associated with cell wall fiber. Therefore, dietary protein in high-fiber feeds may not be available until the large intestine. This becomes important with respect to digestibility of feedstuffs to ensure adequate amino acids are available for absorption in the small intestine. High-quality protein sources will, therefore, be both highly digestible and have optimal amino acid profiles. Absorption of intact amino acids from the equine large intestine is likely small (Bochroder et al., 1994), though the presence of lysine transporters within the colon have been reported (Woodward et al., 2003). Whereas small intestinal digestion results in the absorption of protein as amino acids, ammonia is an important product of the digestion of protein in the large intestine. The absorbed ammonia can be used to synthesize nonessential amino acids in horses, or it can be excreted as urea in the urine.

Along with the digestion and absorption of energetic substrates, the absorption of vitamins, minerals, and water necessary for athletic function is a vital function of the digestive tract. Water is absorbed from the large intestine, along with a large portion of the electrolytes (Argenzio et al., 1974a). The large intestine may act as a reservoir for fluid, particularly during dehydration, and is influenced by the amount of soluble fiber in the diet (Warren et al., 1999a). Sodium is absorbed along with glucose and water by SLGT-1, giving justification for the inclusion of glucose with electrolyte-replenishing beverages and supplements (Shirazi-Beechey et al., 2008). Further, salt water offered following a 45-km ride has been shown to assist with recovery of lost body weight (Butudom et al., 2002).

Careful attention should be paid not only to the amount of mineral in the diet but also to the ratios of particular minerals (e.g., calcium:phophorus [Ca:P] and zinc:copper [Zn:Cu]) and their sources (organic versus inorganic; presence of interfering substances). Most minerals, including calcium and magnesium, are absorbed within the small intestine, although phosphorus is absorbed within the large intestine. Calcium absorption largely depends on calcium status in the body, and its absorption is regulated by parathyroid hormone and vitamin D. Calcium absorption can be further affected by phosphorus, phytate, and oxalates in the diet. Phosphorus absorption is also affected by the presence of high levels of calcium and oxalates in the diet. Much of the phosphorus in typical feeds is found as phytate phosphorus, which may be less digestible than nonphytate phosphorus. Fat-soluble vitamins are absorbed in the small intestine and may be influenced by the degree of fat absorption (Kronfeld et al., 2004). A considerable amount of B vitamin synthesis occurs in the large intestine (Linerode, 1966), and absorption from the large intestine has been demonstrated for several B vitamins.

Nutrient Metabolism

At Rest

Following a meal, the process of digestion and absorption will result in an increase in the circulating levels of several nutrients. The effects of diet and feeding schedule on glucose metabolism have received the most attention from researchers because of the relationship between glucose and insulin. When a horse receives a meal consisting of a concentrate feed (such as corn), blood glucose levels will rise from a prefeeding concentration of approximately 4 to 5.5 millimoles per liter (mmol/L) to a concentration of 6.5 to 7.5 mmol/L within 2 hours of feeding. The extent of the increase in blood glucose concentration will depend on meal size and composition. In human nutrition, the glycemic index (GI) has been used to characterize the extent of the increase in blood glucose that occurs in response to specific foods. The GI is the area under the glucose curve after consumption of a test food expressed as a percentage of the response to a standard amount of a standard food. An alternative term, the glycemic load (GL), accounts for the available carbohydrate in the meal. There has been some interest in applying the GI or GL to equine diets (Kronfeld et al., 2004), but the relevance of a GI or GL to equine athletic performance has not been well defined. In addition, application of the GI or GL to equine diets is difficult, in part due to the inconsistency of the reference feed, differences in consumption rates, and potential interactions among ingredients. For example, a study showed that when fat was added to a grain mix, the GI for the grain mix tended to decrease (Pagan et al., 2000). Other studies have shown that processing can affect the glucose response to a specific feed (Healey et al., 1995; Hoekstra et al., 1999), but in some other studies, processing had no effect on glucose or insulin responses to feeding (Vervuert et al., 2003;2004). However, when a high-roughage meal is consumed, the increase in blood glucose is much smaller than when a grain meal is consumed (Stull and Rodiek, 1988).

In humans, it is well recognized that absorbed triglycerides are packaged into chylomicrons for transport to sites of metabolism or storage. The enzyme lipoprotein lipase facilitates the hydrolysis of triglycerides to fatty acids, thus allowing movement into the cell. Once inside the cell, fatty acids may be used to resynthesize triglycerides. The postabsorptive fate of fat in equines is not well understood, as attempts to isolate chylomicrons following a fat load have failed (Watson et al., 1992). Also, it is interesting to note that horses have a relatively slow clearance rate of triglycerides following a load (Moser et al., 1993). This slow clearance occurs despite an apparent increase in lipoprotein lipase activity with increased substrate, such as with hyperlipemia in ponies (Watson et al., 1992). Conversely, it appears that chronic fat feeding affects the metabolism of fat such that flux of triglycerides from blood to muscle is increased (Geelen et al., 1999), likely through the activity of lipoprotein lipase (Geelen et al., 2001).

Following a meal, plasma concentrations of amino acids also will increase. Peak levels of most amino acids in the plasma have been shown to occur within 2 hours of feeding, with decreases seen by 4 hours. Most amino acids will return to baseline concentrations within 8 hours (Hackl et al., 2006). It has been suggested that even after a 10-hour period of withholding feed, plasma amino acid concentrations are highly variable, so care should be taken with interpretation of data (Hackl et al., 2006). Similar to glucose, insulin stimulates amino acid uptake by skeletal muscle and other tissues.

Unlike triglycerides, the VFA are taken directly into the bloodstream and delivered to tissues without being packaged into chylomicrons or other lipoproteins. VFAs are absorbed from the large intestine quickly, within 30 minutes of being infused directly into the cecum (Glinsky et al., 1976). Lieb (1971) also detected VFAs in the portal blood 30 minutes after infusion into the cecum. However, as peak production rates occur 2 to 8 hours after feeding (Argenzio et al., 1974b), one would expect blood concentrations to peak shortly thereafter. The VFAs are transported in the portal system to the liver, where butyrate and propionate are taken up (Lieb, 1971). Acetate appears to remain in blood for delivery to systemic tissues. Propionate is largely glucogenic, and it has been reported that between 50% and 61% of blood glucose is derived from colonic propionate, depending on diet (Simmons and Ford, 1991). Butyrate is converted to acetate and ultimately acetyl-CoA (coenzyme A), which can then be oxidized via Kreb's cycle or used for fat synthesis.

Acetate is quantitatively the most important VFA. On an all-roughage ration, the molar percentage of VFAs in cecal or colon fluid is about 70% acetate, 17% propionate, 8% butyrate, and 5% others (isobutyrate, isovalerate, and valerate) (Glinsky et al., 1976; Hintz et al., 1971). When horses are fed high-concentrate diets, the proportion of acetate decreases, and the proportion of propionate increases. As expected, dietary composition affects blood VFAs accordingly; Pethick and coworkers (1993) and Doreau et al. (1992) reported higher concentrations of blood acetate with higher-forage diets.

During the process of fermentation, some energy is lost and is unavailable to the horse. Nonetheless, fiber fermentation and the VFAs produced can generate significant energy for the horse. Using labeled VFAs, it was estimated that 30% of the horse's total digestible energy intake was derived from cecal VFA production when fed a high-forage diet (Glinsky et al., 1976). Assuming substantial VFA production within the colon as well, it is assumed that an even greater proportion of the horse's energy needs would be met by VFAs. Studying arterial-venous differences, Pethick and others (1993) found that acetate accounted for up to 32% of the total substrate oxidation within the hindlimb at rest.

The storage or metabolism of energetic substrates available following a meal is directed, in part, by insulin. Insulin acts to shift metabolic processes toward anabolism, to create fuel reserves for future use. One of insulin's key functions is to facilitate the movement of glucose from the bloodstream into tissues, by stimulating the translocation of glucose transporter 4 (GLUT4) to the cell membrane. Though details are lacking, insulin binds to its receptor, which triggers a series of reactions and secondary messenger signals, ultimately resulting in the fusion of intracellular vesicles containing GLUT4, with the cell membrane. Also, through these secondary messenger systems, insulin activates glycogen synthase, the key enzyme of glycogen synthesis, thus promoting storage of glucose. At adipose tissue, insulin activates lipoprotein lipase, thereby facilitating the shuttling of triglycerides from within chylomicrons and lipoproteins to adipose tissue for fat storage. Insulin also has a negative effect on gluconeogenesis, amino acid catabolism, and lipolysis.

Following the decline of blood glucose concentrations, insulin secretion from the pancreas is decreased. In the hours following a meal, low blood glucose concentrations stimulate the release of glucagon, another hormone from the pancreas. Glucagon functions to antagonize the effects of insulin; as insulin promotes the storage of energetic substrates, glucagon acts to mobilize energetic substrates. In the horse, feed deprivation of 54 hours results in significantly elevated glucagon concentrations (Sticker et al., 1996). At the liver, through activation of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA), glucagon ultimately functions to activate glycogen phosphorylase. This enzyme stimulates glycogenolysis, and glucose is released into the bloodstream. Glucagon also activates hormone-sensitive lipase within adipocytes, promoting the hydrolysis and subsequent release of free fatty acids (FFAs) for mobilization and metabolism. Frank et al. (2002) reported a 16-fold increase in plasma FFA concentration following feed restriction for 36 hours.

With prolonged feed restriction, liver glycogen stores decrease, and the need for gluconeogenesis increases. As triglycerides are hydrolyzed in adipose tissue, the liberated glycerol becomes a key component for gluconeogenesis. Amino acids can also provide carbon skeletons for oxidation or glucose synthesis. Most of the amino acids are considered glucogenic, except leucine and lysine, which can only be metabolized for energy through conversion to acetyl-CoA or acetoacetyl-CoA. When amino acids are catabolized, the amino group must be removed. Typically, the amino group is transferred to another carbohydrate compound in a transamination reaction. Pyruvate, α-ketoglutarate, and glutamate can be aminated to form alanine, glutamate, and glutamine, respectively. When amino acid catabolism occurs in muscle, alanine and glutamine serve to transport the amino group to the liver, where urea synthesis can occur. In the liver, the carbon skeletons from alanine and glutamine can be used for gluconeogenesis.

Prolonged fasting in humans results in the adaptation to utilize ketones, which are fatty acid metabolites, for energy production, especially within the central nervous system. In horses, extreme feed deprivation often results in hyperlipemia, characterized by accumulation of very-low-density lipoproteins (VLDL) and plasma triglyceride concentrations >500 milligram per deciliter (mg/dL), though there is significant variation among horses (Frank et al., 2002). The response to prolonged fasting suggests that lipid metabolism in horses may not be identical to lipid metabolism in humans. It is possible that hormone-sensitive lipase activity in horses is impaired (Watson et al., 1998), particularly if accompanied by insulin resistance (Jeffcott and Field, 1985). Further research will be necessary before fat metabolism in the equine athlete is fully understood.

The contribution of various energetic substrates to metabolism at rest appears to largely depend on the diet; however, overall resting metabolism will be primarily supported through aerobic pathways. Recently, glucose tracers and modeling computations were used to compare glucose metabolism in horses. Horses consumed either a diet with the majority of calories coming from starch and sugar or a diet with most calories coming from fat and fiber (Treiber at al., 2008). It was concluded that increased availability of glucose in the starch and sugar type diets resulted in an increased flux of glucose, suggesting increased usage. Several other methods are available to determine substrate contribution to overall energy production. Indirect calorimetry is a method of estimating energy expenditure from the measurement of respiratory gases. The respiratory exchange ratio (RER) is the ratio of carbon dioxide exhaled to the oxygen inhaled, and this closely matches the respiratory quotient (RQ), which refers to the ratio of cellular production of carbon dioxide to oxygen consumption. The RER and the RQ are approximately equal under steady state conditions. On the basis of the chemical structure of carbohydrate and fat, oxidation of each substrate affects the RER such that oxidation of carbohydrate results in an RER of 1.0 and oxidation of fat results in an RER closer to 0.7. The effect of diet on the RQ or the RER has not been well studied in resting horses, but it would seem that there would be greater shift toward carbohydrate metabolism (and, therefore, an RQ closer to 1.0) if horses were fed higher-starch and higher-sugar types of diets.

During Exercise

With exercise, energetic substrates are mobilized and used to generate adenosine triphosphate (ATP) for skeletal muscle. However, the specific substrates mobilized and utilized for energy production depend on several factors, including the intensity of the exercise, duration of the exercise, fitness of the individual, habitual diet, and feeding state.

The main substrates of energy production are carbohydrate and fat. Sources of carbohydrate include blood glucose (from a recent meal or glycogenolysis or gluconeogenesis in the liver) and muscle glycogen. Muscle contraction mediates the movement of GLUT4 to the muscle membrane (independent of insulin) to facilitate glucose uptake into muscle during exercise. Fat sources include FFAs hydrolyzed from adipose tissue, blood triglycerides, or muscle triglyceride stores. Protein can be utilized for energy production, though its overall contribution to energy production is minimal except during endurance types of exercise. In the horse, it is possible that VFAs contribute to energy production during exercise. Pethick et al. (1993) showed that acetate contributes significantly to energy production at the muscle at rest, and Pratt et al. (2005) showed greater clearance of acetate during low-intensity exercise compared with rest, suggesting it was used for energy production.

Exercise intensity has the greatest effect on substrate use. In general, as the intensity of the exercise increases, there is a shift toward a greater percentage of energy being derived from

carbohydrate sources and less energy derived from fat. This change in major energetic substrate is often referred to as the *crossover concept*. It should be noted, however, that at all times, there is combined metabolism of fat, carbohydrate, and even protein, just at different contributions.

At rest and during low-intensity type of exercise, a majority of the ATP produced will be through fat oxidation. As the intensity of the exercise increases through the 30% to 50% $\dot{V}O_{2max}$ range, there is an increase in the absolute oxidation of fat, with maximum fat oxidation peaking at approximately 55% to 65% $\dot{V}O_{2max}$ (maximal oxygen consumption) in humans (Achten et al., 2002). As exercise intensity reaches 65% $\dot{V}O_{2max}$ and higher, the relative contribution of fat to overall oxidation declines. Carbohydrate oxidation is relatively low (30%–40% of total energy expenditure) during lower-intensity exercise and increases to nearly 100% of energy expenditure with very-high-intensity exercise. Work by Romijn et al. (1993) reported RER values of 0.73, 0.83, and 0.91 at exercise intensities of 25%, 65%, and 85% $\dot{V}O_{2max}$, respectively, in humans. Also, using tracers, this group was able to determine the contribution of plasma glucose, plasma FFAs, muscle glycogen, and muscle triglyceride. At the lower intensities of exercise, plasma FFAs likely make up a significant portion of the relative energy expenditure, whereas at the higher intensity of exercise, muscle glycogen is the primary source of energy (Figure 4-1).

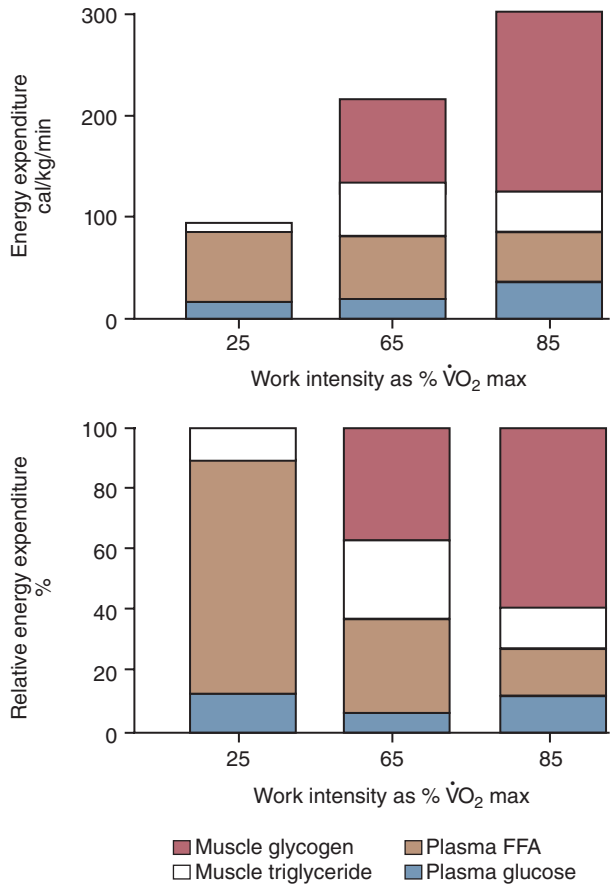

FIGURE 4-1 Total and relative energy expenditure at 25%, 65%, and 85% maximal oxygen capacity ($\dot{V}O_{2max}$) in humans. *(Adapted from Romijn JA, Coyle EF, Sidossis LS et al: Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration,* Am J Physiol Endocrinol Metab *265:E380, 1993.)*

In the horse, the oxidative substrates for energy production also largely depend on exercise intensity. For example, Geor et al. (2000b) showed that muscle glycogen (and lactate) accounted for approximately 30% of the total energy expenditure at 30% of $\dot{V}O_{2max}$, and about 65% of total energy expenditure at 60% of $\dot{V}O_{2max}$. As expected, at lower intensities of work, fat oxidation accounts for a larger percentage of relative energy expenditure (~55%) compared with that at higher intensity (fat oxidation accounting for 25% of energy expenditure). At both exercise intensities, blood glucose contributed 12% of the relative energy expenditure at 30% of $\dot{V}O_{2max}$ and 7% of energy expenditure at 60% $\dot{V}O_{2max}$. It should be noted that there may be differences in substrate utilization among different breeds (Prince et al., 2002) and that other factors such as fitness, age, and gender can contribute to individual differences among horses.

Substrate selection at a given exercise intensity is influenced by hormones, blood flow, and local factors such as the energy status of the cell. The onset of exercise is met with an increase in epinephrine concentration. Epinephrine acts at the adipose tissue and hormone-sensitive lipase to mobilize triglycerides into FFAs and glycerol. Thus, at the start of exercise, particularly low- to moderate-intensity exercise, a rise in FFAs is observed. It has been shown that the contribution of FFAs to energy production during low-intensity exercise is largely dependent on supply. For example, a rise in fatty acid concentrations from the administration of heparin increases fat oxidation and reduces glycogen use during moderate exercise (70% $\dot{V}O_{2max}$; Costill et al., 1977). The concept that fat supply and metabolism regulates carbohydrate metabolism is termed "the Randle Effect" (Randle et al., 1964). The Randle Effect states that glucose oxidation is impaired in the presence of fatty acids. Specifically, increases in fatty acids were shown to increase acetyl-CoA and citrate, which inhibits pyruvate dehydrogenase (PDH), phosphofructokinase (PFK), and hexokinase activities, ultimately decreasing glucose metabolism (Randle et al., 1964). Thus, at lower intensities of exercise, fat supply to muscle is high, so fat oxidation will be greater than carbohydrate metabolism. It should be noted that in the effort for fat to be oxidized, carbohydrate intermediates are required to maintain the citric acid cycle ("fat burns in a carbohydrate flame"). Pyruvate is a major contributor to maintain the cycle, and amino acids (glutamine and aspartate) play a role in anaplerosis as well. Thus, even with substantial fat stores, carbohydrate availability can limit exercise at lower intensities.

As exercise intensity increases, blood flow is shunted away from adipose tissue and directed more at muscle, thereby decreasing the delivery of FFAs from adipose tissue to muscle. Because of reduced substrate supply, energy status of the cell decreases (that is, ATP is reduced, and AMP and inorganic phosphate are increased). Increases in AMP stimulate one of the two key enzymes regulating carbohydrate metabolism, glycogen phosphorylase (Howlett et al., 1998). The other key enzyme, pyruvate dehydrogenase (PDH), is also upregulated with increasing exercise intensity, largely due to contraction mediated increases in calcium (Howlett et al., 1998). Increases in carbohydrate flux decrease fat oxidation, likely through the limitation of fat transport into the mitochondria. The transport of fat is mediated by carnitine palmotoyl transferase (CPT-1), an enzyme inhibited by malonyl-CoA, which

is increased with increased carbohydrate flux (Coyle et al., 1997). Furthermore, if fatty acid availability is increased pharmacologically, such as via infusion of a fat emulsion (Intralipid), fat oxidation by muscle is still hindered at higher intensities (above 65% $\dot{V}O_{2max}$). It has been suggested that transport into mitochondria by CPT-1 is inhibited by lactate (Starritt et al., 2000), potentially contributing to the rationale behind the reduction in fat oxidation at higher intensities. At higher intensities, there will also be a further increase in catecholamine response. In addition to its effects on lipolysis, epinephrine is a significant stimulator of glycogen phosphorylase, the enzyme that stimulates glycogenolysis. Epinephrine affects glycogenolysis to a greater extent than it does lipolysis, contributing to the greater dependency on glycogen at higher exercise intensities.

Thus, it appears that in contrast to the Randle Effect, at higher intensities of exercise, carbohydrate metabolism regulates fat oxidation (Coyle et al., 1997). In the horse, the infusion of glucose (and consequential increase in carbohydrate flux) prior to exercise at 55% $\dot{V}O_{2max}$ reduced fat oxidation, which supports this theory (Geor et al., 2000a). In summary, it is likely a combination of reduced fat availability and increased glycolytic flux that result in the increase in carbohydrate oxidation at higher intensities of exercise. Furthermore, with increasing exercise intensity, the recruitment of type II muscle fibers increases compared with the recruitment of type I fibers. Because type II fibers are more glycolytic, it stands to reason that there would be increased rates of glycogen and glucose use for energy production and a lower dependency on fat oxidation at higher intensities of exercise.

As indicated above, although both fat and carbohydrate are utilized in different amounts, depending on exercise intensity and duration, at any given time, there will be a combination of these substrates generating ATP. However, because of the relatively large energetic storage supply of fat (primarily triglycerides stored in adipose), carbohydrate availability, in particular muscle glycogen, is thought to be limiting to exercise. During high-intensity exercise, glycogen will be the primary energetic substrate. However, because the duration of such exercise is relatively short, overall glycogen use is minimal. Studies in Thoroughbreds (in 800-m to 2000-m exercise) and Standardbreds (in 1600-m exercise) have found glycogen depletion rates around 20% to 35% (Harris et al., 1997; Lindholm et al., 1974). During lower-intensity endurance exercise (e.g., 80-km to 160-km rides), there is substantial glycogen depletion (between 50% and 75%), despite a lower rate of use (Snow et al., 1981; Snow et al., 1982).

Adequate stores of muscle glycogen are important for both short-term, high-intensity exercise as well as for endurance (low intensity) exercise. It has been shown that reduced glycogen stores (to approximately 30% of resting values) can negatively affect subsequent exercise bouts and impair performance (Lacombe et al., 2001; Lacombe et al., 2003). In humans (Coyle and Coggan, 1984) as well as in horses (Snow et al., 1981), fatigue following endurance exercise (>80-km ride in horses) is associated with a depletion of both liver and muscle glycogen stores. Hypoglycemia is often reported following such exercise (Essen-Gustavsson et al., 1984), where hepatic glucose production cannot meet the increased demands of exercise. It is possible that when the muscle glycogen concentration is reduced, blood glucose uptake into muscle is increased to accommodate metabolism, thereby reducing the amount of glucose available to the brain (Hargreaves, 1997). It is believed that endurance exercise that results in hypoglycemia causes a decline in central nervous system function, or "central fatigue" (Davis et al., 1992; Nybo, 2003) and that maintenance of euglycemia through endogenous supplementation can attenuate fatigue (Davis et al., 1992; Farris et al., 1998). Not only is glucose directly required for adequate cerebral function, but increases in fatty acid concentration with exercise promote the production of serotonin in the brain, which contributes to fatigue (Farris et al., 1998). Hypoglycemia may also play a role in peripheral fatigue, particularly when muscle glycogen is depleted, as carbohydrate intermediates are required to maintain flux through the citric acid cycle. Administration of exogenous glucose is therefore also believed to provide substrate for energy production while preserving muscle glycogen (Coyle and Coggan, 1984; Geor et al., 2000b). In the horse, it is possible that propionate (derived from microbial fermentation in the hindgut) serves as anaplerotic substrate (Kasumov et al., 2007), whereas acetate and butyrate are easily metabolized to acetyl-CoA (Knowles, 1974), though only acetate has been studied as a source of energy for the horse (Pethick et al., 1993; Pratt et al., 2005; Waller et al., 2009a). Therefore, for equine sports such as endurance racing, horses should be fed periodically during the event to maintain adequate substrate levels, through direct absorption of glucose as well as VFA production from microbial fermentation (Harris, 2009).

It has been shown that in both humans and horses, exercise conditioning that results in improved fitness causes a move toward reduced glucose flux and increased fat oxidation at a given intensity (Geor et al., 2002). It is likely that increases in oxidative enzyme capacity and reduced catecholamine response to exercise favor this shift in substrate use (Korzeniewski and Zoladz, 2003). Similarly, adaptation to a high-fat diet increases fat oxidation while reducing dependency on carbohydrate metabolism (Pagan et al., 2002; Sloet van Oldruitenborgh-Oosterbaan et al., 2002). The consumption of a diet that provided 29% of the digestible energy as fat reduced carbohydrate utilization during a low-intensity exercise test (at 35% $\dot{V}O_{2max}$) (Pagan et al., 2002). Fat adaptation also resulted in reduced lactate accumulation with a standardized stepwise exercise test (Sloet van Oldruitenborgh-Oosterbaan et al., 2002). Glucose kinetics were investigated in horses adapted to either a diet high in starch and sugar (SS) (SS: 3.3% fat dry matter [DM], 44.7% non-structural carbohydrate [NSC]) compared with a diet high in fat and fiber (FF) (FF: 10.6% fat DM,13.1% NSC DM)(Treiber et al., 2008). This study found increased glucose use during exercise for horses on the SS diet and subsequently lower glucose use during exercise for horses on the FF diet. It was suggested that dietary fat adaptation increases metabolic flexibility and could contribute to the ability to spare muscle glycogen.

During Recovery from Exercise

Following exercise, the body works to replenish substrates used during the exercise bout, particularly muscle glycogen. Rapid resynthesis of glycogen is important for the horse, especially for events such as 3-day eventing, where strenuous exercise bouts are performed on consecutive days. Glycogen is formed via the actions of the enzyme glycogen synthase (GS). GS is regulated both allostearically and covalently (Nielsen and Richter, 2003). Depending on substrate

availability, namely, glucose-6-phosphate, glycogen synthase is activated. GS is also highly regulated by insulin. With exercise, insulin concentrations decrease, facilitating the mobilization (rather than storage) of energetic substrates. However, because of increased blood flow, it is likely that a significant amount of insulin actually reaches the muscle. In humans and rodents, insulin sensitivity is high following exercise, promoting glycogen synthesis. Furthermore, contraction-mediated increases in glucose uptake (insulin independent) remain high after exercise, providing substrate for glycogen synthesis.

GS activity also appears to be affected by the amount of glycogen present such that following exercise, when muscle glycogen concentrations are low, glycogen synthase activity is increased. It is believed that the enzyme may reside within granules of glycogen and is freed upon glycogen usage (Nielsen et al., 2001). Due to increased muscle contraction–stimulated glucose uptake, increased delivery of insulin, and reduced glycogen concentrations, GS is active following exercise. The degree of GS activation is largely dependent on the intensity of the exercise and the extent of glycogen depletion. Studies in humans (Nielsen and Richter, 2003) and horses (Pratt et al., 2007) have found inverse correlations between GS activity and glycogen concentrations.

In humans, the replenishment of glycogen following exercise is rapid and depends largely on glucose supply to muscle. It is recommended that athletes consume carbohydrate (1 gram per kilogram body weight [g/kgBW]) immediately after glycogen depleting exercise and then every 2 hours for a total of 6 hours to maximize glycogen resynthesis (Ivy, 1998). With this program, glycogen concentrations are replenished within 4 to 6 hours, and supraphysiologic concentrations of glycogen can be achieved. In horses, however, glycogen replenishment following exercise is much slower. It has been shown that oral administration of carbohydrate (3 g/kgBW over 6 hours) following glycogen-depleting exercise results in only 56% of pre-exercise glycogen concentrations after 6 hours (Geor et al., 2006). However, the venous infusion of glucose (at 0.5 g/kgBW) for 6 hours (for a total of 3 g/kgBW) following glycogen-depleting exercise resulted in more rapid glycogen resynthesis, with glycogen concentrations returning to approximately 75% of baseline by 6 hours (Geor et al., 2006). Despite intravenous (IV) glucose, supraphysiologic concentrations of glycogen are not attained. Similarly, starch consumption (2.8 kg of corn) following glycogen-depleting exercise resulted in muscle glycogen of only 52% of resting concentrations at 24 hours following exercise (Jose-Cunilleras et al., 2006). Interestingly, it has been shown that electrolyte supplementation along with hay and grain feeding results in greater glycogen replenishment than does feeding alone (Waller et al., 2009b). Therefore, it is likely that factors beyond substrate availability influence glycogen resynthesis in the horse. In fact, the provision of acetate following glycogen-depleting exercise, which can be oxidized directly, may preserve any glucose derived from the diet for use in glycogen resynthesis (Waller et al., 2009a).

The differences in glycogen metabolism between horses and other mammals (i.e., humans and rodents) may be attributed to differences in glycogen content and insulin sensitivity. For example, muscle glycogen stores in horses range from 500 to 650 mmol glucosyl units per kilogram (units/kg), whereas in humans stores range between 320 and 400 mmol glucosyl units/kg. Further, glycogen itself may be different between species, with proglycogen and macroglycogen being repleted at different rates (Brujer et al., 2006). As indicated above, an increase in insulin sensitivity is observed in most species following glycogen-depleting exercise, which is believed to hasten glycogen resynthesis when carbohydrate is consumed (Holloszy, 2005). In horses, however, no such increase in insulin sensitivity exists following glycogen-depleting exercise to approximately 60% of original stores (Pratt et al., 2007). In fact, insulin sensitivity was lower when assessed 30 minutes following exercise compared with baseline insulin sensitivity (no prior exercise). Furthermore, although GS activity was increased following exercise, it was not to the same extent as observed in other species (Pratt et al., 2007). It is possible that these discrepancies contribute to the relatively slow glycogen resynthesis following exercise that is observed in horses. It should be noted that patients with McArdle's disease, a disease characterized by high glycogen concentrations in humans, have low insulin sensitivity and GS activity (Nielsen et al., 2002).

With high-intensity exercise, blood lactate concentrations in horses can rise dramatically, to levels higher than those seen in other species. Lactic acid is produced during anaerobic metabolism in muscle, and it is transported out of muscle into blood in an attempt to maintain muscle pH. Red blood cells (RBCs) act as a sink for lactate, facilitating its transport out of muscle. The key transporter regulating lactate uptake into RBCs is the monocarboxylate transporter (MCT1). Following exercise, lactate is primarily delivered to the liver, where it can be converted back to glucose via gluconeogenesis or can be oxidized (Brooks et al., 1995). Plasma glucose concentrations also increase with high-intensity exercise. This is, in part, caused by glycogenolysis at the liver and return to baseline within 60 minutes (Gordon et al., 2007). Mobilization of fatty acids from adipose tissue continues to increase following exercise, as evidenced by the increase in glycerol concentrations (Poso et al., 1989). Fatty acids are either oxidized or may be re-esterified into triglycerides (Poso et al., 1989).

Low-intensity endurance-type exercise results in substantially lower lactate concentrations because of reduced dependence on anaerobic metabolism to support energy production. Furthermore, blood glucose concentrations tend to not peak, in part because of reduced liver glycogen concentrations to support glycogenolysis (Lindholm et al., 1974). In fact, if no carbohydrate is consumed during or following endurance exercise, the animal may become hypoglycemic (Coyle and Coggan, 1984). Fatty acids will be used for oxidation, whereas glycerol will be an important component of gluconeogenesis. Muscle protein catabolism may also support gluconeogenesis, and increased alanine concentrations have been observed following endurance exercise in the horse (Trottier et al., 2002).

Nutrient Requirements of Performance Horses

Exercise type, climate, and individual horse characteristics will affect the nutrient requirements of each equine athlete. It is impossible to define the requirements of every type of equine athlete, so broad categories of exercise levels are often used. The National Research Council (NRC, 2007) has suggested that mature horses may be grouped into four exercise categories: (1) light, (2) moderate, (3) heavy, and (4) very heavy. Light exercise is described as 1 to 3 hours of imposed exercise each week that consists of mostly walking and

trotting. Many horses used for recreational purposes would be included in this category. Moderate exercise is described as 3 to 5 hours of imposed exercise each week that consists of mostly trotting with some cantering and skills work. Horses used for riding lessons, horse shows, ranch work, and possibly polo would fit into this category. This category could also include horses that are beginning training programs for racing or other strenuous events. Horses that compete at the upper levels of strenuous sports such as 3-day eventing, endurance racing, polo, flat racing, steeplechasing, or harness racing would be included in the heavy and very heavy exercise categories. These horses may be exercised for long periods each week or for relatively short periods at a high intensity. In addition, many of these horses are transported frequently or over long distances and are often housed in unfamiliar environments, all factors which may contribute to increased use of some nutrients. The suggested nutrient requirements (NRC, 2007) for a 550-kg horse at each exercise level are shown in Table 4-1. These values should be used as a starting place for individual horses, rather than as absolutes. The following material discusses some of the sources of variation for each nutrient category.

TABLE 4–1

Recommended Daily Nutrient Intakes of 550-kg Mature Horses Performing Light, Moderate, Heavy, and Very Heavy Work*

	Light	Moderate	Heavy	Very Heavy
DE (Mcal/d)	22.0	25.6	29.3	37.9
Crude protein (CP) (g/d)	769	845	948	1105
Lysine (g/d)	33	36	41	48
Calcium (Ca) (g/d)	33	39	44	44
Phosphorus (P) (g/d)	20	23	32	32
Sodium (Na) (g/d)	15	20	28	45
Chloride (Cl) (g/d)	51	59	73	102
Potassium (K) (g/d)	31	35	43	58
Magnesium (Mg) (g/d)	10.5	12.7	16.5	16.5
Copper (Cu) (mg/d)	110	124	138	138
Iodine (mg/d)	3.8	4.3	4.8	4.8
Iron (Fe) (mg/d)	440	495	550	550
Manganese (mg/d)	440	495	550	550
Zinc (Zn) (mg/d)	440	495	550	550
Selenium (mg/d0	1.1	1.2	1.4	1.4
Vitamin A (IU/d)	24,750	24,750	24,750	24,750
Vitamin D (IU/d)	3630	3630	3630	3630
Vitamin E (IU/d)	880	990	1100	1100
Thiamin (mg/d)	33	62	69	69
Riboflavin (mg/d)	22	25	28	28

*Adapted from NRC (2007). Sodium, potassium, and chlorine recommendations apply to mild climates. In cool or hot climates, sodium, potassium, and chlorine amounts should be decreased or increased, respectively.

Energy

Dietary energy requirements can be expressed in a variety of ways. Gross energy is the total amount of energy in a feed. Digestible energy (DE) is determined as the difference between the gross energy that is consumed and the energy excreted in feces. Thus, DE approximates the amount of energy absorbed by the horse. In the United States and several other countries, the dietary energy requirements of horses are commonly expressed in units (either megacalories or megajoules) of DE. However, daily energy requirements may also be expressed in units of net energy. A system that utilizes net energy as the basis of requirements has been developed in France, but it expresses feeding guidelines in relation to the amount of net energy contained in a standard feed (Martin-Rosset et al., 1994; Martin-Rosset and Vermorel, 2004). The French system more accurately accounts for the efficiency of use of different energy sources, but it has not been completely validated for exercising horses, and many horse owners are unfamiliar with it. Therefore, energy requirements in this chapter will be expressed as units of DE, unless otherwise noted.

The daily energy requirement of an equine athlete is the sum of the calories needed to maintain the horse at rest and the calories needed to replace the energy used during exercise. The caloric needs for maintenance are variable. The NRC (2007) described three levels of maintenance energy requirements for adult horses: (1) minimum, (2) average, and (3) elevated. The elevated maintenance requirement applies to horses with alert temperaments and above-average levels of voluntary activity, which would include many elite performance horses. Other factors that can affect maintenance energy requirements include climate, housing conditions, and transportation.

The caloric debt created by exercise will depend on several factors including the duration of the exercise and the intensity of the exercise. Exercise intensity is often equated with speed, and many studies have shown that energy utilization increases with speed for exercising horses (Eaton et al., 1994; Eaton et al., 1995; Hiraga et al., 1995 Pagan and Hintz, 1986). However, the effect of speed on energy use does not appear to be linear; and horse-related factors such as fitness and gait will affect the relationship (Eaton et al., 1999; Hoyt and Taylor, 1981; Katz et al., 2000). Other factors such as hills, jumps, or ground conditions can also increase energy use at a given speed. The weight of the rider must also be considered. Thus, although speed of travel is an important characteristic of work effort, it may not adequately describe the intensity of a work bout unless other factors are considered as well. In most of the studies cited above, oxygen utilization was measured as a means of estimating energy use, but it is difficult to measure oxygen utilization under most practical conditions.

Heart rate (HR) is closely related to oxygen utilization during submaximal exercise (Coenen, 2005; Eaton et al., 1995), and it is relatively easy to monitor heart rate in practical situations. Therefore, HR may provide a relatively simple but comprehensive assessment of exercise intensity (Coenen, 2005; NRC, 2007). The NRC (2007) reported that Coenen had reviewed data from 87 studies to arrive at the following equation relating HR to oxygen utilization in exercising horses:

$$\text{Oxygen utilization (mL } O_2\text{/kgBW/min)} = 0.0019 \times \text{HR } 2.0653$$

This equation can be used to estimate the liters of oxygen utilized during an exercise bout. When combined with an

estimate of the caloric equivalent for each liter of oxygen (approximately 4.86 kcal/L; NRC 2007) the equation can be used to calculate the calories burned during the exercise bout. For example, if a 490-kg horse with a 60-kg rider (combined BW of 550 kg) is exercised at an HR of 90 beats per minute (beats/min) for 30 minutes, it will consume approximately 350 L of oxygen. If each liter of oxygen has an energetic equivalent of 4.86 kilocalories (kcal), then energy use during that exercise bout will be about 1.7 megacalories (Mcal). To arrive at the amount of DE that must be consumed to replace this energy, the efficiency of DE use for exercise must be known. Pagan and Hintz (1986) estimated that the efficiency of DE use for exercise was about 57%. However, a summary of subsequent studies suggests that the efficiency is lower, especially for high-intensity exercise (NRC, 2007). If the efficiency of DE use is 40% for the type of exercise described above, then about 4 Mcal of DE must be added to the daily diet to replace the energy expended in the exercise described above.

Few horses perform exactly the same amount of exercise every day, but it is not practical to change the diet every day. Feeding programs can be based on weekly (or even monthly) work averages, especially if the horse is in a steady state of fitness. The NRC (2007) recommendations for DE intakes in exercising horses are based on weekly averages. These broad categories of light, moderate, heavy, and very heavy exercise represent increased DE intakes that are 20%, 40%, 60%, and 90% above maintenance, respectively. The maintenance requirement for horses in light, moderate, and heavy work has been estimated at 33.3 kcal/kgBW/day; however, the maintenance requirement for horses in very heavy work has been estimated at 36.3 kcal/kgBW/day. The higher maintenance requirement was applied to horses in very heavy work because it is anticipated that they will have more voluntary activity, higher lean body mass and higher feed intakes; all factors that can increase the maintenance component.

The weekly work loads suggested by the NRC (2007) for each exercise category will not exactly match the work loads of specific performance horses, so it should be expected that actual feeding programs will have to be adjusted to reflect the real work loads on an individual basis. It is relatively easy to evaluate whether a performance horse is receiving an appropriate amount of energy from its diet. If daily energy expenditure exceeds energy intake, then the horse will lose weight. Conversely, if energy intake exceeds energy expenditure, then the horse will gain weight. Skilled horsemen can often detect a small change in a horse's body weight, but the most reliable method of assessing weight change is to weigh the horse regularly. The most consistent results are obtained if the horse is always weighed at the same time of day relative to feeding and exercise.

In human athletics, body composition profiles have been developed for different types of athletes. There is limited information about the ideal body composition for horses engaged in various activities, at least in part because it is difficult to measure body composition in live horses. Ultrasonic measurement of subcutaneous fat over the rump has been used by some researchers to assess body composition in equine athletes. It would be expected that equine athletes would have less body fat compared with sedentary horses. Using ultrasonic equipment to estimate rump fat, Lawrence and coworkers (1992b) reported that competitive endurance horses had a mean body fat of 7.9% ($n = 61$). For comparison, broodmares in a separate study were reported to have 10% to 15% body fat (Lawrence et al., 1992a). Kearns et al. (2002) reported that high-quality Standardbred race horses had a mean body fat of 7.4% for males ($n = 6$) and 9.9% for females ($n = 8$). However, the relationship between rump fat thickness and carcass fat is not particularly strong (Kane et al., 1987; Westervelt et al., 1976), and none of the available equations relating rump fat thickness to body composition were derived with athletic (fit) horses. Therefore, the estimates of total body fat that exist for horses may illustrate qualitative differences in fatness within groups of equine athletes but the specific estimates may not be quantitatively accurate. Body condition scoring has also been used to assess horses. Henneke and coworkers (1983) developed a condition scoring system for broodmares that has been applied to performance horses. This system uses a nine-point scale, where 1 is extremely thin and 9 is extremely fat. Endurance horses tend to have condition scores below 5 (Garlinghouse and Burrill, 1999; Lawrence et al., 1992a). Gallagher et al. (1992) reported a mean condition score of 5 for Thoroughbreds in race training. Similarly, horses used for competitive polo have condition scores of about 5, but horses used for dressage and as show hunters may have condition scores above 6 (Pagan et al., 2009).

Phased dietary programs for humans that change energy supply and source with training status and event have been explored (Schroder et al., 2008). Phased feeding programs that manipulate calorie amount and type during training may hold some potential for equine athletes as well, but additional research is needed to define optimal programs for horses performing in different events. Regular monitoring of DE intakes is warranted for equine athletes as they progress through different stages of training and competition. More frequent monitoring should be considered for horses that are less than 4 years old, as these horses will be growing, training, and competing at the same time.

As discussed previously, adequate muscle and liver glycogen stores are important for optimal performance. Horses are capable of synthesizing glucose from propionate that is produced by microbial fermentation, but glucose may be obtained more efficiently from dietary sugar and dietary starch. Most feeds are relatively low in simple sugars; therefore, starch is the primary source of absorbable glucose in horse feeds. The optimal level of dietary starch has not been determined. It is possible that the level of dietary starch that is optimal for an endurance horse is different from the amount that is optimal for a race horse. Starch is most effective as an energy source if it is digested in the small intestine and absorbed as glucose. Starch that escapes the small intestine will be fermented to VFA in the large intestine. The amount of ATP produced from absorbed glucose will be greater than the amount of ATP generated from the VFA produced from glucose that reaches the large intestine. Several factors affect the susceptibility of starch to small intestinal digestion, including the size of the meal, the type of starch, and the processing of the starch prior to ingestion. Oat starch is digested more easily than corn starch, and small meals are digested more easily than large meals. In addition, processing of cereal grains has been shown to increase the availability of starch to digestion in the small intestine. Although starch is an important energy source in the diets of most performance horses, dietary fiber and dietary fat are also important. Dietary fibers that are fermented to VFA provide energy, and as noted above, propionate can be used to synthesize glucose. Fats,

particularly vegetable oils, are often added to the diets of performance horses. Fats contain more than twice as much DE as starch on an equivalent weight basis. Fats are often used to increase total calorie intake when feed intake is limited. In addition, horses that have been adapted to supplemental fat in the diet appear to have an increased capacity to utilize fat during exercise and to spare carbohydrate use (Dunnett et al., 2002; Pagan et al., 2002).

Protein and Amino Acids

The importance of protein to athletic performance has been pondered for decades, possibly for centuries. Even ancient Greek athletes may have believed that the addition of meat to their diet would improve performance (Hickson and Wolinski, 1989). Today, it is recognized that dietary protein plays an important role in the maintenance of tissue but that it is a relatively minor energy source in comparison with carbohydrate or fat. Most amino acids in the body are found in structural proteins and are, therefore, not readily available for energy production. However, there are some free amino acids in plasma and some labile proteins in the liver that could provide amino acids to fuel energy production. In other species, evidence for exercise-induced protein catabolism comes from the observation of metabolic changes during exercise and from studies using isotope-labeled amino acids. Several studies with horses have documented that plasma alanine concentrations increase with moderate exercise (Essen-Gustavson et al., 1991; Miller-Graber et al., 1991a, b; Miller and Lawrence, 1988). Plasma urea concentrations may also increase in response to long-term exercise (Snow et al., 1982). No studies with labeled amino acids have been performed to examine whether certain amino acids are preferentially catabolized in exercising horses, but it can be inferred from studies with other species that leucine might be used to a greater extent by muscle compared with other essential amino acids.

Regular exercise can alter the percentage of lean body mass in humans, and certain types of exercise can increase absolute lean body mass. An increase in absolute lean body mass in horses may be more likely with strength training than with endurance training, but hypertrophy of the longissimus dorsi has been reported in response to aerobic training in horses (D'Angelis et al., 2007). Regular exercise has been reported to enhance nitrogen retention in horses (Freeman et al., 1988; Wickens et al., 2003), which could be related to increased lean body mass. Performance horses will have increased nitrogen losses in sweat, and there may be increased endogenous fecal losses when feed intakes are high. Collectively, these observations support the concept that exercising horses have higher protein requirements compared with sedentary horses.

There is general agreement that regular exercise can elevate the protein requirement of horses, but there has been much debate regarding the magnitude of the increase. In 1989, the NRC suggested that horses engaged in intense exercise (such as racing) should receive twice as much dietary crude protein (CP) as would a sedentary horse of the same body weight. This recommendation was arrived at by maintaining the same protein:calorie ratio in the diet of the intensely working horse that was suggested for the sedentary horse. This recommendation was a practical approach to meeting the protein needs of exercising horses, but it was not based on actual estimates of protein needs in exercising horses. In 2007, the NRC substantially reduced the dietary protein recommendations for exercising horses. For example, it is currently recommended that 550-kg horses performing light, moderate, heavy, and very heavy work receive 769 g CP/day, 845 g CP/day, 948 g CP/day, and 1105 g CP/day, respectively; compared with the previous recommendations (NRC, 1989) of 895 g CP/day, 1074 g CP/day, and 1432 g CP/day for light, moderate and intense work, respectively. The current recommendations apply to diets with highly digestible protein sources, whereas previous estimates were based on lower estimates of dietary crude protein digestibility. The 2007 NRC recommendations for crude protein intakes of exercising horses account for the crude protein needed for lean tissue accretion during exercise, the crude protein needed to replace nitrogen losses in sweat, and the crude protein needed for normal maintenance. The 2007 NRC recommendations do not maintain a constant protein:calorie ratio across all levels of work effort (light, moderate, heavy, and very heavy). The additional dietary protein needed for muscle gain was estimated as 0.089 g CP/kgBW, 0.177 g CP/kgBW, 0.266 g CP/kgBW, and 0.354 g CP/kgBW, for light, moderate, heavy, and very heavy exercise, respectively (NRC, 2007). The crude protein needed to replace nitrogen losses in sweat was calculated from estimates of the nitrogen losses in sweat with adjustments for the efficiency of digestible protein use and crude protein digestibility. Sweat losses were estimated at 0.25%, 0.5%, 1%, and 2% of BW per day for light, moderate, heavy, and very heavy exercise, respectively, and the nitrogen content of sweat was estimated as 1.25 g/kg sweat (NRC, 2007). The estimates used by the NRC (2007) to calculate the amount of dietary protein needed to support lean tissue accretion were based on limited experimental data. In addition, sweat losses can be extremely variable depending on environmental conditions. Therefore, the current recommendations probably do not apply to every horse in every situation. However, the current recommendations are consistent with studies that have examined the effect of exercise on nitrogen retention in horses (Freeman et al., 1988; Wickens et al., 2003). Diets providing 12% to 15% of the total energy as protein or 1.2 g to 1.7 g protein/kgBW have been suggested to be adequate for human athletes (ADA, 2000; Rennie and Tipton, 2000); the current NRC recommendations are consistent with these allowances.

There are no demonstrated benefits to feeding very high levels of crude protein to exercising horses. Meyer (1987) suggested that digestible protein intakes above 2 g/kgBW per day should be avoided in endurance horses because of effects on water balance and urea and ammonia metabolism. If dietary protein is 79% digestible (NRC, 2007), Meyer's recommendation would set an upper limit of about 2.5 g CP/kgBW. Exercising horses consuming approximately 3.2 g CP/kgBW per day excreted more urea in sweat and had higher plasma urea concentrations than horses consuming approximately 1.6 g CP/kgBW (Miller et al., 1991a). An increase in post-exercise orotic acid excretion in the horses receiving a high-protein diet was interpreted to suggest that the high-protein diet exceeded the capacity of the urea cycle. It has been suggested that high-protein diets are acidogenic and may affect systemic acid–base balance in exercising horses (Graham-Thiers et al.,1999; 2001). As a dietary energy source, protein is metabolized to net energy less efficiently than is starch or fat; thus, if protein is added to a diet at the expense of another calorie source, there could be a negative effect on net energy

intake. If protein replaces starch in the diet of exercising horses, muscle glycogen storage may be reduced (Pagan et al., 1987), although this response has not been observed in all studies (Miller-Graber et al., 1991b). Although high-protein diets are not recommended, extremely-low-protein diets should also be avoided. Low-protein diets may not provide adequate amounts of essential amino acids. In addition, the microbial population in the equine large intestine will require adequate nitrogen for optimal function.

In other species, the term "ideal protein" is used to describe a dietary protein with an array of amino acids that closely matches the amino acids required by the animal. It is presumed that horses require the same amino acids in the diet as other monogastrics, but few studies have investigated the amino acid requirements of athletic horses. A summary of available data suggested that a 500-kg horse doing moderate to heavy work needs approximately 34 g of lysine per day (NRC, 2007). The NRC (2007) crude protein requirements for athletic horses are based on a diet containing good-quality protein containing at least 4.3% lysine (as a percentage of the crude protein). If a lower-quality protein is fed, more total protein will be needed to ensure that the lysine requirement is met. Lysine is usually considered the first limiting amino acid, so if lysine needs are met, it is likely that other amino acid requirements will be met as well. However, experimental evidence that verifies this assumption is not available.

The incorporation of various amino acids in equine supplements is common. A review of studies evaluating the ergogenic benefits of these supplements can be found later in this chapter. Although not generally considered an ergogenic amino acid, glutamine is an important amino acid for enterocyte and leukocyte metabolism. There is some evidence that glutamine supplementation may help maintain gut health in challenged animals (Sukhotnik et al., 2007; Wu 2009), but studies in horses have not been conducted. Exercise or training may decrease plasma glutamine concentrations in humans, and a decrease in glutamine availability to leukocytes had been suggested as a mechanism for exercise-induced immune suppression. Muscle and plasma glutamine concentrations do not appear to change in response to acute exercise in horses (Beaunoyer et al., 1991; Miller-Graber et al., 1990; Russell et al., 1986), but studies in heavily trained horses have not been conducted.

Aerobic capacity is closely related to lean body mass; therefore, the maintenance of lean body tissue is probably important for optimal athletic performance in horses. Diets that do not meet the amino acid needs of the horse may lead to loss of lean body mass; therefore, it is important to provide an adequate amount of high-quality protein in the diets of performance horses. Some human athletes consume high-protein or amino acid supplements, in an attempt to stimulate muscle synthesis or to reduce the effects of exercise-induced muscle damage. It appears that adequate protein intake will help preserve lean body mass in exercising individuals that consume energy-deficient diets (Pikosky et al., 2008). Inadequate feed consumption during rigorous training can result in negative energy balance and weight loss. If consumption of a higher-protein diet during this time would help preserve lean body mass and favor fat loss, then the effects of negative energy balance on the horse might be partially ameliorated. Unfortunately, there have been no studies with horses that test this hypothesis.

Studies with human athletes have suggested that the timing of ingestion of a protein or amino acid supplement may affect whether a beneficial effect is observed. Consuming protein during resistance-type exercise has been reported to enhance protein synthesis in humans (Beelen et al., 2008). Consuming protein or amino acid supplements shortly after exercise has been reported to upregulate markers of protein synthesis (Dreyer et al., 2008; Willoughby et al., 2007). Recent literature reviews suggest that muscle soreness and indicators of exercise-induced muscle damage in humans may be reduced when protein or amino acid supplements are consumed before, during, or after exercise, although the results are not always consistent (Howatson and van Someren, 2008; Negro et al., 2008). The effects of administering protein or amino acid supplements to horses in the period immediately before or after exercise may deserve study. It is possible that studies performed in the past did not provide protein or amino acid supplements at the most optimal time.

Minerals

Calcium and phosphorus are the major minerals in the equine skeleton, and they also play important roles in energy metabolism and muscle contraction. Exercise programs that mimic race training have been reported to alter bone density in horses (Nielsen et al., 1997). However, changes in bone density were not observed in response to 60 km of endurance exercise (trotting and cantering; Spooner et al., 2008). The current recommendations for calcium and phosphorus intakes of mature equine athletes engaged in heavy and very heavy exercise are about double the recommended intakes for sedentary horses (NRC, 2007). Calcium and phosphorus intakes of horses engaged in light and moderate exercise are also increased above maintenance, but the magnitude of the increase is less than for horses in heavy and very heavy work (see Table 4-1). The NRC (2007) recommendations do not make allowances for the effect of exercise on the calcium and phosphorus requirements of young horses (Table 4-2), possibly because the calcium and phosphorus allowances for growing horses are already quite high.

Owners and trainers sometimes feed extra amounts of calcium and phosphorus in an attempt to produce or maintain stronger bone. Porr et al. (1998) studied the effect of dietary

TABLE 4–2

Calcium and Phosphorus Requirements* in Mature and 24-Month Old Horses with a Current Weight of 450 kg

		Calcium (g/d)	Phosphorus (g/d)
Mature	No Exercise	18.0	12.6
	Moderate Exercise	31.5	18.9
	Heavy Exercise	36.0	26.1
24-month old	No Exercise	39.0	21.6
	Moderate Exercise	39.0	21.6
	Heavy Exercise	39.0	21.6

*Source: NRC (National Research Council): *Nutrient requirements of horses,* Washington, DC, 2007, National Academy Press.

calcium level on changes in bone density when horses stopped receiving regular exercise. A reduction in bone density occurred when regular exercise was discontinued; however, dietary calcium level (58 g versus 34 g/day) had no effect on the decrease. In growing horses, low-calcium diets can affect bone mineralization, but once the calcium requirement is reached, further improvements in bone density are not likely. A diet that was deficient in calcium (0.2% of dry matter) reduced bone mineral accrual in growing horses compared with calcium-adequate diets containing 0.4% or 0.7% calcium, but there was no difference in bone response between the two calcium-adequate diets. In addition, feeding a very-high-calcium diet (2.5% of dry matter) did not provide any benefit to growing horses when compared with diets containing 0.44% or 0.7% calcium (Thompson et al., 1988). Nielsen et al. (1998a, b) reported that 2-year-old Quarter-horses in race training receiving a diet containing about 0.3% calcium had lower bone density compared with horses fed a diet with about 0.4% calcium. The daily calcium intakes in that study (approximately 25 g/day and 34 g/day for the low-calcium and high-calcium diets, respectively) were lower than those suggested for growing horses at all levels of work (see Table 4-2).

The current dietary recommendations in Tables 4-1 and 4-2 are calculated using a calcium digestibility of 50% and a phosphorus digestibility of 45% in horse feeds. The true digestibility of calcium in most feeds is much higher than 50% (Pagan, 1994), so it is likely that the current calcium allowances for exercising horses of all ages are adequate. When horses are fed diets with calcium digestibility below 50%, the daily calcium allowances should be increased. Some tropical forages may be high in oxalates, which can reduce calcium absorption by horses. In addition, excess dietary phosphorus can reduce calcium digestibility, so it is recommended that the concentration of phosphorus in the diet should not exceed the concentration of calcium in the diet. The true digestibility of phosphorus in typical horse feeds appears to be quite variable. The NRC (2007) recommendations for young horses are based on diets containing inorganic phosphorus sources (i.e., dicalcium phosphate; monosodium phosphate) which are believed to be more digestible than some plant-based phosphorus sources.

Diets containing inadequate levels of calcium or phosphorus can result in abnormal bone physiology. Diets that are low in calcium and high in phosphorus can cause nutritional secondary hyperparathyroidism which can result in demineralization of bone and, in extreme cases, lameness (NRC, 2007). As noted above, high dietary phosphorus can reduce calcium availability, so even if a diet contains adequate calcium, a high phosphorus intake may result in a deficiency in the amount of calcium absorbed by the horse. Calcium and phosphorus deficiencies, excesses, or imbalances can occur in practical horse feeding situations. Grass hays (timothy, orchardgrass, bromegrass, Bermudagrass) tend to be low to moderate in calcium and phosphorus, whereas legume hays (alfalfa, clover) tend to be high in calcium and low to moderate in phosphorus. Soil conditions can affect the mineral content of forages, so there can be great regional variation in hay or pasture from different areas. Plain cereal grains (oats, barley, corn) and most cereal grain byproducts (wheat middlings, wheat bran, rice bran) are very low in calcium and moderate to high in phosphorus. Horses receiving grass hay and plain cereal grains or a mixture of a cereal grain and a grain byproduct may be consuming inadequate calcium or excess phosphorus. This problem is less common when horses are fed legume or legume-mix hays. Because cereal grains and their byproducts are likely to be low in calcium and moderate to high in phosphorus, commercial concentrate mixes are usually fortified with adequate amounts of calcium and phosphorus to produce a balanced feed. Hand mixing cereal grains and cereal grain byproducts with fortified commercial feeds may unintentionally alter the ratio of calcium to phosphorus in the diet.

Calcium and phosphorus are the major minerals in bone but the effects of other mineral or dietary supplements on bone quality have been examined. Sodium zeolite A, a silicon containing supplement, has been fed to horses in several studies. Nielsen et al. (1993) reported that horses fed sodium zeolite A had fewer musculoskeletal injuries compared with control horses. The mechanism for the decrease in injuries has not been elucidated, and the trial has not been repeated to confirm the results. Supplemental silicon may affect calcium retention (O'Connor et al., 2008), but there appear to be minimal effects on bone density (Frey et al., 1992). Recently, a dietary supplement containing a calcium proteinate was studied, but the details of the preliminary study were insufficient to assess any benefits (Atwood et al., 2007). Many studies have evaluated the effect of dietary copper on bone development in growing horses with variable results (Gee et al., 2005; Knight et al., 1990b; Pearce et al., 1998). The effect of copper supplementation on bone metabolism in exercising horses has not been studied.

Sodium, chlorine, and potassium play essential roles in nerve function, ion transport across cell membranes, and acid–base balance. The maintenance requirements of sedentary adult horses have been estimated at 20 mg/kgBW, 50 mg/kgBW, and 80 mg/kgBW for sodium, potassium, and chloride, respectively (NRC, 2007). Conditions that increase sweat losses, such as exercise or hot environments will increase the daily requirement for these minerals. The amounts of sodium, potassium, and chloride that are lost in sweat can be calculated from sweat composition and sweat volume. However, many factors can affect sweat loss; consequently, the sodium, potassium, and chloride requirements shown in Table 4-1 should be used only as a guide. It is likely that these values overestimate the needs of horses in cold environments and underestimate the requirements of horses in hot environments. Horses that receive furosemide prior to racing or training may also have increased electrolyte needs.

Chronic consumption of low-sodium diets can result in reduced feed intake and reduced sweating rates in exercised horses (Lindner et al., 1983; Meyer et al., 1985). Although horses may not regulate their own sodium balance precisely, sodium-depleted horses will voluntarily increase their salt intake if a salt block (lick) is available (Houpt et al., 1991). Many common feed ingredients are low in sodium and chloride; consequently, salt is often added to commercially manufactured concentrate mixes. The amount of salt added to commercial manufactured concentrate mixes may not be sufficient for horses performing heavy or very heavy work, especially in hot environments. Low-potassium diets may also result in reduced feed intake, but most forages are high in potassium, so most equine diets will meet the potassium needs for horses engaged in light or moderate exercise without additional supplementation.

The relationship of cations to anions in the diet can affect systemic acid–base balance in other species (Patience et al., 1987; Tucker et al., 1988). Dietary cation–anion difference (DCAD) is often defined as follows:

$$DCAB = mEq[(Na + K) - Cl]/kg\ diet\ DM$$

Other minerals that may be used to calculate DCAD are calcium (Ca), magnesium (Mg), phosphorus (P), and sulfur (S). When diets containing DCADs of 21, 125, 231, and 350 were fed to sedentary horses, the lowest DCAD resulted in decreased arterial and venous pH and decreased urine pH when compared with the two highest DCAD diets (Baker et al., 1992). In a subsequent experiment, when trained horses were fed diets containing DCAD of 10, 131, 206, and 323, lactate levels following a high-intensity exercise test were higher in horses receiving the highest DCAD compared with those in horses receiving the lowest DCAD (Popplewell et al., 1993). In that study, sulfur was included in the equation to calculate DCAD. In addition to altering systemic acid–base balance, DCAD may affect other physiologic processes of importance. DCAD has been shown to affect calcium metabolism in dairy cows. Wall and coworkers (1992) reported that lowering DCAD to 5 resulted in increased calcium excretion and suggested that increased calcium excretion could result in negative calcium balance in horses receiving low levels of dietary calcium.

Thus far, the available data suggest that diets containing low DCADs should be avoided. Fortunately, many common horse rations will have at least a moderate DCAD, particularly if they contain at least 50% hay. For example, a ration consisting of 5 kg timothy hay, 5 kg oat grain, and 50 g salt would have a DCAD of about 200 (using sodium [Na], potassium [K], and chloride [Cl] in the equation). The amount of hay in the ration will affect DCAD because most hays are fairly high in potassium. Diets with low DCADs might be more common in situations where hay intake is limited. Unmollassed beet pulp is much lower in potassium content compared with most hays; therefore, a diet that depends on beet pulp as the roughage source might have a lower DCAD.

Very few studies have investigated the trace mineral requirements of performance horses. Recommendations for daily intakes have been based on studies of horses in other physiologic states (maintenance, growth, lactation), on extrapolation from studies with other animals, and the absence of clinical deficiency signs when horses are fed typical diets. Therefore, whether the recommendations for trace mineral intakes are sufficient for optimal performance in horses is not known. Conversely, there is little information to suggest that supplementation of trace minerals above the current recommended levels is necessary or beneficial for performance.

The trace minerals of most interest in performance horses are those associated with red blood cell formation or metabolism, such as iron, copper, and zinc. In human athletes, iron deficiency will progress through three stages: (1) depletion of iron stores, (2) diminished erythropoiesis, and (3) reduced hemoglobin production resulting in anemia (Weaver and Rajaram, 1992). It should be noted that decreased hemoglobin concentration is not apparent until the later stages of iron deficiency. It is generally agreed that exercise performance is reduced in individuals with anemia, but it is less clear whether performance is impaired during the earlier stages of iron deficiency (Lukaski, 2004). Iron deficiency develops when daily losses exceed daily intake. Daily iron losses have not been measured in horses during training, but racehorses might be at highest risk for uncompensated losses, as they are susceptible to a high incidence of gastric ulcers and exercise-induced pulmonary hemorrhage. The recommended iron intakes for 550-kg horses range from 440 mg for light work to 550 mg for very heavy training (NRC, 2007). The daily recommended iron intakes for athletic men and women are 8 mg/day and 18 mg/day, respectively (Lukaski, 2004). If the requirements for horses and humans are compared on a body weight basis, the recommended intakes for horses appear generous.

Iron supplements, alone or in combination with copper, zinc, and several vitamins, are often provided to horses in an effort to increase the oxygen-carrying capacity of blood. Benefits of this practice have not been documented. In controlled studies, the use of iron supplements has failed to produce increases in hemoglobin or packed cell volume in horses (Kirkham et al., 1971; Lawrence et al., 1987). However, as noted above, there are several stages of iron deficiency that develop before anemia becomes evident. Additional research on the iron requirements of exercising horses is warranted.

Selenium deficiency causes white muscle disease in foals, and it has been suggested that low selenium status is linked to exertional myopathies in adult horses. No scientific studies support a relationship between selenium status and exertional rhabdomyolysis or exercise-induced muscle damage. Forages grown in areas with low selenium soils will contain concentrations of selenium below the proposed dietary requirement. However, selenium is commonly added to commercial concentrates at levels that will compensate for any deficiency of selenium in the forage portion of the diet. Therefore, if a horse is receiving a commercially manufactured fortified concentrate, it is unlikely that additional selenium supplementation will be necessary. Most commercially manufactured concentrates are fortified with other trace minerals, including copper, zinc, manganese, iodine, and iron. Indiscriminate use of additional trace mineral supplements should be avoided because of the potential for toxicity and negative interactions with other nutrients.

Vitamins

Many common horse feeds are good sources of vitamins. In addition, some vitamins are synthesized and absorbed in the large intestine. However, the requirements of performance horses for most vitamins have not been studied, and the adequacy of typical diets for optimal performance is unknown.

Vitamin E functions as a biologic antioxidant that protects membranes against damage from free radicals. This function may be particularly important during exercise, when the formation of damaging compounds such as peroxide and hydroxyl radicals may be increased. In rats with vitamin E deficiency, exercise performance was impaired (Gohil et al., 1986), but studies in horses have failed to show a clear relationship between vitamin E intake and performance. Petersson and coworkers (1991) could not demonstrate differences in any indicators of membrane integrity between exercised and nonexercised horses receiving a diet low in vitamin E (<10 mg/kg) for 4 months. In addition, there did not appear to be any effect of dietary vitamin E level on the horses' response to a standardized exercise test. Siciliano et al. (1997) found that vitamin E status declined in exercising horses when they received approximately 1.6 international units (IU) of

vitamin E per kilogram body weight but not when they received 6 IU/kgBW. It has been suggested that the vitamin E requirements in Table 4-1 may not be optimal for all performance horses (NRC, 2007), but objective studies defining the requirements are not available. Vitamin E is usually added to horse feeds as an ester of α-tocopherol. However, there are several naturally occurring compounds with vitamin E activity, including four tocopherols and four tocotrienols (NRC, 2007). Gansen and coworkers (1995) did not find a beneficial effect of a supplement containing natural vitamin E on the exercise response of Thoroughbred horses.

Vitamin C is another nutrient that functions as an antioxidant. It is generally believed that horses are capable of synthesizing adequate vitamin C. However, low plasma ascorbic acid concentrations in horses compared with other species have caused speculation that horses may require supplementation of this vitamin (Snow et al., 1989). In addition, exercise has been shown to alter plasma or serum ascorbic acid concentration in horses (Hargreaves et al., 2002; Marlin et al., 2002). Studies defining a requirement or a benefit of supplementation have not been conducted. More information regarding the relationship between antioxidants and exercise performance can be found in the section of this chapter related to supplements.

As discussed earlier in this chapter, B vitamins may be obtained from the diet or from microbial synthesis in the large intestine. Whether horses can synthesize adequate quantities of B vitamins to meet the needs of heavy exercise has been questioned (Frape, 1989). The B vitamins play important roles in RBC physiology and in energy metabolism and are, therefore, key nutrients for exercising horses. In other species, deficiencies of folacin or vitamin B_{12} can result in megaloblastic anemia, but this situation has not been documented in the horse. Studies that have examined the effect of vitamin B_{12} or folacin supplementation on RBC numbers in performance horses have not been conducted. Alterations in blood levels of these vitamins in performance horses have been reported, but the significance of such changes to performance is not known. The NRC currently makes no recommendation for desirable dietary folacin or vitamin B_{12} levels.

Thiamin (vitamin B_1), riboflavin (vitamin B_2), niacin, pyridoxine (vitamin B_6), pantothenic acid, and biotin are involved in energy metabolism, usually as cofactors in enzymatic reactions. Because exercise increases energy expenditure, the requirements for these vitamins may be increased. When exercising horses were fed diets containing 2 mg, 4 mg, or 28 mg thiamin per kilogram of diet dry matter, blood lactate levels were lowest in the horses receiving 28 mg/kg (Topliff et al., 1981). The results of this study may be interpreted to suggest that the thiamin requirement of working horses is between 4 mg/kg and 28 mg/kg.

The riboflavin requirement for exercise has not been studied in horses, but it has been suggested that exercising horses should be fed riboflavin 0.05 mg/kg BW. This recommendation is actually higher than the recommendation for humans when differences in body weight are considered. The NRC does not make recommendations for any other B vitamins or for vitamin C. Biotin supplementation (up to 30 mg/day) is believed to improve hoof condition in some horses (NRC, 2007), but biotin supplementation (50 mg/day) did not affect the response of Thoroughbreds to an exercise test (Lindner et al., 1992).

Several nutrients, including vitamin E, selenium, vitamin A, and zinc, are known to be important for optimal immune function. Deficiencies of these nutrients have been linked to impaired immune responses in other species and occasionally in horses (Knight et al., 1990a). Rigorous exercise may lead to depressed immune function, at least in human athletes (Malm, 2004). The requirements of horses for optimal immune function have not been determined but the interactions among nutrition, immune function, and exercise deserve consideration.

Use of Dietary Supplements for Performance Horses

Owners and trainers often look to dietary supplements to enhance the performance of their horses. An ergogenic aid is a substance that increases or improves work performance. By definition, an ergogenic aid is not limited to nutritional or dietary compounds but may include drugs or mechanical aids such as nasal strips. This enhancement of performance can be measured as increased speed, power, endurance, or all of these and is obtained through increased exercise capacity, enhanced physiologic processes, and reduced psychological inhibition or through providing a mechanical advantage (Brooks et al., 1995). Specifically, ergogenic aids are believed to have psychological benefits, decrease energy requirements, increase muscle mass, increase efficiency of movement, provide fuel, increase stored energy, improve metabolic efficiency, reduce substrate depletion, improve high energy phosphate ratios, reduce accumulation of end-products, and reduce damage to tissues (Harris and Harris, 2005).

Several dietary supplements are commonly considered ergogenic aids, including caffeine, creatine, and carnitine, but vitamins and amino acids may also fit the classification. In fact, under the broadest definition, carbohydrates and fats could be considered ergogenic aids as they can significantly enhance the ability of an animal to exercise through the provision of fuel. Many ergogenic aids are non-nutritional such as narcotics and stimulants but may also include compounds such as erythropoietin, furosemide, and anabolic steroids. The use of any ergogenic products in equine sport is highly controversial, and many countries and horse organizations attempt to ban substances with such performance-enhancing properties, including some dietary supplements.

The numerous dietary supplements that are available make claims of athletic-enhancing properties. However, these claims are often unfounded when researched thoroughly. Measurement of performance is difficult, though the use of treadmills (to control work intensity) and the measurement of oxygen uptake and metabolites (as indicators of athletic capacity such as $\dot{V}O_{2max}$ and the lactate threshold) facilitate the ability to obtain quantitative data. However, even such well-controlled laboratory studies do not predict the potential effects of a supplement in the field. Complicating the matter is that a statistically insignificant effect in a research study may be very significant in competition (Snow, 1994). It is well-recognized that it is expensive and difficult to adequately verify beneficial effects of dietary supplements on performance. At a minimum however, ergogenic products and nutritional supplements, should be tested for safety.

Table 4-3 lists a number of dietary supplements commonly used in equine sport and their proposed metabolic effects. Dietary supplements will be discussed further; however, compounds such as hormones and diuretics are beyond the scope of this section.

TABLE 4–3

Dietary Supplements Used as Ergogenic Aids in Horses

Substance	Proposed Effect	Reference*
Vitamin E	Antioxidant	McMeniman and Hintz, 1992 Siciliano et al., 1997 Williams and Carlucci, 2006
Vitamin C	Antioxidant	White et al., 1991 Williams et al., 2004
Coenzyme Q10	Antioxidant, electron transport	Rathgeber-Lawrence et al., 1991
Carnitine	Increase fatty acid transport	Harris et al., 1995 Zeyner and Harmeyer, 1999 Rivero et al., 2002 Niemeyer et al., 2005
Creatine	Improve high energy phosphate ratio	Sewell and Harris, 1995 Schuback et al., 2000 D'Angelis et al., 2005
Omega-3 Fatty Acids	Improve cardiovascular efficiency, antioxidant	O'Connor et al., 2004 De Moffarts et al., 2007
Chromium	Improve glucose utilization	Pagan et al., 1995
B Vitamins	Improve energy metabolism	Lindner et al., 1992 Turner et al., 2006
Branched-Chain Amino Acids	Energy source, reduce central fatigue	Glade, 1991 Casini et al., 2000 Stefanon et al., 2000
Methylsulphonylmethane (MSM)	Antioxidant/Anti-inflammatory	Maranon et al., 2008
L-histidine	Precursor to carnosine myocyte buffer and β-alanine	Powell et al., 1991 Dunnett and Harris, 1999
Dimethylglycine (DMG), Betaine	Reduce lactic acid	Rose et al., 1989 Warren et al., 1999

*The cited studies investigated the effects of the specified supplement but did not necessarily find benefits. Please see the list of References at the end of the chapter.

Antioxidants

With exercise metabolism, there is the formation of free radicals and reactive oxygen species. The accumulation of these compounds is normally controlled through the body's antioxidant and free radical scavenging systems (for a review, see Kirschvink et al., 2008). However, with intense exercise, it is believed that these defense systems are overwhelmed, and muscle damage can occur (Kirschvink et al., 2008). Thus, the supplementation of various compounds with proposed antioxidant properties is commonplace for athletes. Antioxidants are purported to reduce the extent of lipid peroxidation and muscle damage, and their efficacy is often measured through the assessment of thiobarbituric acid reactive substances (TBARS), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities, and the extent of muscle damage is reflected by measurement of creatine kinase (CK) and aspartate transaminase (AST) activity (Kirschvink et al., 2008). Vitamin E is the most common antioxidant fed to horses, despite conflicting evidence for its effectiveness in reducing oxidative damage from exercise (McMeniman and Hintz, 1992; Siciliano et al., 1997; Williams and Carlucci, 2006). Nonetheless, vitamin E status has been shown to be affected by exercise, so exercising horses should be fed above basal levels (NRC, 2007). Although vitamin E is relatively nontoxic, large doses of vitamin E given to intensely working horses have been shown to negatively affect the status of β-carotene, another antioxidant (Williams and Carlucci, 2006). Vitamin C (ascorbic acid) has been shown to prevent exercise-induced increases in thiobarbituric acid reactive substances (TBARS) and maintain antioxidant status in blood in Thoroughbred racehorses, though serum CK was not affected by supplementation (White et al., 2001). Another study compared vitamin E supplementation, alone or with vitamin C, in endurance horses (Williams et al., 2004). Despite an increase in plasma ascorbate, there was no benefit of vitamin C supplementation on CK or AST activities. Both treatment groups showed oxidative stress despite antioxidant supplementation. Coenzyme Q10, or ubiquinone, is part of the electron transport chain of mitochondria but is also considered an antioxidant. One study in horses failed to show any effect of Q10 on exercise performance (Rathgeber-Lawrence et al., 1991). The omega-3 fatty acids may also have antioxidative properties in their ability to affect the membrane fluidity of RBCs. When fish oil extract (containing eicosapentaenoic acid and docosahexaenoic acid [EPA and DHA]) was fed to horses in combination with vitamin E and copper, erythrocyte membrane fluidity was maintained following exercise, though there was no effect on the oxidant–antioxidant balance (De Moffarts et al., 2007).

Fish oil supplementation was also shown to reduce HR in exercising horses (O'Connor et al., 2004). Methyl-sulphonyl-methane (MSM) is an organic source of sulfur, a component of glutathione. Recently, MSM fed alone or in combination with vitamin C reduced the formation of lipid hydroperoxides induced by training and maintained glutathione levels in plasma following training (Maranon et al., 2008).

Creatine

Creatine (methylguanidine-acetic acid) is an amino acid derivative found in skeletal muscle, primarily as creatine phosphate. Muscle creatine concentrations are relatively low (100–150 millimoles per kilogram [mmol/kg] dry weight). Creatine phosphate is utilized by the muscle as a high energy phosphate donor to regenerate ATP from ADP in the early stages of exercise. Because of creatine's ability to maintain energy status, as well as its ability to buffer the hydrogen ions produced during high intensity exercise, attempts to increase muscle creatine content through supplementation are common. In humans, creatine is consumed through meat but may also be produced by the kidneys or liver (Bemben and Lamont, 2005; Schoch et al., 2006). In the horse, it is likely that the only source of creatine is via synthesis in the liver, and there is no dietary requirement of creatine. Similar to vegetarians who show benefits from creatine supplementation (Venderley and Campbell, 2006), it is possible that horses may benefit as well. However, when supplemented orally, creatine must first be absorbed from the digestive tract and then transported to the site of action, the skeletal muscle. Absorption from the digestive tract is thought to be either through the creatine transporter 1 (CT1) carrier system or through paracrine action (Orsenigo et al., 2005). The transporter into the muscle, CreaT appears to be regulated by diet and physical conditioning (Schoch et al., 2006). In humans, oral doses of 250 mg/kgBW have been shown to increase muscle creatine concentrations and force production during exercise (Bemben and Lamonte, 2005). Studies in horses have not shown any athletic benefits to oral creatine supplementation (D'Angelis et al., 2005; Schuback et al., 2000; Sewell and Harris, 1995), possibly because it is poorly absorbed (Sewell and Harris, 1995). It is unknown if intravenous supplementation would be beneficial to horses.

Carnitine

L-Carnitine is required for the transfer of long chain fatty acids across the inner mitochondrial membrane into the mitochondrial matrix for oxidation. Carnitine is a constituent of several enzymes involved in this transfer; carnitine–palmityl transferase I, carnitine–palmityltransferase II, and carnitine–acylcarnitine translocase (Zeyner and Harmeyer, 1999). The transportation of fatty acyl chains into the mitochondrial matrix are considered a rate-limiting step for fat oxidation, and thus any increase in enzymatic transfer capacity has the potential to enhance fat oxidation. The carnitine complex also acts as an acetyl-buffer by accepting acetyl groups from acetyl-CoA to form acetyl carnitine. This maintains CoA available for substrate flux through the citric acid cycle and pyruvate dehydrogenase reactions (Zeyner and Harmeyer, 1999). Oral carnitine administration increases plasma carnitine in the horse, but bioavailability is low (Harris et al., 1995). In addition, it appears that there is minimal effect of oral carnitine supplementation on muscle carnitine concentration (Foster et al., 1988). Despite this, muscle adaptations, including increases in the percentage of type IIA muscle fibers and a rise in the capillary to fiber ratio, were observed in horses when supplemented with oral L-carnitine during 5 weeks of exercise training (Rivero et al., 2002). However, there was no effect of such supplementation on responses to exercise such as HR (Niemeyer et al., 2005).

Amino Acids

Amino acids may be used directly as a source of energy for exercise, for muscle protein synthesis or for the synthesis of intermediary enzymes. The branched chain amino acids (BCAA; leucine, isoleucine, and valine) are believed to be related to the onset of central fatigue. The proposed role of BCAA in central fatigue is based on their interaction with tryptophan metabolism. Tryptophan is used for the synthesis of 5-hydroxytryptamine (also known as serotonin) in the brain. High levels of 5-hydroxytryptamine can induce sleep, thus it has been suggested that an increase in tryptophan in the brain could contribute to central fatigue. The BCAA can regulate tryptophan uptake into the brain at the blood–brain barrier, such that an increase in BCAA reduces tryptophan uptake, potentially reducing serotonin and fatigue. Tryptophan administration (IV 100 mg/kgBW), resulting in plasma concentrations nine times the levels at resting, does appear to decrease exercise performance in horses (Farris et al., 1998). However, while pharmacological amounts of tryptophan affect exercise performance, tryptophan concentrations only double during a controlled 72-km ride (Assenza et al., 2004); thus, it is unknown if physiologic levels of tryptophan affect performance. Similarly, the tryptophan: BCAA ratio increases with endurance riding (72 km), although it is unknown if exercise potential was affected (Assenza et al., 2004). To this point, administration of BCAA to horses does not appear to affect exercise performance (Casini et al., 2000; Stefanon et al., 2000), though one study did report reduced lactate concentrations in horses undergoing light exercise when supplemented with BCAA (Glade, 1989).

There is significant interest in the amino acids L-histidine and β-alanine because of their role in the synthesis of carnosine. Carnosine is found in relatively high amounts compared with other athletic species such as greyhound and man and functions as a buffer within muscle cells (Harris et al., 1990). In man, β-alanine supplementation was shown to increase muscle carnosine concentrations and improved muscle torque during repeated isometric contractions (Derave et al., 2007). In the horse, histidine, alone (Powell et al., 1991) or in combination with β-alanine (Dunnett and Harris, 1999), has resulted in inconclusive results. In both studies, muscle carnosine increased with supplementation; however, these increases were not significant overall. Nonetheless, Dunnett and Harris (1999) did find that horses that had increased carnosine concentration also had increased buffering capacity, and Powell et al. (1991) reported a similar but nonsignificant trend for increased buffering.

Several other amino acids, including glycine, have been purported to have ergogenic effects. Specifically, dimethylglycine (DMG) is a component of choline metabolism and serves as a methyl donor. DMG was initially believed to be beneficial for exercise through the reduction of lactate concentrations; however, DMG supplementation to Thoroughbred horses completing a standardized exercise test to intensities of 100%

$\dot{V}O_{2max}$ had no effect on plasma lactate, HR, or blood pH (Rose et al., 1989). Trimethylglycine (betaine) was shown to reduce lactate in untrained horses but not in trained horses (Warren et al., 1999b). Lysine and threonine supplementation was found to increase subjective muscle mass scores in young (<10 years of age) and aged (>20 years of age) horses, with no apparent detrimental effects, though exercise performance was not tested (Graham-Thiers et al., 2005).

Other Supplements

Several other micronutrients and feed components are reported to have some ergogenic benefits. A horse deficient in any nutrient will show benefit (ergogenic and otherwise) to supplementation. However, nutrients normally required in the diet, fed at amounts higher than recommended, generally do not produce ergogenic benefits. Chromium is believed to function as the glucose tolerance factor to assist insulin functions, specifically with respect to glucose metabolism, anabolism, and muscle gain. In humans, reports of increased muscle mass are unfounded; in fact, chromium may alter iron status (Wolinsky, 1998). One study in trained horses found a small decrease in lactate concentrations during a standardized exercise test (Pagan et al., 1995), though this may have been related to reduced chromium status. Iron status is highly related to exercise capacity because of its importance in oxygen-carrying capacity. However, supplemental iron has no beneficial effect on RBC count or hemoglobin concentration, and the detrimental effects of iron (toxicity and interactions with other minerals such as zinc) should dissuade horse owners from utilizing iron as an ergogenic aid (Lawrence et al., 1987; Lindner et al., 1992). The B vitamins, because of their general functions associated with carbohydrate and lipid metabolism, are often purported to have benefits to exercise. Biotin is the only B vitamin studied specifically in the horse with respect to exercise performance, and it was found to have no effect on lactate concentrations (Lindner et al., 1992). Recently, however, a bee pollen product, reported to contain high amounts of thiamin and other B vitamins was found to increase feed intake in exercised horses; however, the study numbers were small, and no effect on athletic performance was recorded (Turner et al., 2006). Herbal products such as Echinacea may have some beneficial effects and are reviewed elsewhere (Williams and Lamprecht, 2008). Other compounds commonly found in equine products include β-hydroxy-β-methyl butyrate (HMB) and γ-oryzanol, though there are no published papers supporting their efficacy.

Feed Selection

Forages

Pasture can be an excellent forage source for horses, but many performance horses have little or no access to pasture. When pasture is not available, some type of conserved forage must be used instead. Many types of conserved forages are acceptable for horses as long as they are clean and free from mold and dust. The most common conserved forages are hay, hay cubes, and haylage. Although haylage is not common in the United States, it is used effectively in many parts of the world. The forages that are available in any geographic area will usually depend on the climate and growing conditions of that area. In most of Europe and North America, forages that are well adapted to cooler climates (cool season plants) are often fed to horses. Common cool season forages include timothy, orchardgrass, bromegrass, perennial ryegrass, alfalfa, and some clovers. In warmer climates, warm season plants may be more common. Bermudagrass, bahiagrass, big bluestem, and lespedeza are examples of forages that are well adapted to warmer climates.

The average nutrient content and chemical composition of forages varies among warm and cool season plants and between grasses and legumes. Soil fertility and growing conditions can also affect the nutrient content of forages, particularly the protein and mineral composition. Soils that are low in nitrogen or certain minerals will often produce forages that are low in those nutrients. One of the most important factors affecting the nutrient content of forages is the stage of maturity of the plant at the time of harvest. Terms used to describe forage maturity include *vegetative* (very early maturity; material is leafy and rapidly growing as in spring pasture); *prebloom* (early maturity; the forage is beginning to make flowers or seed heads but they have not emerged); *bloom* (midmaturity; the forage has flowers or seed heads); *postbloom* (late maturity; seed heads are mature). The forages with the highest nutrient density are usually legume or legume–grass mixes that are harvested at an early stage of plant maturity (Table 4-4). The forages with the lowest nutrient density are usually grasses that are harvested at a late stage of maturity. Mature forages have seed heads, thick stems, and relatively high fiber concentrations. Forages are commonly analyzed for two categories of fiber: (1) acid detergent fiber

TABLE 4–4

Nutrient Composition of Various Hays (90% Dry Matter Basis)

	Category	DE (Mcal/kg)	% NDF	%ADF	%CP	%Ca	%P
Alfalfa Hay – Early Maturity	Legume	2.2	35.4	28.7	17.9	1.3	0.2
Alfalfa Hay – Late Maturity	Legume	1.9	43.9	34.8	15.3	1.1	0.2
Timothy Hay – Early Maturity	Cool season grass	1.9	55.3	31.7	9.7	0.5	0.3
Timothy Hay – Late Maturity	Cool season grass	1.6	62.9	37.6	7.0	0.3	0.2
Bermudagrass Hay – Early Maturity	Warm season grass	1.9	65.7	30.6	10.8	0.4	0.3
Bermudagrass Hay – Late Maturity	Warm season grass	1.7	68.9	34.5	7.0	0.3	0.2

ADF, acid detergent fiber; *Ca*, calcium; *CP*, crude protein; *DE*, digestible energy; *NDF*, neutral detergent fiber; *P*, phosphorus.

Source: NRC (National Research Council): *Nutrient requirements of horses*, Washington, DC, 1989, National Academy Press.

(ADF) and (2) neutral detergent fiber (NDF). The ADF fraction contains cellulose and lignin. The NDF fiber fraction contains the ADF fraction as well as hemicellulose. From a nutritional perspective, the NDF and ADF concentrations in forages are important because they negatively influence digestibility and also possibly palatability. The microbial population in the large intestine can digest much of the cellulose and hemicellulose in forages, but lignin is essentially nondigestible. Cellulose, hemicellulose, and lignin compose the rigid portion of the plant cell walls, and in general, as the plant matures, the concentrations of these compounds increase. Grasses usually have higher levels of NDF than legumes but the ADF content can be relatively similar between legumes and grasses.

Concentrates

Concentrates are used to increase energy intake and also to supply any nutrients that are not adequate in the forage. Plain cereal grains (such as oats, barley, and corn) are good sources of energy but are deficient in some nutrients. Many feed companies manufacture fortified concentrates for performance that contain cereal grains, as well as protein, vitamin, and mineral supplements. These concentrates may be in the form of a "sweet feed" (also called a *textured feed*) or a "pelleted feed." Concentrate feeds that are made using extrusion technology may also be available.

Concentrates should be selected and fed in amounts that complement the forage in the diet and the nutrient needs of the horse. When horses are receiving mature grass hay, the amount of nutrients that must be supplied by the concentrate will be higher than when horses are receiving legume hay. For example, when a performance horse is fed a legume hay (>15% crude protein), a concentrate containing 10% to 12% crude protein may be sufficient. However, if a mature grass hay containing less than 10% crude protein is used, then a concentrate containing more than 12% crude protein should be selected. Similarly, the concentrate must provide more digestible energy when mature grass hay is fed than when early maturity alfalfa hay is used.

Many commercial concentrates are marketed on the basis of protein quantity, but diets should meet amino acid requirements as well as crude protein requirements. The NRC (2007) recommendations for crude protein requirements are based on a lysine percentage in the crude protein of 4.3%. Lysine is typically the most limiting amino acid; therefore, if the diet is adequate in lysine, it is usually considered to be adequate in other amino acids. If the lysine level in crude protein is lower than 4.3%, then the amount of crude protein in the diet should be increased to ensure that an adequate amount of lysine is consumed by the horse. The lysine levels of several feed ingredients are shown in Table 4-5. Some feed ingredients are adequate in crude protein but may be low in specific amino acids; or the amino acids may not be available for absorption from the small intestine. When these ingredients are incorporated into horse feeds, feed companies may increase the amino acid content of the feed by incorporating an amino acid supplement into the formulation.

Most commercial horse feed manufacturers offer at least one, and often several, concentrate feeds that have been formulated for equine athletes. Cereal grains (oats, corn, barley, etc.) are the traditional source of digestible energy in performance horse feeds. In recent years, cereal grains have been replaced in some commercial concentrate feeds by other ingredients, including beet pulp, soybean hulls, wheat middlings, and rice bran. High-fat ingredients such as vegetable oil, rice bran, whole roasted soybeans, and sunflower seeds are often added to increase the energy density (Mcal of DE/kg feed), to alter the form of energy absorbed by the horse, or both. As discussed previously, diet composition may influence the availability and utilization of energy substrates during exercise. Diets containing elevated levels of fat may induce changes that allow horses to utilize more fat during exercise, thus sparing carbohydrate stores. This type of adaptation may be more important for horses performing endurance-type exercise than for horses that perform at maximal intensity for brief periods, such as race horses.

Ingredients such as beet pulp and soybean hulls provide additional fiber in the diet, which may be important to gastrointestinal health, particularly if hay intake is limited. These fiber sources are relatively well digested and thus can be a useful source of digestible energy in the diet. When these ingredients replace cereal grains in the concentrate, the level of starch in the diet is usually reduced. Concentrates with added fat, increased fiber, and reduced starch levels may be desirable for horses that must consume large amounts of concentrate to maintain body weight. These feeds provide adequate calories and can reduce the risk of starch bypass to the large intestine. In addition, reduced starch diets may convey some benefits to horses with recurrent exertional rhabdomyolysis (MacLeay et al., 1999), although management of this disorder is not usually accomplished by diet alone (McKenzie et al., 2002).

Some commercially manufactured concentrate feeds include products described as probiotics or prebiotics. Probiotics and prebiotics are usually intended to influence the microbial population of the gastrointestinal tract, enhance digestion, or improve gastrointestinal health. Some of these products may include live microbes, and/or the fermentation products of live cultures from *Lactobaccillus* Sp, *Saccharomyces cerevisiae, Aspergillus oryzae*, or other organisms. Although there have been many research trials with various probiotic and prebiotic

TABLE 4–5

Crude Protein and Lysine Content of Some Common Feed Ingredients

	% Crude Protein	% Lysine	% Lysine in Crude Protein
Alfalfa Meal	17	0.74	4.3
Oats	11.5	0.4	3.5
Barley	11.3	0.41	3.6
Corn	8.3	0.26	3.1
Wheat Middlings	15.9	0.57	3.6
Wheat Bran	15.7	0.64	4.1
Distillers' Dried Grains with Sol	27.7	0.74	2.7
Soybean Meal	43.8	2.83	6.5
Flax Meal	33.6	1.24	3.7

*Source: NRC (1989)

Source: NRC (National Research Council): *Nutrient requirements of horses,* Washington, DC, 1989, National Academy Press.

products, the benefits to performance horses have not been well documented. Various factors could be important to the success of these products, including the diet of the horse, the overall health of the horse's gastrointestinal tract, and the actual composition of the product. Most research trials use normal horses in relatively unstressed environments and additional research with animals under competition stress is probably warranted.

Nutritional Supplements

Nutrient supplements should be added to the diet only when there is a clear need. Indiscriminate use of supplements may result in unbalanced diets or possibly toxicities. Most commercial feeds are fortified with enough vitamins and trace minerals that additional nutrient supplementation is not likely to be a benefit. Possible exceptions would be biotin for horses with poor hoof quality and electrolytes for horses in warm environments.

Although most commercially manufactured performance horse feeds do not require additional supplements, concentrates entirely composed of cereal grains will need to be supplemented with a variety of nutrients. Oats, corn, and barley are deficient in calcium and are low in many trace minerals and vitamins. Some feed manufacturers sell a supplement referred to as a *balancer pellet*, which can be used when horses are receiving diets consisting of only hay and oats or other cereal grains.

Feeding Management

A mature idle horse can eat between 2 kg and 2.5 kg of dry matter for each 100 kg of body weight. Hay and concentrate are about 90% dry matter, so maximum total daily feed intake for a 500-kg horse will range from 11 kg to 14 kg per day. Forage will comprise the majority of the idle horse's diet (Table 4-6). As nutrient needs increase with increasing work level, forage is gradually replaced with concentrate. At the highest levels of work, performance horses will need to consume relatively high levels of concentrate, even if the forage quality is high (see Table 4-6). When total feed intake drops below 2 kg/100 kgBW, energy requirements for heavy and very heavy work can only be met if the diet contains mostly concentrate. High concentrate intakes may compromise gastrointestinal health, so feeding management should focus on maintaining normal feed intake.

The type and form of the available forage can affect feed intake in horses. Todd and coworkers (1995) reported that horses consumed more dry matter when alfalfa was offered as a cube than when it was offered as long-stemmed hay.

Several studies have observed that horses will consume alfalfa hay at a higher rate than grass hay (Crozier et al; 1997; Dulphy et al., 1997; LaCasha et al., 1999). The NDF concentration of forages may be an important determinant of voluntary dry matter consumption. In general, horses will consume less total forage if the forage is higher in NDF (St. Lawrence et al., 2000). Forages that are high in NDF include warm and cool grasses that were harvested in a late stage of maturity.

It is expected that voluntary forage intake will be reduced when low quality forages are used. Reduced forage consumption may not be a problem for horses performing light exercise because a small amount of added concentrate will compensate for any nutrient deficits. However, reduced forage palatability or consumption can be problematic for horses performing very heavy exercise. When horses consume mature forages with lower nutrient density, excessive amounts of concentrate will be needed to meet the horse's nutrient needs for very heavy exercise (see Table 4-6).

Most performance horses are fed two or three meals of concentrate per day. Concentrate meals should be as evenly spaced as possible, and meal size should be determined by the horse's appetite and also the composition of the concentrate. Most horses can consume 1 to 2 kg of concentrate in 10 to 15 minutes. In some parts of the world, chaff is mixed with concentrate to slow the rate of consumption and also to increase the intake of dietary fiber. As meal size increases, horses may take longer to finish the concentrate. If a horse fails to consume all of the concentrate offered in a meal, it may be necessary to reduce the meal size and increase the number of meals offered per day. Meal size may also be influenced by the chemical composition of the concentrate. The overflow of rapidly fermented carbohydrates to the large intestine will be increased when a single meal provides more than 2 to 3 g/kgBW of nonstructural carbohydrate (NSC: starch + simple sugars). For a 500-kg horse, this would be a maximum of 1 to 1.5 kg of NSC per meal. If a concentrate contains 40% NSC, then the maximum meal size would be 2.5 to 3.7 kg. If the concentrate contains 60% NSC, then the maximum meal size would be only 1.7 to 2.5 kg. The amount of NSC in a concentrate will be determined by the type and inclusion rates of the various ingredients; consequently, the amount of NSC in commercially manufactured feeds can vary widely. In the United States, feed companies are not required to report the level of NSC on the feed tag or bag, but many

TABLE 4–6

Effect of Hay Quality on Concentrate Intakes for A 500-kg Horse at Different Levels of Work*

	Idle	Light Work	Moderate Work	Heavy Work	Very Heavy Work
Maximum expected total daily feed intake (as fed)	11–14 kg	11–14 kg	11–14 kg	11–14 kg	11–14 kg
Early Maturity Alfalfa-timothy Hay*	8–9 kg	9–10 kg	9–10 kg	8–9 kg	6–7 kg
Concentrate*	0 kg	0–1 kg	1–2 kg	3–4 kg	6–7 kg
Late Maturity* Timothy Hay	10–11 kg hay	9–10 kg	8–9 kg	7–8 kg	5 kg
Concentrate*	0 kg	1–2 kg	3–4 kg	4–5 kg	8–9 kg**

*Early maturity alfalfa-timothy hay is estimated to contain (as fed basis): 2.0 megacalories (Mcal) digestible energy per kilogram (DE/kg), 14% crude protein (CP), 0.7% calcium (Ca) and 0.3% phosphorus (P); late maturity timothy hay is estimated to contain 1.6 Mcal DE/kg; 7% CP, 0.3% Ca, 0.2% P; concentrate is estimated to contain 3.0 Mcal DE/kg, 13% CP, 0.7% Ca, 0.5% P.

**This diet is not recommended. To avoid excessive concentrate intakes, diets of horses engaged in very heavy work should utilize high-quality hay.

feed companies will provide the information if requested, or a feed can be submitted to a laboratory for analysis. This information can then be used to make better feeding decisions. There is a common misconception that sweet feeds are higher in NSC compared with pelleted feeds because they contain molasses. The amount of molasses added to sweet feeds is usually small (5%–8% of the total mix) and the amount of NSC contributed by the molasses is minor in comparison with the amount contributed by cereal grains or cereal grain byproducts. It is not uncommon for a sweet feed containing beet pulp or another fiber source to be lower in NSC compared with a pelleted feed that is based on cereal grains or their byproducts.

Concentrates with reduced levels of NSC usually contain increased levels of digestible fiber sources and relatively high levels of added fat (6%–10%). Fat supplemented feeds have been reported to be well accepted and well digested by horses in research trials (Bowman et al., 1979; Duren et al., 1987; Kronfeld et al., 2004). However, anecdotal reports from owners and trainers suggest that some feeds with elevated fat and fiber levels are poorly accepted by some horses. If a feed is poorly consumed, it will not provide the calories needed to maintain body weight. The choice between a traditional grain-based concentrate and a reduced NSC concentrate with higher fiber and fat levels may be dictated by the dietary preferences of individual horses.

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CHAPTER

5 Hematology and Biochemistry

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For more than 50 years, evaluation of the hemogram and plasma or serum biochemistry has been a cornerstone in the assessment of the athletic horse. Initially, investigations focused on the hemogram, which was assessed manually using the hemocytometer. Currently, automated techniques are available for both hematologic and plasma or serum biochemical measurements, resulting in a wider range of available measurements as well as less expense per test. This has given rise to "profiles," where a large number of measurements are performed on each blood sample with the ability to provide access to the function of a range of body systems to screen for "fitness" to race and to screen for disease that may affect performance. Despite much investigation, there has been little evidence to suggest that any routine clinicopathologic tests in the resting state can detect athletic fitness or performance potential. However, the role of clinical pathology in detection or support of subtle or subclinical disease and overtraining that can have a large impact on performance cannot be underestimated.

Although abnormalities may be found, in many cases, it is difficult to interpret the findings in the absence of a detailed physical examination. It also must be remembered that with a large number of analyses, one could be outside the normal range by chance and not because of any particular pathology. It is useful, therefore, to view minor abnormalities found on a "profile" critically, since they may have no pathologic significance. Particular care must be taken in interpretation of changes in the red blood cell (RBC) indices (erythrocyte numbers, hematocrit, and hemoglobin) because of the labile nature of the RBC pool.

In this chapter, we will examine some of the factors that can affect the interpretation of the hemogram, leucogram, and biochemical profile, including technical considerations, as well as the influence of exercise and training.

RESTING HEMOGRAM, LEUCOGRAM, AND SERUM OR PLASMA BIOCHEMICAL VARIABLES

BLOOD COLLECTION TECHNIQUES

Blood samples are usually collected from the jugular vein by using evacuated collection tubes (Vacutainer, Beeton-Dickinson, Cockeysville, MD) with double-ended needles. This system allows quick and simple sample collection. For hematology, blood is collected into tubes containing ethylenediarninetetraacetic acid (EDTA) as an anticoagulant, whereas lithium or ammonium heparin is used if plasma biochemical measurements are to be performed. Tubes containing fluoride oxalate as an anticoagulant are preferred when either plasma glucose or lactate values are to be measured, or this cannot be performed immediately. With the latter anticoagulant, hemolysis is common, and plasma samples cannot be used for other measurements. Some laboratories prefer serum to plasma samples, and plain evacuated tubes are available for serum collection.

Sample Handling

Although there have been some reports that the use of evacuated blood collection tubes could damage erythrocytes, extensive experience has shown that provided needles no smaller than 21-gauge diameter are used, the evacuated tubes are quite satisfactory.

Following blood collection, blood smears for cytologic examination should be made as soon as possible and preferably within 6 hours of sample collection. If blood is stored overnight prior to analysis, there may be a small increase in the hematocrit (HCT) and mean cell hemoglobin (Hb) associated with enlargement of the erythrocytes. This increase is usually limited to no more than 0.01 to 0.02 liter per liter (L/L; 1% to 2%) in HCT values.

Exposure of the blood samples to high environmental temperatures, such as may be found if blood samples are stored in a car exposed to direct sunlight, may result in some sample hemolysis, causing serum or plasma potassium values to be falsely elevated. Even without obvious hemolysis, sample storage can cause increases in serum or plasma potassium, and therefore, elevated potassium values should first be investigated as a methodologic problem rather than a pathologic one.

Sample Processing and Accuracy

The precision of measurements of Hb, HCT, erythrocyte counts, and leukocyte counts has been found to be ±5% in 36 duplicate measurements. Thus, in the normal ranges for each of these measurements when sequential sampling is performed, changes would have to be greater than 0.02 L/L (2%) for HCT, 7.5 gram per liter (g/L; 0.75 gram per deciliter [g/dL]) for Hb, 0.5×10^9/L (500/μL [microliter]) for leukocyte counts, and $0.5 \times l0^{12}$/liter (0.5×10^6/μl) for erythrocyte counts before the changes could be regarded as clinically significant. In addition to the precision related to the measuring equipment, daily variation in the RBC indices in individual horses must be taken into account. Persson (1975a) reported up to a 30% variation in the resting Hb values of three Standardbred trotters that had daily blood samples collected for 7 days. From these findings, it is clear that some caution is required when interpreting erythrocyte indices,

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particularly from a single blood sample. Repeated measurements may permit greater confidence in the findings.

Plasma and serum biochemical measurements are generally performed using autoanalyzers, and the accuracy of such equipment is generally ±5%. Measurement of electrolytes is generally performed using ion-selective electrodes in autoanalyzers rather than flame photometry, used previously. Carlson (1989) has noted that because ion-selective electrodes measure the electrolyte concentration in the water component of plasma or serum, values are 6% to 7% higher than those of electrolytes measured by flame photometry. Flame photometry measures electrolyte concentrations in millimoles per liter (mmol/L) of plasma or serum, where the water content is 93% to 94%.

RESTING HEMOGRAM

RBCs containing Hb play a vital role in the pathway transporting oxygen from the lungs to the muscles and tissues that are used in performance (Weibel et al., 1991). The correlation between $\dot{V}O_{2max}$ and Hb concentration was shown experimentally in human athletes using autologous blood transfusions and blood loss (Ekblom et al., 1972; 1976). The relationship between an acute increase in blood Hb concentration and performance has been well studied in humans, with researchers showing increases in endurance time, greater speed and mean power output, and lower blood lactate concentration in standardized exercise tests (Calbet et al., 2006). Conversely, although perhaps less well studied, reduced Hb concentration reduces endurance performance (Calbet et al., 2006).

Similarly in horses, a relationship has been found between RBC volume, training, and performance. RBC volume increased with the horse's age and training and also was correlated with racing performance in normally performing horses (Persson 1968; 1983a). An Australian study indicated that Thoroughbred horses with RBC indices falling by more than 1 standard deviation (SD) below the mean did not win races at city race courses (Stewart and Steel, 1975). More recent work has shown that administering low-dose erythropoietin three times a week for 3 weeks to horses resulted in significant increases in packed cell volume (PCV; resting and peak), Hb, and RBC volume, as well as a resultant increase in $\dot{V}O_{2max}$ (McKeever et al., 2006), further supporting the relationship between RBC volume and performance.

As a result, the main focus of the hemogram of a racehorse is the RBC count, Hb concentration, and PCV or HCT. However, these data have notoriously failed to detect differences in fitness or performance at rest because of low reproducibility and variations associated with other factors, including degree of excitement, time since last exercise, feeding status, and transport (Snow, 1983). Of particular importance is the fact that routinely measured values of PCV or HCT, Hb, and RBC number may not reflect the true RBC volume. This is principally attributed to the uneven distribution of RBCs in the circulatory system, with varying degrees of splenic storage (Persson, 1983a).

Normal Ranges, Monitoring, and Performance

Although the normal range for an individual horse may be quite narrow, normal values for a breed fall into a broad range (Table 5-1). The normal ranges for adult Thoroughbred and Standardbred horses in training are given in Table 5-2. Most of the hematologic values for the different breeds are similar.

TABLE 5–1

Normal Hematologic Values (Mean or Mean ± SD) Reported for Adult Horses at Rest

Breed and Training State	RBC (x 10^{12}/L)	HB (g/L)	PCV (L/L)	WBC (x 10^{9}/L)
THOROUGHBREDS				
Macleod and Ponder				
2- and 3-year olds	10.8	141	–	–
More than 3 years old	11.6	154	–	–
Irvine				
2-year old, not in training	–	125	–	–
More than 3 years old, not in training	8.1	134	0.43	–
2-year-old, in training	7.4	117	0.39	–
More than 3 years old, in training	6.7	114	0.36	–
Archer and Miller				
In training	9.5 + 1.1	147 ± 9	0.41 ± 0.07	8.4 ± 2.2
Brenon				
In training	6.8	139	0.43	–
Steel and Whitlock				
In training	9.7 ± 1.3	134 ± 19	0.42 ± 0.05	10.4
Sykes				
2-year-old, in training less than 1 month	10.2	136	0.40	–
2-year-old, in training 3 to 6 months	11.0	153	0.46	–
2-year-old, in training more than 6 months	11.1	155	0.46	–

Continued

TABLE 5–1

Normal Hematologic Values (Mean or Mean ± SD) Reported for Adult Horses at Rest—cont'd

Breed and Training State	RBC (x 10^{12}/L)	HB (g/L)	PCV (L/L)	WBC (x 10^{9}/L)
3-year-old, in training less than 1 month	10.5	145	0.43	–
3-year-old, in training 3 to 6 months	11.0	157	0.47	–
3-year-old, in training more than 6 months	11.0	156	0.46	–
More than 4 years old, in training less than 1 month	10.6	148	0.44	–
More than 4 years old, in training 3 to 6 months	10.9	151	0.45	–
More than 4 years old, in training more than 6 months	10.9	152	0.45	–
Tasker				
In training	-	145 ± 11	0.40 ± 0.04	–
Stewart, Clarkson, and Steel				
In training	10.3 ± 1.5	157 ± 18	0.40 ± 0.05	–
Allen, Archer, and Archer				
2-year-old	9.9 ± 1.0	146 ± 14	0.40 ± 0.04	–
3-year-old	9.7 ± 1.1	151 ± 15	0.41 ± 0.04	–
4-year-old	9.3 ± 1.0	150 ± 17	0.41 ± 0.05	–
More than 4 years old	8.8 ± 1.1	146 ± 16	0.40 ± 0.05	–
Stewart and Steel				
In training	9.5 ± 1.3	150 ± 20	0.40 ± 0.06	–
Schalm et al.	9.6 ± 1.1	152 ± 14	0.44 ± 0.04	9.8 ± 1.4
Stewart, Riddle, and Salmon				
In training	9.1 ± 1.0	142 ± 14	0.40 ± 0.04	8.4 ± 1.2
Allen and Powell				
Before training	9.2 ± 0.8	136 ± 10	0.37 ± 0.02	9.8 ± 1.3
After 5 months of training	10.2 ± 1.2	152 ± 17	0.41 ± 0.04	9.6 ± 1.1
Revington				
Racing	9.6 ± 0.9	151 ± 10	0.42 ± 0.03	8.9 ± 1.3
STANDARDBREDS				
Steel and Whitlock	8.7 ± 1.4	124 ± 19	0.39 ± 0.04	9.8
Tasker	–	149 ± 15	0.39 ± 0.04	–
Schalm et al.	8.3 ± 0.7	137 ± 9	0.39 ± 0.03	7.9 ± 1.0
ARABIAN				
Schalm et al.	8.4 ± 1.2	138 ± 21	0.39 ± 0.05	9.5 ± 2.3
QUARTERHORSE				
Tasker	–	139 ± 22	0.38 ± 0.05	–
Schalm et al.	9.1 ± 1.4	138 ± 17	0.40 ± 0.05	9.7 ± 1.3
EQUITATION AND POLO HORSES				
Tasker	–	132 ± 16	0.37 ± 0.05	–
ENDURANCE HORSES				
Carlson	7.3	–	0.35	7.7
Carlson et al.	–	–	0.36 ± 0.03	7.5 ± 1.2
Rose	7.9 ± 0.5	130 ± 11	0.37 ± 0.03	8.8 ± 1.9
COLD-BLOODED BREEDS				
Schalm et al.	7.5	115	0.35	8.5

From Rose RJ, Allen RJ: Hematologic responses to exercise and training. *Vet Clin North Am Equine Pract* 1:465, 1985.

TABLE 5–2

Published Ranges for Resting Hematological Variables in Racehorses

	Standardbred	Thoroughbred
PCV (HCT) liter/liter (L/L)	32–39	32–43
Maximal PCV	48–65	60–65
Red blood cells ($\times 10^{12}$/L)	6.8–9.8	7.8– 9.7
Hb (gram per liter [g/L])	115–146	124–180
MCV ([fl])	45–45.5	39.4–47.2
MCH ([pg])	14.3–16.6	13.8–16.6
MCHC (g/L)	314–372	315–361

Hb, hemoglobin; *HCT*, hematocrit; *MCH*, mean corpuscular hemoglobin; *MCHC*, mean corpuscular hemoglobin concentration; *MCV*, maximal cell volume; *PCV*, packed cell volume.

However, as groups, the RBC indices from Standardbred pacers and Endurance horses are lower than those for Thoroughbred racehorses. This may, in part, be due to differences in plasma volumes, since plasma volume expansion may occur during training in Standardbred and Endurance horses because of the extensive submaximal training that is included in their work schedules.

Virtually all mature athletic horses that do not have clinical abnormalities will have hemogram values within the ranges reported in Table 5-2. Some veterinarians advocate regular collection of blood samples every 1 to 2 weeks from horses in training. This may be helpful in the diagnosis of subclinical abnormalities because individual normal values will fall within a much narrower range compared with values for the breed. Thus, an HCT value of 0.34 L/L may be of clinical significance in a horse that normally has values between 0.38 and 0.42 L/L, whereas no clinical significance could be attributed to an isolated blood sample with an HCT value of 0.34 L/L.

However, it is tempting for clinicians who cannot find any other abnormalities to falsely ascribe the cause of poor performance to minor deviations from breed means. Several studies in Thoroughbred racehorses have failed to demonstrate any relationship between HCT values prior to racing and subsequent racing performance (Laufenstein-Duffy, 1971; Revington 1983). Carlson and colleagues (1983) demonstrated that horses presented with anemia (2 SD below the mean) were most likely to have intercurrent disease rather than primary anemia. Therefore, if the RBC indices are low and there is no history of blood loss, low-grade infectious or inflammatory disease should be suspected rather than a primary disorder in RBC production.

Maximal Packed Cell Volume or Hematocrit

Although there is some variation in the resting RBC indices, samples collected after fast exercise or epinephrine administration show little variation on repeated sampling (Persson, 1975a). During exercise, under the influence of catecholamines, contraction of the spleen and release of erythrocytes occur, increasing PCV by as much as 20 L/L to 25 L/L (Snow et al., 1983a) (see Table 5-2). Because of the wide variations of normal resting PCV, maximal HCT may be a more reliable indicator of true RBC volume in horses (Persson, 1983a). However, it should be noted that part of this increase is from fluids shifts, with 15% increase in plasma protein recorded following racing (Snow et al., 1983a). Further, there may be up to a 10% individual horse variation in plasma volume (Persson, 1983a). These variations in plasma volume may significantly affect maximal HCT and its ability to represent RBC volume in horses.

FACTORS AFFECTING THE RESTING HEMOGRAM

Hematologic measurements are affected by a variety of factors, including the time of day, the relationship to feeding, the demeanor of the horse, and the relationship to exercise, whereas plasma or serum biochemical values are less subject to change as a result of diurnal variations or feeding.

Attitude of the Horse

The horse's demeanor and degree of excitement can have a significant effect on the resting hemogram because of the release of catecholamines causing splenic contraction and mobilization of erythrocytes. Increases of PCV between 6 L/L and 13 L/L have been reported in horses before exercise after simply being walked to the racecourse (Revington, 1983; Snow et al., 1983a). In one study of Thoroughbred horses, the animals were classified as either placid, timid (forceful jugular pulse and elevated heart rate), apprehensive (horse pulled back during blood collection), or excited (resisted blood collection and moved about). Although only the excited horses had increases in the erythrocyte and leukocyte counts when compared with the placid group (Stewart et al., 1977), we have noted small increases in RBC indices when horses show only slight degrees of apprehension. The critical factor appears to be the time required to collect the blood sample, since Persson and colleagues (1973) found that erythrocytes were mobilized from the spleen 30 to 60 seconds after the intravenous injection of epinephrine in a study using ^{15}Cr-labeled erythrocytes. Thus, it would seem that slight temperament changes will have little influence on the hemogram, provided blood samples are collected within 30 seconds of entering a box stall. However, it is important to note that in one study of Endurance horses, in which samples were collected every 2 weeks throughout 12 weeks of training, a group of five horses that were considered apprehensive during blood collection had HCTs that were 21% higher than in six quiet horses (Rose and Hodgson, 1982). Thus, care must be taken when interpreting changes in erythrocytes and leukocytes, particularly when values are higher than normal. It also should be anticipated that very placid horses may have values for RBC indices that are lower than mean values for the breed.

Effect of Feeding

When hay is fed, there may be substantial increases in HCT and plasma total protein, probably associated with substantial salivary fluid production (Kerr and Snow, 1982). HCT and total protein remain elevated for several hours. In a study in which a multiple regimen (feeding every 4 hours) was used, HCT and total protein remained constant, whereas a single large meal resulted in substantial fluid shifts out of the extracellular space (Clarke et al., 1988). In combination with the fluid shifts after the single feed, increases were found in plasma sodium and decreases in plasma potassium for several hours after feeding. Thus, blood sampling should be avoided

for at least 3 hours after feeding, particularly when a large feed is given or when there is access to substantial amounts of hay.

Exercise

The RBC pool is under the direct influence of catecholamine concentrations, so exercise has a variable effect on RBC indices, depending on the speed and duration of the exercise bout. Horses have large splenic reserves of up to 50% of the RBC volume that is able to greatly influence the exercising blood volume (Persson and Lyndin, 1973). The splenic capacity for RBC storage and subsequent release during exercise is related to the type of horse; draught horses having much lower relative splenic weights than Thoroughbred horses (Kline et al., 1991). Splenic capacity also appears to alter in response to increasing age, since several studies in trotters have found that postexercise HCT and total circulating Hb increase progressively from 1 to 3 years of age (Persson, 1967; 1975b; 1983b).

There is a linear increase in HCT with increasing exercise intensity, up to exercise intensities approaching three-quarter pace (90% to 100% $\dot{V}O_{2max}$) (Rose and Allen, 1985). In adult athletic horses, maximal HCT is usually in the range 0.60 L/L to 0.65 L/L, the mean value being 0.61 L/L in Thoroughbred racehorses. Although most of this increase is related to splenic erythrocyte release, there are also substantial fluid shifts out of the plasma during exercise, and therefore, some of the increase in HCT is from fluid movement (Carlson, 1983).

The increase in oxygen transport capacity associated with the erythrocyte release during exercise is one of the important factors in the horse's high aerobic capacity. However, an upper point must be reached where the improved oxygen-carrying capacity is offset by an increase in blood viscosity, probably accounting for the dramatic reduction in performance in horses with RBC hypervolemia (Persson, 1968). Studies in splenectomized horses have shown a considerable reduction in exercise capacity (Persson and Bergsten, 1975; Persson and Lyndin, 1973). Because of the reduced cardiac output in splenectomized horses, Persson and Bergsten (1975) proposed that the spleen acts as a cardiovascular reserve to maintain ventricular filling at high heart rates.

Effect of Prior Exercise and Diurnal Variation

Exercise obviously has a major impact on RBC indices, with the mobilization of erythrocytes from the spleen under the influence of catecholamines. Following exercise, there is a gradual decrease in circulating erythrocytes over a period of 1 to 2 hours to return to pre-exercise values.

Overtraining

There has been a long association of RBC hypervolemia and poor performance in Swedish Standardbred trotters, with higher-than-normal RBC volumes found in horses that performed poorly (Persson, 1968). Horses diagnosed with RBC hypervolemia had lower earnings and increased race times in the year of diagnosis compared with their previous performance, although, interestingly, many had been superior athletes prior to diagnosis (Persson and Österberg, 1999). Despite the association between RBC hypervolemia and overtraining in Swedish Standardbred trotters, this association has not been repeated in horses elsewhere. In Standardbred horses overtrained in a treadmill study in Australia, there was actually a significant reduction in measured RBC volume and maximal PCV in the overtrained horses compared with the controls (Golland et al., 2003). In these overtrained horses, the reduction in RBC volume was only weakly reflected as small but not significant decreases in PCV, RBC number, and Hb compared with controls (Tyler-McGowan et al., 1999). In another study of overtraining in Standardbred horses in New Zealand, there was decrease in maximal PCV in overtrained horses, but no change in RBC volume (Hamlin et al., 2002).

Erythrocyte Changes with Intense Exercise

Changes may occur in the erythrocytes themselves, there being small decreases in mean corpuscular volume and increases in mean corpuscular Hb and mean corpuscular Hb concentration. Elevations in serum Hb and bilirubin, associated increases in erythrocyte fragility, and the presence of abnormal erythrocytes (echinocytes) have all been reported in the post-race recovery period (Poso et al., 1983). Their presence may be related to both the release of senescent erythrocytes from the splenic pool and the high pressures and turbulence associated with intense exercise (Carlson, 1987). However, evidence suggests that increased fragility of RBCs is associated with decreased blood pH and increased temperature with more intense exercise or exercise stress (Hanzawa et al., 1999). Despite the presence of abnormal erythrocytes, their role in performance has not been established.

LEUCOGRAM

The leucogram is also frequently monitored in racehorses, particular total and differential leucocyte cell count, the latter referring principally to the neutrophil:lymphocyte ratio. Although it has not been linked to performance or fitness, changes in the leucogram may be indicative of subclincial disease or stress. The detection of these potentially performance-limiting problems is clearly of interest to the racehorse trainer. However, resting leucocyte counts only represent the circulating pool of leucocytes. Approximately 50% of the total neutrophils are sequestered in the spleen and capillary beds and are referred to as the *marginated* or *marginal pool*. Marginated neutrophils can be mobilized under certain conditions, including excitement, exercise, stress, transport, and exogenous corticosteroid or catecholamine administration, causing alteration to the leucogram (Carlson, 1987; Snow, 1983). The normal ranges for the Thoroughbred are given in Table 5-3.

TABLE 5–3

Values for the Resting Hemogram in Normal Adult Thoroughbred Horses

Value	Range	Mean
Leukocytes ($\times 10^9$/L)	6.0–11.0	8.5
Neutrophils ($\times 10^9$/L)	2.5–6.5	4.5
Lymphocytes ($\times 10^9$/L)	2.0–5.5	3.5
Monocytes ($\times 10^9$/L)	0.2–0.8	0.5
Eosinophils ($\times 10^9$/L)	0.1–0.4	0.2
Basophils ($\times 10^9$/L)	0–0.3	0.1

FACTORS AFFECTING THE RESTING LEUCOGRAM

Excitement and Intense Exercise (Effect of Catecholamines)

In man and most other animal species, exercise results in physiologic leucocytosis associated with a mobilization of marginated neutrophils to the circulating pool and moderate to marked neutrophilia (Carlson, 1987; Snow, 1983). In the horse, the mobilization of leucocytes from excitement and exercise is masked by the concomitant increase in erythrocytes and blood volume as a result of splenic contraction. Immediately following intense exercise or as a result of prerace excitement, there may be no change in total leucocyte count or only a moderate leucocytosis (Revington, 1983; Snow, 1983; Snow et al., 1983a). However, both prerace excitement and intense exercise (racing or training gallops) have been shown to result in a reduced neutrophil:lymphocyte ratio as relatively more lymphocytes than neutrophils are released into the circulation from the spleen. This altered ratio takes up to 6 hours to return to baseline values (Revington, 1983; Snow et al., 1983a). Allen and Powell (1983) reported that in Thoroughbreds after morning exercise, blood samples collected at 4:00 P.M. had higher leukocyte numbers as well as a higher proportion of neutrophils than samples collected in the morning prior to exercise.

Stress and Moderate Intensity Exercise (Effect of Corticosteroids)

Lower-intensity exercise produces a different change in leucogram with a marked leucocytosis due to neutrophilia, lymphopenia, and eosinopenia (Rose and Allen, 1985). The total leukocyte count increases by 10% to 30%, depending on the intensity and duration of exercise, but the extent of the increase is not as dramatic as for the erythrocyte indices. This is a result of the effects of cortisol associated with exercise, which is correlated with increases in the neutrophil:lymphocyte ratio (Wong et al., 1992). Elevated plasma cortisol concentration results in decreased marginated neutrophils, an increased neutrophil output from bone marrow, and decreased lymphocytes and eosinophils (Snow, 1983). Although the degree of leucocytosis may not vary, there is greater neutrophilia and lymphopenia with increased stress, for example, increased speed or signs of exhaustion that may last for more than 24 hours in prolonged endurance exercise (Rose, 1982). Under conditions of severe stress, such as is found in exhausted Endurance horses, there is not only a greater degree of neutrophilia but also the appearance of band-form neutrophils (Rose, 1982) (see Figure 5-1).

The effects of moderate exercise in a typical racehorse program have been shown to have similar effects, albeit of shorter duration. Trotting exercise for 15 minutes on an inclined treadmill was associated with an increase in total leucocyte count and neutrophils with a decrease in lymphocytes. However, values had returned to normal by 1 hour after exercise (Rose et al., 1983a).

Overtraining

Some veterinarians at the racetrack regard an increase in neutrophil:lymphocyte ratio as an indicator of overtraining or "training off." Although such changes often appear to provide an indication of "stress," reflecting increased plasma cortisol (Rossdale et al., 1982), care must be taken in interpreting these changes because a number of factors such as exercise and time of collection can affect the results.

Eosinopenia has been reported in overtrained horses without systemic illness but not in a control group (Tyler-McGowan et al., 1999). This suggests that eosinophils may be

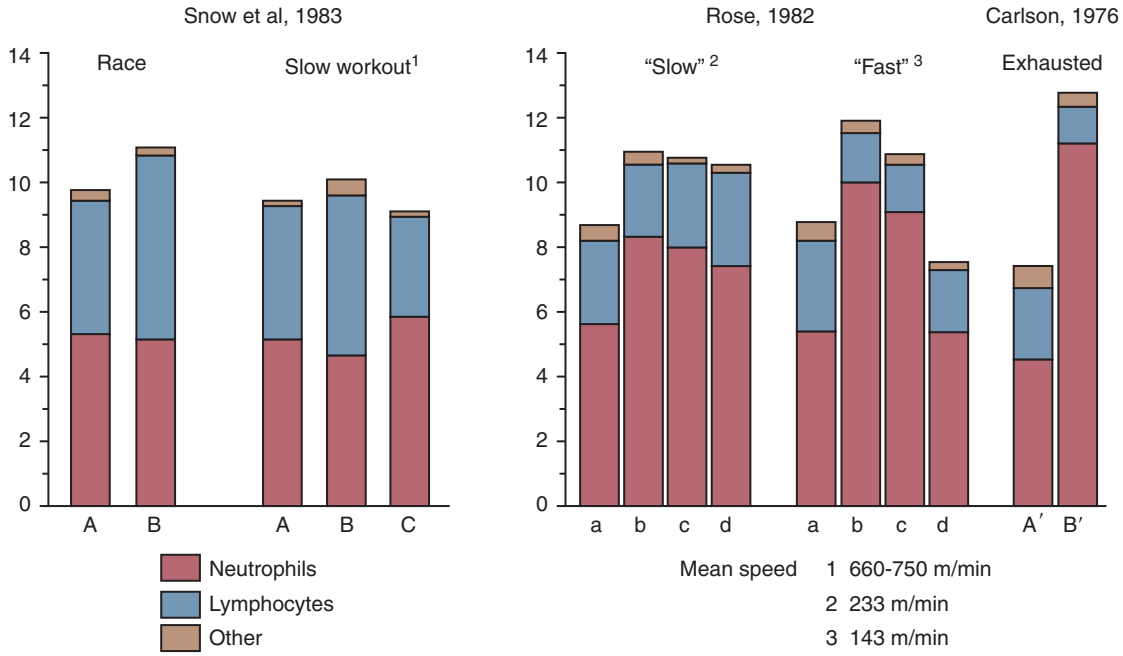

FIGURE 5-1 Changes in leukocyte count after exercise. **1**, Data from Snow and colleagues (1983) in Thoroughbreds: A = before exercise; B = immediately after exercise; e = 3 hours after exercise. **2**, Data from Rose (1982) in Endurance horses: a = before ride; b = immediately after the ride; c = 30 minutes after ride, d = 24 hours after the ride. **3**, Data from Carlson and colleagues (1976) in Endurance horses A' = rest; B' = exhausted horses, after the ride.

a more sensitive indicator of training stress than neutrophils or lymphocytes. In the above study on overtraining, there was a small, gradual increase in the neutrophil:lymphocyte ratio during the 32-week training period for both overtrained and control horses (Tyler-McGowan et al., 1999). However, this was possibly associated with prolonged activity of the cortisol response to exercise as shown by Rose (1982), as horses in this study were trained intensively 6 days a week.

Subclinical Disease

Decreases in neutrophil counts may be an indication of systemic or respiratory disease. More prolonged or purulent inflammatory conditions may result in the opposite effect with neutrophilic leucocytosis (Carlson, 1987). In studies in Hong Kong, horses with equine herpesvirus type 1 infections had monocyte counts higher than 0.5×10^9/L, together with higher neutrophil and lower lymphocyte counts than normal, during the first 1 to 2 days (Mason et al., 1989; 1990). Within the first 4 to 5 days, the neutrophil count decreased and the lymphocytes increased, whereas the monocytes continued to remain elevated. The other notable finding was an increase in plasma viscosity, which, together with changes in monocyte numbers, persisted for several months in some horses following infection with equine herpesvirus.

NORMAL RESTING SERUM OR PLASMA BIOCHEMISTRY

The role of serum biochemistry in assessment of the athletic horse is principally to investigate for subclinical disease that can cause poor performance. Traditionally, this has included markers of fluid and electrolyte balance and renal function, including urea, creatinine, electrolytes, and fractional excretion of electrolytes; protein analysis, including total protein, globulins, and acute phase proteins as markers of inflammation (serum amyloid A and fibrinogen); and muscle and liver enzyme analysis.

A number of measurements are included in the usual biochemical "profile" to determine whether some of the key body systems are dysfunctioning. In this section, we will discuss the most common measurements and the significance of abnormalities for athletic performance. The normal ranges for plasma or serum biochemical indices are given in Table 5-4.

TABLE 5–4

Normal Ranges for Plasma Biochemical Measurements in Mature Performance Horses

Measurement	Normal Range (SI units)	Normal Range (traditional units)
Sodium	134–144 mmol/L	133–144 mEq/L
Potassium	3.2–4.2 mmol/L	3.2–4.2 mEq/L
Chloride	94–104 mmol/L	94–104 mEq/L
Total CO_2	26–34 mmol/L	26–34 mEq/L
Total Protein	55–75 g/L	5.5–7.5 g/dL
Albumin	26–38 g/L	2.6–3.8 g/dL
Globulins	20–35 g/L	2.0–3.5 g/dL
Fibrinogen	<4 g/L	<400 mg/dL
AST (Units/L)	150–400	150–400
CK (Units/L)	100–300	100–300
LDH (Units/L)	<250	<250
Glucose	4–8 mmol/L	70–140 mg/dL
GGT (Units/L)	10–40	10–40
AP (Units/L)	70–210	70–210
Urea	4–8 mmol/L	24–48 mg/dL
Creatinine	100–160 µmol/L	1.1–1.8 mg/dL
Calcium	2.7–3.3 mmol/L	10.8–13.2 mg/dL
Phosphate	0.75–1.25 mmol/L	2.3–3.9 mg/dL
Serum Amyloid A	<2.9 mg/L	<2.9 µg/mL

AP, alkaline phosphatase; *AST*, Aspartate amino transferase; *CK*, Creatine kinase; *CO_2*, carbon dioxide; *GGT*, Gamma -glutamyl transferase; *LDH*, Lactate dehydrogenase.

Electrolytes

Abnormal electrolyte levels in the plasma will adversely affect athletic performance, and there have been some reports that even small deviations from a narrow concentration of serum electrolytes are associated with poor racing performance (Williamson, 1975). However, most electrolyte disturbances are associated with clinical diseases such as diarrhea, renal disease, and electrolyte losses in sweat. Harris and Snow (1991) showed that some horses with recurrent rhabdomyolysis had abnormal creatinine clearance ratios for various electrolytes, although plasma concentrations were within the normal ranges. Thus measurement of clearance ratios for the different electrolytes should be considered in cases of recurrent "tying up."

Sodium

Concentrations are maintained within narrow limits in the plasma, and abnormalities tend to reflect relative water excess (decreased plasma sodium) or relative water deficit (increased plasma sodium) rather than net changes in sodium balance. Sodium values in plasma are affected by the total exchangeable sodium and potassium concentrations as well as the total-body water (Edelman et al., 1958). Under conditions of marginal sodium intake, horses show excellent renal sodium conservation (Tasker, 1967a). However, a study in exercised ponies demonstrated that decreased sweat production and a greater decrease in plasma volume occurred when there was dietary sodium restriction (5 milligram per kilogram [mg/kg]) compared with diets in which sodium was available at 25 mg/kg of body weight (Lindner et al., 1983). Therefore, it is important to ensure that adequate sodium is available in the diets of most athletic horses, and salt licks or electrolyte supplements would seem to be important.

Potassium

Potassium is a critical electrolyte because it is involved in a range of body functions, in particular neuromuscular activity. As a grass eater, the horse has evolved ingesting large amounts of potassium, about two thirds of which is excreted in urine (Tasker, 1967a). Therefore, when a horse is in full training on a high-grain diet, it is possible for potassium deficiencies to develop. Less than 2% of the total-body potassium is contained in the extracellular fluid, and therefore, serum or plasma potassium values may not reflect changes in total-body potassium. For example, in a study by Tasker (1967b), food and water restriction resulted in a total-body potassium loss of 4500 mmol (16% of total exchangeable potassium), but serum potassium decreased to only 3.5 mmol/L. However,

in general, plasma or serum potassium values less than 3 mmol/L indicate decreases in whole-body potassium content. Hyperkalemia is an unusual disorder in athletic horses, and if values greater than 4.5 mmol/L are found, hemolysis or incorrect sample handling should be suspected first before a pathologic disorder is diagnosed. However, in a Quarterhorse, the syndrome of periodic hyperkalemic paralysis, which is a familial disorder now relatively common in the United States, should be at the top of the list of differential diagnoses.

It is important to note that substantial variation can occur in plasma potassium values during the course of a day, particularly in horses that may be fed only twice daily. A study by Clarke and colleagues (1988) showed that 1 hour after a large meal (4 kg), the mean serum potassium values decreased from 3.5 to 2.9 mmol/L, returned to prefeeding values by 4 hours, but at 5 and 7 hours after feeding increased to values of 4.0 and 4.2 mmol/L, respectively. From these results, it is clear that to interpret plasma or serum potassium values correctly, it is important to collect the blood samples at the same time of the day and under the same feeding conditions.

Plasma or serum potassium values may decrease substantially below normal resting values 1 to 2 hours after exercise, and following endurance exercise, they may decrease to values below 3 mmol/L (Rose et al., 1983b).

Chloride

Chloride is the major anion of the extracellular fluid (ECF) and in sweat. Most of the chloride ingested each day, which may reach 3000 mmol on alfalfa diets (Groenendyk et al., 1988), is excreted in urine. Primary alterations in athletic horses result from losses of chloride in sweat, particularly in horses involved in prolonged exercise. Thus, hypochloremia found in a resting blood sample would most likely be from sweat electrolyte losses. Hyperchloremia is seldom found but is most common in situations that produce metabolic acidosis because plasma concentrations of bicarbonate and chloride are inversely related. Large increases in plasma chloride (>110 mmol/L) are most common in cases of renal tubular acidosis (Aleman et al., 2001).

Most laboratories that use autoanalyzers measure *bicarbonate* as *total carbon dioxide* (TCO_2), which, on average, is about 5% higher than actual bicarbonate values because TCO_2 includes dissolved carbon dioxide (CO_2). In general, low TCO_2 values indicate metabolic acidosis, and high values signify metabolic alkalosis. Acid–base disturbances are extremely rare in resting samples from athletic horses. Low TCO_2 values are occasionally found if blood samples are collected within 90 minutes of high-intensity exercise because of elevated lactate concentrations. High TCO_2 values are mostly found as a result of excessive feeding of sodium bicarbonate or because of administration of bicarbonate as a "milkshake" to horses prior to racing (Rose and Lloyd, 1992). Metabolic alkalosis can sometimes be found in Endurance horses following extensive sweat losses in response to the depletion of extracellular chloride.

Calcium and Phosphate

Calcium and phosphate are maintained within a very narrow range, and normal plasma or serum concentrations tend to be maintained, even in the face of severe dietary calcium–phosphorus imbalances. Calcium is also lost in sweat, although the extent of the losses is much less than the other major electrolytes. Changes in these electrolytes are found mostly in cases of intestinal or renal disease.

Muscle-Derived Enzymes

The most common enzymes that are used to indicate muscle damage are aspartate amino transferase (AST) and creatine kinase (CK). In addition, lactate dehydrogenase (LDH), which is a commonly available enzyme measurement on autoanalyzers, also increases following muscle damage, although it is less specific than AST or CK. Changes in CK are more rapid than those in AST, where the long half life in plasma may lead to values being increased for several weeks after a single bout of muscle damage. In contrast, CK values may decrease quickly over a period of 6 to 48 hours (Cardinet et al., 1967). Although it has been generally assumed that increases in CK and AST indicate muscle damage because elevations in these enzymes are found in horses with rhabdomyolysis, some studies have suggested that the increases may be related to the exercise load.

Serum or plasma muscle enzyme activities have been shown to increase following racing in galloping and trotting races (Poso et al., 1983; Snow et al., 1983b). Despite the increases, values still remained within normal limits, and generally, there is only a 50% increase in plasma enzyme activity, partly due to a 10% to 20% decrease in plasma volume (Snow and Harris, 1988). Consistent changes in muscle enzyme activity with training have not been observed. Some elevations during monitoring of horses over prolonged periods of training have been observed but, when examined closely, were found to represent individuals with muscle damage or periods of increased training intensity (Robertson et al., 1996; Snow and Harris, 1988).

Muscle enzyme increases following submaximal exercise have not been observed. Trotting exercise for 15 minutes on an inclined treadmill was not associated with increased muscle enzymes CK or AST during or up to 1 hour after exercise or associated with 7 weeks of training (Rose et al., 1983a). Similarly, Standardbred training protocols on a track, including jogging and fast work, did not result in increased CK or AST immediately following exercise or following a period of 9 weeks training (Milne et al., 1976). Part of the lack of response to exercise in submaximal studies may have been the amount of exercise performed, as moderate increases in CK and AST have been observed following endurance exercise (Grosskopf et al., 1983; Kerr and Snow, 1983a). Interestingly, and possibly more importantly, the *individual* horses identified in each of the studies had very high increases associated with suspected pathologic muscle damage (see below).

Overtraining and Subclinical Muscle Damage

Overtraining has been shown to result in significant increases in mean AST activity in overtrained horses compared with controls (Tyler-McGowan et al., 1999). Overtraining in humans has also been associated with muscle overload, eccentric muscle damage, and delayed onset muscle soreness (Fry et al., 1991). However, similar effects are also seen with intense training without overtraining indicating that the effects of overtraining and subclinical muscle damage are hard to separate. In the overtraining study in horses, it appears that some of the increase in muscle enzymes was also associated with muscle damage with three cases of clinically evident gluteal muscle injury and four cases of subclinical muscle damage in overtrained horse during the latter part of their training program, whereas none were observed in the controls (Tyler-McGowan et al., 1999). This study was treadmill based, with horses exercising daily on a 10% inclined treadmill; some of the damage could be associated with exercising on a slope, as

this effect was not seen in another study of overtraining in horses trained on a track (Hamlin et al., 2002). However, decreased coordination and fatigue associated with overtraining (Fry et al., 1991) may have played a part in the susceptibility to injury in this group, since the injury or increases in CK and AST were not seen in the control horses exercising in the same inclined treadmill environment.

Muscle Disease Recurrent Rhabdomyolysis and Delayed Onset Muscle Soreness

Subclinical or clinical muscle disease may also occur in racehorses during training from recurrent exertional rhabdomyolysis (RER), whereas in Quarterhorses and other breeds, such disease may be caused by polysaccharide storage myopathy (see also Chapter 12). Most of the affected horses show clinical signs of recurrent rhabdomyolysis episodes, although clinical signs can be mild or subclinical and difficult to detect without muscle enzyme activity analysis. It is a common cause of poor performance and lost training days, affecting up to 6% to 7% of racing Thoroughbreds, with 2-year-old fillies most likely to be affected (MacLeay et al., 1999; McGowan et al., 2002; Snow and Harris, 1988; Upjohn et al., 2005).

Elevations in muscle enzyme activity may also occur as a result of other causes of muscle damage, including viral myositis associated with viral respiratory disease, and muscle injury (Snow and Harris, 1988). Less reported in horses, and probably underdiagnosed is delayed onset muscle soreness (DOMS). This is highly prevalent in human athletes (and nonathletes) following intense or unaccustomed exercise (Kuipers, 1994; MacIntyre et al., 1995). There have been reported unexplained elevations of muscle enzymes of horses starting to be trained for the first time that was hypothesized to be due to DOMS (Kirby and McGowan, 2004); and on closer scrutiny of some of the long-term training studies, there may be evidence of DOMS occurring with seasonal increases in muscle enzymes associated with the beginning of training or racing season (Mullen et al., 1979; Snow and Harris, 1988).

Although genetic markers of some forms of muscle disease are available, accurate diagnosis of the cause of subclinical elevations of muscle enzymes may still be difficult in many cases. However, horses with elevated muscle enzymes are unlikely to perform optimally, and screening for elevations following exercise will be valuable in the detection of poor performance (Snow and Harris, 1988).

Liver Enzymes

The most commonly measured indicator of liver dysfunction is gamma-glutamyl transferase (GGT), although both alkaline phosphatase (AP) and sorbitol dehydrogenase (SDH) are also useful measurements when liver disease is suspected. In the acute stages of hepatocellular dysfunction as well as in biliary tract obstruction, liver enzymes will become elevated, AP showing the greatest elevation when there is biliary obstruction. Liver disease is uncommon in athletic horses but can be a cause of weight loss and poor performance in areas where horses have access to plants with high concentrations of pyrrolizidine alkaloids. Unlike muscle enzymes, few effects of intense or submaximal exercise are seen on liver enzymes. However, bilirubin has been reported to increases postrace in horses, possibly associated with RBC breakdown as mentioned above (Hanzawa et al., 1999; Poso et al., 1983).

Protein Measurements

Measurement of total protein, albumin, globulins, serum amyloid A, and fibrinogen provide an index of hydration status, as well as indices of infection, inflammation, increased protein loss, or decreased protein production. Hyperproteinemia usually is the result of dehydration in athletic horses, but because of the large normal range (see Table 5-4), it may be difficult to detect protein increases in horses that have normal protein values in the range 55 to 60 g/L. During submaximal and maximal exercise, there is an increase in total plasma protein and plasma albumin concentration as a result of intercompartmental fluid shifts, with greater increases associated with maximal exercise (Hargreaves et al., 1999; Judson et al., 1983). Although these increases in plasma protein associated with short duration exercise generally return to baseline by 30 minutes after exercise, with more prolonged endurance exercise or with excessive sweating, these fluids shifts may be more substantial and prolonged. Therefore, evaluation of total serum protein and serum albumin is important in determining hydration status in horses and may be particularly important during the recovery period after intense exercise or in hot conditions.

It is important to remember that a high plasma protein concentration also may be caused by elevations in globulins or fibrinogen. Hypoproteinemia is uncommon in athletic horses, and if it occurs, horses should be investigated by seeking possible sites of protein loss (gastrointestinal tract, kidney), which is a much more common cause of hypoproteinemia than decreased protein production.

Acute-phase proteins are more important as markers of inflammation and can be used to support evidence in the leucogram of subclinical or clinical disease that may affect performance. Fibrinogen is a sensitive index of inflammatory foci within the body and is a useful screening measurement in any athletic horse with poor performance to ensure that a low-grade infection is not the cause of the problem. Serum amyloid A is considered a more sensitive acute-phase protein and may be a more sensitive marker of subclinical inflammation or even repetitive microtrauma that may play a role in overtraining syndromes. Small, but significant increases have been detected post intense exercise in horses (Ducharme et al., 2009).

Measurements of Renal Function

Creatinine and urea are indices of renal function, but both measurements can be increased in response to prerenal factors, particularly dehydration and exercise. Alterations in resting levels of creatinine and urea are unusual in athletic horses, and some changes occur with training, there being modest increases in plasma or serum urea concentrations (1 to 2 mmol/L), probably as a result of increases in dietary protein.

CHANGES IN PLASMA OR SERUM BIOCHEMICAL VALUES ASSOCIATED WITH EXERCISE

ELECTROLYTES AND ACID–BASE STATUS

Maximal Exercise

With brief periods of high-intensity exercise, there are transient changes in plasma electrolyte concentrations, some of which may be quite marked. Following racing over distances varying from 1200 to 2400 meters (m), similar changes occur in electrolyte and acid–base status. Sodium increases largely as a result of fluid movement out of the extracellular space. Potassium increases dramatically at high exercise loads, reaching

values greater than 10 mmol/L (Harris and Snow, 1988; 1992). This increase in potassium has been thought to be mainly from an accumulation of hydrogen ions in active muscle, resulting in a decrease in the reuptake by the fibers and an impairment of the sodium–potassium–adenosine triphosphatase (Na^+- K^+ -ATPase) pump. However, the increased plasma potassium is transient, and most studies where samples have been collected several minutes after maximal exercise have shown potassium values within the normal resting range (Judson et al., 1983; Keenan, 1979; Snow et al., 1983b). Nonetheless, it has been proposed that the decreased muscle membrane potential resulting from the decreased intracellular potassium concentration, together with the increased extracellular concentration, may be a contributing factor to fatigue during high-intensity exercise (Sahlin and Broberg, 1989).

A consistent finding in most of the studies of high-intensity exercise is that despite the increase in total protein and plasma sodium, reflecting fluid movement out of the extracellular fluid, no change occurs in chloride values (Judson et al., 1983; Keenan, 1979; Snow et al., 1983b). This may be caused by large increases in lactate, another strong anion, with exchange of chloride across muscle cell membranes.

Bicarbonate or total carbon dioxide concentrations decrease following racing to the range 5 to 10 mmol/L. This decrease in bicarbonate is coincident with a large increase in hydrogen ion concentration, the venous blood pH decreasing to values less than 7.0 (Snow et al., 1983b). However, lactate metabolism after exercise results in a rapid increase in pH and bicarbonate, with values returning to within the normal range by 90 minutes after exercise.

Prolonged Low-Intensity Exercise

Endurance exercise results in substantial sweat losses of electrolytes because horse sweat is hypertonic relative to plasma (Carlson and Ocen, 1979; Kerr and Snow, 1983b). During the course of an endurance ride in hot conditions, it is likely that horses may lose between 5% and 10% of their body weight (Rose, 1986). The sweat electrolyte losses result in variable changes in plasma or serum electrolyte concentrations because of the associated alterations in total body water. Plasma or serum electrolyte changes are known to be influenced by alterations in exchangeable sodium and potassium as well as total-body water. Plasma sodium has been reported to be unchanged, decreased, or increased, depending on the conditions and duration of the endurance ride (Carlson and Mansmann, 1974; Lucke and Hall, 1978; 1980a; 1980b; Rose et al., 1977; Snow et al., 1982). In one study, despite an estimated 5900-mmol loss of sodium, chiefly in sweat, plasma sodium concentrations increased from 140 to 148 mmol/L (Snow et al., 1982). Moderate decreases in plasma or serum potassium have been reported following endurance exercise, although small increases also have been found (Carlson and Mansmann, 1974; Deldar et al., 1982; Lucke and Hall 1978; 1980a, 1980b; Rose et al., 1977; Snow et al., 1982). Despite the modest changes in plasma or serum potassium values, decreases in whole-body potassium content are likely to occur when there are substantial sweat losses, although the falls are probably only 25% to 50% of the decline in total exchangeable sodium. Chloride losses during endurance exercise may be substantial because it is the principal anion lost in the sweat. During endurance exercise, most investigators have reported a decrease in plasma or serum chloride values, which, in some cases, may result in decreases of 10 to 15 mmol/L (Rose et al., 1980a). Because of the substantial chloride losses, there may be renal retention of bicarbonate, giving rise to metabolic alkalosis (Rose et al., 1979). The maximal increase in bicarbonate concentration usually is in the range 3 to 5 mmol/L. In other cases, no change in acid–base status or a slight metabolic acidosis occurs, resulting from small increases in plasma lactate (Lucke and Hall, 1978). These studies led to the conclusion that administration of bicarbonate, either as a treatment or as a feed supplement, was contraindicated in endurance horses.

Speed and Endurance Phase (Day 2) of a 3-Day Event

Although considerably shortened since 2004 (see Chapter 23), during the speed and endurance phases of long format 3-day events, horses exercised over distances of around 23 to 25 km at speeds varying from 200 to 700 m/min. The electrolyte alterations found were similar to those during endurance exercise, there being variable sweat losses of fluid and electrolytes (Rose et al. 1980b). Acid–base changes reflect the different intensities of exercise during a 3-day event, there being a slight metabolic alkalosis after the second roads-and-track phases (Rose et al. 1980c). At the conclusion of the cross-country, horses had a mean base deficit of 8.5 mmol/liter due to lactic acidosis (Rose et al. 1980c).

RENAL RESPONSES TO EXERCISE

Increases in both urea and creatinine are found in response to high- and low-intensity exercise (Judson et al., 1983; Lucke and Hall, 1980c; Rose et al., 1977; 1983a; 1983b). These traditional indices of renal function are also affected by prerenal factors such as hemoconcentration. Additionally, creatinine increases during exercise as a result of increased phosphocreatine turnover, and therefore, increases in plasma or serum creatinine cannot be used as an indication of reduced glomerular filtration rate. After maximal exercise, creatinine concentrations remain elevated for 60 minutes, even though plasma total protein concentrations return to normal (Judson et al., 1983). For more information on renal responses to exercise, see Chapter 6.

BLOOD LACTATE AND GLUCOSE CHANGES WITH EXERCISE

Lactate increases in plasma or blood because of diffusion, active transport from active skeletal muscle, or both. Lactate is usually measured in plasma, and concentrations are about 40% to 50% higher than those in blood, although the relationship is quite variable. During all types of exercise, lactate is produced in working muscle, but high lactate concentrations do not occur until higher exercise intensities are reached. Increases in lactate occur in muscle when there is insufficient oxygen available to oxidize pyruvate in the mitochondria. To free nicotinamide-adenine-dinucleotide ($NADH_2$) of its hydrogen, pyruvate can accept the H_2 to form lactate. Lactate increases also occur when there is stimulation of glycogenolysis, with the result that an increase in pyruvate causes a rise in lactate because of a mass-action effect (Gollnick and Saltin, 1982). Thus, plasma lactate increases do not necessarily signify a lack of oxygen availability.

With maximal exercise, such as Thoroughbred racing, there is substantial lactate production. Over distances of 1200 to 2400 m, there is little difference in plasma lactate concentrations after racing, values ranging from 25 to 30 mmol/L (Snow et al., 1983b). Values after trotting races are usually a

little lower (Krzywanek, 1975). In contrast, endurance riding results in little increase in plasma lactate concentrations, values usually being less than 2 mmol/L (Grosskopf et al., 1983; Lucke and Hall, 1978; 1980a; 1980b). After the cross-country phase of a long format 3-day event, blood lactate values of 8 mmol/L were found (Rose et al., 1980c). However, the lactate concentrations after the second roads-and-track phase were only 2 mmol/L, indicating that aerobic metabolism was predominant for most of the exercise on day 2 of long format 3-day events.

Plasma glucose generally increases with all forms of exercise because of stimulation of hepatic glycogenolysis. However, with prolonged exercise, glucose concentrations will decrease as a result of liver glycogen depletion (Rose et al., 1977). After short-term exercise, the degree of increase in plasma glucose concentrations is related to the intensity of exercise, with peak values in the range of 10 to 12 mmol/L (180 to 206 mg/dL) found after racing (Judson et al., 1983; Snow et al., 1983b). The extent of the increase in plasma glucose concentration is probably related to the degree of sympathetic activity, which is related to the intensity of exercise.

HEMATOLOGIC AND BIOCHEMICAL CHANGES ASSOCIATED WITH TRAINING

HEMATOLOGIC CHANGES WITH TRAINING

Racehorse Training

Training results in an increase in the total erythrocyte pool. It has been shown in longitudinal studies in Standardbred horses that training produces an increase in red cell volume (Persson, 1968) and the routine hemogram (Tyler-McGowan et al., 1999). Persson (1975a) estimated that the total hemoglobin, measured using Evans blue dye dilution, increases by 30% in 2-year-old trotters during the training period prior to their first race. Although some of this increase may be an age effect, it is clear that training produces an increase in the total-body capacity for oxygen carriage. The increase in total hemoglobin is not necessarily reflected in increases in resting RBC indices.

In Thoroughbreds, training has been reported to result in increases in the resting HCT, Hb, and RBC count (Allen and Powell, 1983; Stewart et al., 1970). However, this effect is more likely to be seen in horses that have had a significant period of detraining or rest prior to starting exercise. In the study by Tyler-McGowan et al. (1999), the increases in RBC volume were apparent from week 1 to week 7, but there were no further increases throughout 32 weeks of training. Similarly, Clarkson (1968) found that differences in the training response of the hemogram were related to the HCT values prior to the beginning of training. Horses with initial HCT values less than 0.40 L/L had significant increases in RBC variables following training, whereas those with HCT values less than 0.40 L/L prior to training had mean resting HCT increases from 0.36 to 0.43 L/L. Mainly, the increases in RBC indices are modest, there being a mean increase in HCT of between 0.04 and 0.06 L/L in most studies. This increase is similar to the daily variation that can be found on repeated sampling, and therefore, the physiologic significance of the finding is questionable. The temperament of the horse may be one factor responsible for the increase in RBC variables because horses often become more excitable as training progresses. Thus, while small increases in resting Hb, HCT, and erythrocyte numbers may occur during training, such changes are too small to be used reliably as an index of increasing fitness.

There are few changes in the leukocytes during racehorse training. The total and differential leukocyte counts are similar before and after training in Thoroughbreds (Allen and Powell, 1983). In a study in which neutrophil and lymphocyte counts were performed before and 4 hours after a standardized exercise test, there was no change as a result of training.

Endurance Training

Endurance horses have lower resting RBC indices than Thoroughbred racehorses. There may be a reduction in HCT with training in endurance horses because of an expansion of plasma volume. McKeever and colleagues (1987) reported an expansion of plasma volume by about 25% after as little as 1 week of training. However, a study by Rose and Hodgson (1982) reported no significant changes in hematology during 12 weeks of endurance training. Neither RBC indices nor the total or differential leukocyte count changed when samples were collected every 2 weeks. Undoubtedly, changes in plasma volume do occur, but these must be masked by similar changes in RBC mass.

Similarly to racehorse training, the total leukocyte count is unchanged with training, and there is no alteration in the proportions of neutrophils, lymphocytes, and monocytes. It is clear that alterations in the proportions of leukocytes indicate little about the stage of fitness.

CHANGES IN PLASMA OR SERUM BIOCHEMICAL VALUES ASSOCIATED WITH TRAINING

There are few changes in resting biochemical values as a result of training. Although some studies have found statistically significant changes in some plasma biochemical measurements, most of these changes are small and of little biologic significance (Milne et al., 1976).

Training has a significant effect on indicators of liver function. In a prospective long-term treadmill training study, serum GGT and total bilirubin both showed an increase with training, whereas alkaline phosphatase (ALP) decreased with training (Tyler-McGowan et al., 1999). Earlier field based studies had also reported increases in total bilirubin and GGT following a period of prolonged training. Allen and Powell (1983) showed increased total bilirubin following a 20-week training period in Thoroughbreds and surmised that this was from reduced caloric intake rather than erythrocyte destruction, as there was no concurrent increase in serum haptoglobin concentration.

Snow and colleagues (1987) found increased serum GGT associated with training. These results and those of a later study (Snow and Harris, 1988) in Thoroughbreds show a linear increase in serum GGT activity with training. Similar findings have been reported in Standardbred horses undergoing prolonged training and overtraining, where prolonged training also resulted in a linear increase in GGT, with this increase being greater in overtrained horses (Tyler-McGowan et al., 1999). In Standardbred horses trained intensely on a treadmill, GGT increased from a mean value of 14 units per liter (U/L) prior to training (after 4 months detraining) to a mean of 51 U/L in the control horses (see Figure 5-2), and 70 U/L in overtrained horses after 32 weeks of training. Similar-magnitude increases have also been observed in the studies on

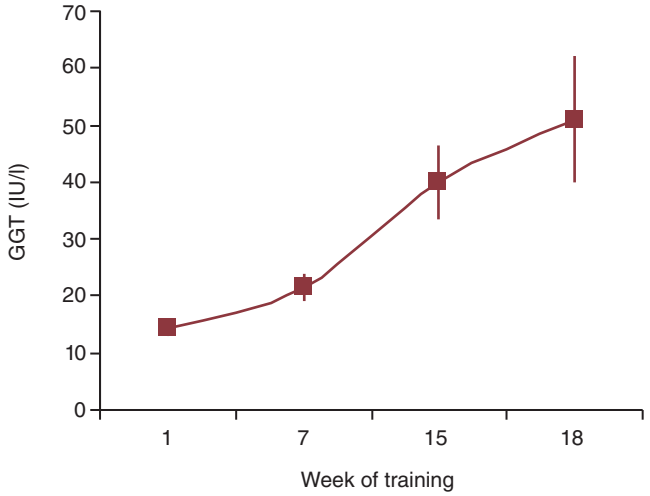

FIGURE 5-2 Changes in gamma-glutamyl transferase (GGT) with training in 13 young Standardbred racehorses undergoing a 22-week treadmill training program from the detrained state. Values are at week 0 (prior to training), week 7 (after 7 weeks of endurance exercise), and weeks 15 and 22 during high intensity training.

Thoroughbreds, although there was a significant effect of training yard (Snow and Harris, 1988).

It appears that basal serum GGT may be one of the few indicators of training in racehorses. Further, detection of very high values may be of importance in the detection of poor performance. In one yard, very high values of GGT were associated with poor performance, with some horses with poor performance having very high values of 100 U/L or more (Snow et al., 1987). Whether these high values are associated with overtraining or subclinical liver disease or other illness would warrant investigation. However, it is of interest that the horses in the stable with high GGT values under investigation showed a markedly reduced incidence of increased GGT following reduction in training intensity in subsequent years (Snow and Harris, 1988).

Resting acid–base and electrolyte status does not change with training status, although postexercise plasma lactate levels decrease following training in response to a standardized submaximal exercise test. Measurement of plasma or whole-blood lactate concentration following such a test is the most reliable biochemical indicator of increasing fitness, although exercise testing on a treadmill is necessary for repeatable results, with measurement of derived indices such as the speed at which lactate reaches a value of 4 mmol/L (V_{LA4}). The V_{LA4}, also called the *point of onset of blood lactate accumulation,* increases with increasing fitness and is a useful objective index of improvements in fitness. The use of lactate measurements in the assessment of fitness is discussed further in Chapter 29.

CONCLUSION

Hematology and plasma or serum biochemical measurements are of vital importance in the assessment of the athletic horse. Blood sampling is simple and relatively inexpensive while providing information about the function of a number of body systems. However, because the physiologic state of the horse can influence many of the measurements, care must be taken in interpretation. Additionally, repeatable results from resting RBC indices are difficult to achieve, although postexercise results are quite consistent. In horses with suspected anemia, sampling of blood following fast exercise would help in determining the significance of resting RBC indices.

Simple guides to the state of fitness and performance capacity cannot be achieved by the use of blood and plasma or serum measurements. However, routine monitoring of hematology and biochemistry during training may provide a mechanism for determining minor disturbances in an individual horse, where the normal range is much narrower than for the general population of horses.

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CHAPTER

Physiology of Acid–Base Balance and Fluid Shifts with Exercise

6

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Food of any specific nature provides the energy needed by any specific species to perform work. This work may be expressed as whole body movement, as the function(s) of individual physiologic systems within the body, or as processes inside a single cell. Regardless of the physiologic "level" at which work is performed, the major byproduct of the transduction of potential energy (food) into kinetic energy (work) is heat (see Chapter 8). Moreover, whether it is intracellular or total body work, the more work that is accomplished in a given period, the more heat is produced (Rowell, 1983; Rowell, 1993).

Normal cellular function requires that temperature be maintained within relatively narrow limits. Temperatures below or above the normal physiologic range can dramatically affect a cell's ability to function and can even lead to cell death. Therefore, every species has its own active and efficient method to maintain core body temperature, the primary concern being the removal of heat produced during work.

The survival of the horse is dependent on its ability to eliminate body heat primarily through the evaporation of sweat (Carlson, 1983; Carlson, 1987; Schott and Hinchcliff, 1993; see Chapter 8). Core temperature in the horse can rise significantly in a matter of minutes. The mere digestion process can raise core temperature in the horse. In the exercising horse, core temperature can exceed 42°C in a matter of minutes. Intracellular heat generated in the horse must be moved to the periphery, where it can be dissipated to the surrounding air. Horses and humans are the only species with physiologic mechanisms in place to allow this process (sweating).

Although sweating, in general, provides an efficient means of heat loss, it can also lead to significant fluid and electrolyte losses (Greenleaf and Morimoto, 1996). The magnitude of these losses is particularly great during exercise of long duration, exercise performed in environments of high temperature and humidity, or both. If uncompensated for, the loss of fluid and electrolytes can lead to thermoregulatory as well as cardiovascular instability (Rowell, 1983; Rowell, 1993) and ultimately death (Geor and McCutcheon, 1998; McConaghy, 1994; McKeever, 1997; McKeever, 2008; Schott and Hinchcliff, 1993). Therefore, an understanding of thermoregulatory mechanisms, as well as appropriate fluid and electrolyte ingestion, is imperative for anyone involved in training equine athletes. This chapter provides a review of the current literature on exercise, fluid balance, and renal function in the horse.

BODY FLUID COMPARTMENTS

As with all animals, the body of the horse is primarily composed of water. The total volume of water in the body is compartmentalized in two areas: (1) inside the cells (intracellular) and (2) outside the cells (extracellular). Carlson (1983; 1987) has done an exceptional job of reviewing these fluid compartments in the horse. Total body water (TBW) is typically estimated to be 50% to 70% of body weight. In a 500-kg horse, this translates into 250 kg to 350 kg of water weight (Carlson 1983; Carlson 1987; McKeever, 1998; McKeever, 2008). Direct measures of TBW may be obtained using various indicator dilution techniques, stable isotope techniques, and bioelectric impedance technologies (Carlson 1983; Carlson 1987; McKeever, 2008).

Cell membranes provide a structural division of TBW into the intracellular fluid (ICF) compartment and the extracellular fluid (ECF) compartment (Carlson 1983; Carlson 1987; McKeever, 2008; Schott and Hinchcliff, 1993). Approximately two thirds (~200 L) of TBW in the horse is ICF. The remaining third (~100 L) resides in the ECF space. According to Carlson (1987), ECF is further compartmentalized into fluid contained within the vascular space, the ICF space, the lymphatics, and transcellular fluids. This last category includes the fluid content of the gastrointestinal tract, which of itself, represents a large reservoir of water (Carlson, 1987).

The fluid occupying the vascular space is known as *total blood volume (BV)*. The actual measure of BV not only includes fluid volume (plasma volume [PV]) but red blood cell volume (RCV) as well. Thus: BV = PV + RCV (Carlson, 1987). In the horse, resting BV varies from breed to breed, ranging from 61 milliliter per kilogram (mL/kg) in draft horses to 137 mL/kg in race horses (Carlson, 1987; McKeever, 1987; Persson, 1967). It is also affected by age, body composition, hydration status, and training status. In an average 500 kg horse at rest with a PCV of 0.40l/L, total BV would be about 40 L, PV about 24 L, and RCV around 16 L (Carlson, 1987; McKeever, 1987; Persson, 1967).

Aerobic performance in the horse is greatly affected by BV. Oxygen delivery to, and uptake by, muscles is dependent on both the PV and RCV components of BV. Optimal PV is needed to ensure proper cardiac filling pressure (preload), and an optimal number of red blood cells (RBCs) must be in place to ensure proper arterial oxygen partial pressure (pO_2). The elegant work of Persson (1967), as well as that of many others (McKeever et al., 1987; McKeever et al., 1993a; Rose and Hodgson, 1994), has shown that there is a strong relationship between RCV and aerobic performance in the horse. However, the interdependence of RCV with PV should not be overlooked. Blood flow can be affected by changes in viscosity. Thus, if RCV is high, PV is low, or both, the resulting increase in viscosity will slow blood flow and the delivery of oxygen to the working muscles.

In the literature, values obtained for BV, PV, and RCV vary with the differing methodologies used to measure and calculate total blood volume. For the most part, studies in the horse have not relied on the direct measurement of BV. Instead, they have generally used dye or indicator dilution techniques to measure PV. The value for PV may then be used along with the hematocrit (HCT) value to estimate BV using the following formula (Formula 1) (Carlson, 1987; McKeever et al., 1987; McKeever et al., 1988; McKeever et al., 1993a; Rose and Hodgson, 1994;).

Formula 1: $BV = (PV/100 - HCT) \times 100$

Dye or indicator dilution techniques require the use of an indicator able to stay within the vascular compartment long enough to reach full steady state distribution without substantial removal by tissues. Ideally, this requires an indicator that binds to a large molecule that does not readily diffuse or transport out of the blood. Two substances commonly used to measure PV in the horse include Indocyanine Green dye (otherwise known as IC-Green or Cardiac green) and Evans Blue dye (Carlson, 1987). Caution must be exercised in the use of IC-Green to measure PV because of its rapid clearance (half life) from the vascular compartment. It is better suited for the repeated injections required during measurement of cardiac output. Conversely, Evans Blue dye binds to albumin and, thus, has a relatively long half life in the vascular compartment. However, albumin can shift out of the vascular compartment if there is an increase in hydrostatic pressure (Figure 6-1 and Figure 6-2). Therefore, measurement of PV using Evans Blue dye requires the horse to be standing quietly, unperturbed by exercise or pharmacologic manipulations during blood sampling (Carlson, 1987; McKeever et al., 1987; McKeever et al., 1988; Rose and Hodgson, 1994).

Anything that disturbs the steady state of the cardiovascular system will affect the distribution of Evans Blue dye. Nonsteady state sampling, particularly in studies employing exercise in their methods, has the potential to cause significant error in the measurement of PV and the resultant calculation of BV and RCV. Therefore, the results from studies reporting BV, PV, and RCV values calculated from postexercise injection samples should be viewed with caution. Injecting the marker after exercise may skew the data in two ways. First, due to increases in hydrostatic pressure, plasma volume can decrease 15% to 20% (see Figure 6-1) after only three 1-minute stages of an incremental exercise test (McKeever et al., 1993a; McKeever, 2004). Such a decrease would effect an artificially high concentration of the dye. Secondly, the same increases in hydrostatic pressure can cause the loss of dye from the vascular compartment due to exercise-induced extrusion of albumin (Carlson, 1987; McKeever et al., 1987; McKeever et al., 1988; McKeever et al., 1993a, 1993b).

Another factor that must be considered in the calculation of BV in the horse is the splenic reserve volume. Most species retain a portion of their BV in the spleen. However, the spleen in the horse is a very capacious and capricious organ. When the horse is at rest, its spleen stores between 6 L and 12 L of blood (16% – 30% of BV) (Carlson, 1987; McKeever et al., 1993b; McKeever and Hinchcliff, 1995; Persson, 1967). In addition, this splenic blood has a typical hematocrit of about 70 to 80 (Carlson, 1987; Persson, 1967) compared with blood in the vascular tree with an HCT of 35 to 40. Thus, the calculation of BV in the horse becomes somewhat problematic.

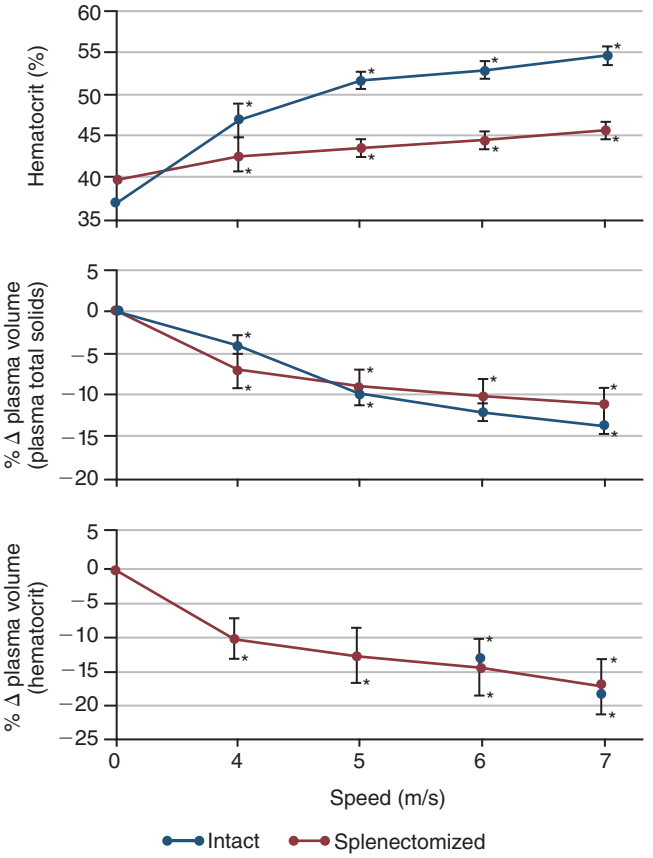

FIGURE 6-1 Changes in hematocrit and plasma volume in the horse during incremental exercise. The second graph depicts the percent change in plasma volume calculated using plasma protein concentration (i.e., total solids). The third graph depicts the percent change in plasma volume calculated using hematocrit and the formula in Box 6-1. *(Adapted from McKeever KH, Hinchcliff KW, Reed SM, et al: Role of decreased plasma volume in hematocrit alterations during incremental treadmill exercise in horses,* Am J Physiol *265:R404, 1993a.)*

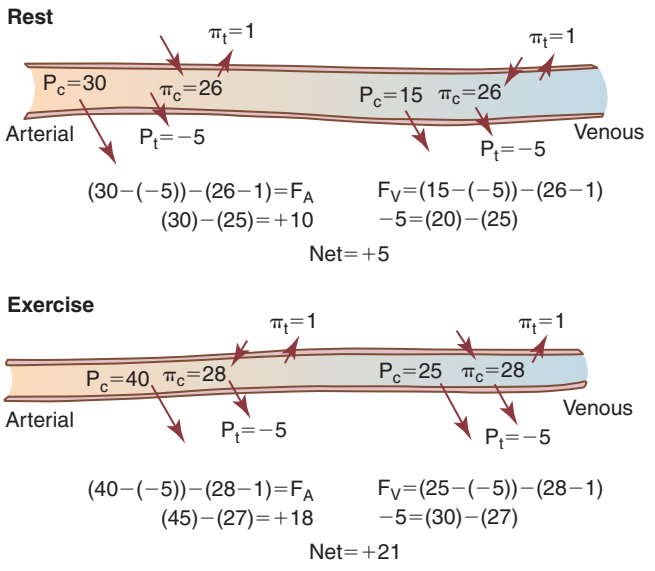

FIGURE 6-2 Starling forces affecting the movement of fluid in and out of the vascular compartment.

To account for the splenic reserve volume, most studies, to date, have used exercise or infusion of adrenaline or an alpha-agonist to cause splenic contraction and thus mobilization of the splenic reserve volume. Once mobilized, accommodation and mixing of this extra volume of blood (RBCs) takes only 1 to 2 minutes (Persson, 1967; Rose and Hodgson, 1994). However, the confounding factor in many studies is the change in hydrostatic pressure resulting from exercise or pharmaceutical manipulation. Although these are accepted viable methods to induce splenic contraction and *estimate* the contribution of the splenic reserve to the total circulating blood volume, if hematocrit samples are drawn after exercise, their values will be skewed upward due to the dynamic fluid shifts that occur with exercise (Persson, 1967; Rose and Hodgson, 1994). Therefore, the hematocrit value used to calculate total BV would reflect both the contribution of splenic reserve mobilization *and* reductions in plasma volume, resulting in an overestimation of BV. This is essentially an offset error that can be controlled for in a study's methodology. Reductions in PV caused by exercise-induced fluid shifts (see Figure 6-1) are linked to exercise intensity (Carlson, 1987; McKeever et al., 1993a; McKeever et al., 1993b; McKeever and Hinchcliff, 1995). The comparison of HCTs only becomes a problem if a study's experimental design employs different exercise intensities. For example, calculating BV using an HCT value obtained at the 10 meters per second (m/s) step of a treadmill test will yield a different result from hematocrit collected at the 11 or 12 m/s step. Therefore, comparisons of BV between treatment groups, or before and after training, must be based on HCT drawn following exercise of the same intensity.

Resting PV can be determined by using Evans Blue dye. However, measurement of PV in the horse during *exercise* is problematic because of mixing time, the requirement for steady state conditions, and the potential for overwhelming the vascular space with dye through repeated injections. Percent changes in PV can be calculated using changes in protein (albumin) concentration (McKeever et al., 1993a). However, because some protein leaves the vascular compartment during exercise, this method tends to underestimate the reduction in PV (McKeever et al., 1993a).

Studies with human athletes have addressed this problem by using changes in HCT rather than albumin to calculate changes in PV. This method is feasible because RBCs do not leave the vascular compartment like protein molecules do, and any change in their concentration must be due to changes in plasma volume (Dill and Costill, 1974; Harrison, 1985; Van Beaumont et al., 1972). For example, in humans, if the pre-exercise hematocrit (HCTb) is 43 and the postexercise hematocrit (HCTa) is 45, then the change in PV calculated using the formula in Box 6-1 is –7.9%. Absolute volume changes in liters are then calculated using the absolute pre-exercise resting PV determined using Evans Blue dye (Dill and Costill, 1974; Harrison, 1985; Van Beaumont et al., 1972). However, it is important to note that the use of this formula requires that there be no addition of RBCs to the central circulation or change in the size of the cells (Dill and Costill, 1974; Harrison, 1985; Van Beaumont et al., 1972). In humans, neither of these factors is a problem if the exercise duration is less than 120 minutes (Harrison, 1985). In the horse, the addition of splenic RBCs to the central circulation during exercise makes the use of this formula problematic. Fortunately, McKeever and coworkers (1993a), who employed splenectomized horses, developed a correction factor for this splenic contraction. They demonstrated that the spleen contracts very rapidly with the onset of exercise and that both the extruded PV and RBCs accommodate with the central circulation within the first 90 seconds of exercise. Changes in HCT from the point of full mixing onward were parallel in splenectomized and intact horses. Therefore, from 2 minutes into exercise and beyond, changes in HCT were caused by decreases in PV in both groups (McKeever et al., 1993a). This meant that the difference between pre-exercise and 2-minute values for HCT in intact horses represented a splenic reserve offset that could be used as a correction factor (McKeever et al., 1993a).

BOX 6–1

Calculation of the Percent Change in Plasma Volume Using Corrected Hematocrit in Horses*

$HCT_b = 35$

$HCT_{raw} = 58$

$HCT_{2\,min} = 55 - 35 = 20$

$HCT_a = HCT_{raw} - HCT_{2\,min} = 58 - 20 = 38$

$$\%\Delta PV = \left(\frac{100}{100 - HCT_b}\right) \times \left[100 \times \frac{(HCT_b - HCT_a)}{HCT_a}\right]$$

$$\%\Delta PV = \left(\frac{100}{100 - 35}\right) \times \left[100 \times \frac{(35 - 38)}{37}\right]$$

$$\%\Delta PV = \left(\frac{100}{65}\right) \times \left[100 \times \frac{(-3)}{37}\right]$$

$$\%\Delta PV = (1.54) \times [-5.41]$$

$$\%\Delta PV = -12.2$$

From McKeever KH, Hinchcliff KW, Reed SM, et al: Role of decreased plasma volume in hematocrit alterations during incremental treadmill exercise in horses, *Am J Physiol* 265:R404, 1993a.

Box 6-1 demonstrates the use of HCT to calculate percent changes in PV in the horse. If a horse has a resting hematocrit (HCTb) of 35, and the hematocrit measured at 2 minutes of exercise (HCT_{2min}) is 55, then the difference between the two would be the correction factor (HCT_{2min}) applied to all the hematocrits drawn after 2 minutes. In Box 6-1, if the uncorrected hematocrit (HCT_{raw}) was 58, then the value for HCTa to be used in the calculation of PV would be obtained by subtracting the correction factor (HCT_{2min}) from the uncorrected hematocrit (HCT_{raw}).

$$HCT_{raw} - HCT_{2min} = HCTa$$

PLASMA OSMOLARITY AND THE CONCENTRATION OF KEY ELECTROLYTES

Osmolality is the total number of dissolved particles in solution, independent of the elemental species making up that solution (Johnson, 1998; Wade et al., 1989). The term *osmolality* is applied when the solute is expressed as weight (kg). Thus, osmolality is the number of osmoles (Osm) of solute per kilogram (kg) of solvent or (Osm/kg). In living organisms, the solvent is expressed in terms of volume

(liters). The relationship is the same, but the term is changed to *osmolarity* (Osm/L). Normal plasma osmolarity in the horse and most other mammals averages 290 mOsm/L and is a reflection of the dissolved particles in both ICF and ECF spaces (Carlson, 1987; Wade et al., 1989). Plasma osmolarity plays a key role in maintaining a normal intracellular environment, a factor that is vital for cellular function (Carlson 1987; Greenleaf and Morimoto, 1996; Nadel et al., 1993; Wade et al., 1989).

All of the electrolytes contribute to osmolarity by affecting the osmotic concentrations in the various fluid compartments of the body. Sodium (Na^+) is the major cation in the ECF space, making it the greatest contributor to osmolarity (Carlson, 1987; Greenleaf and Morimoto, 1996; Nadel et al., 1993; Wade et al., 1989). This large molecule in solution exerts osmotic force across semipermeable membranes such as capillaries and cell membranes. This force, or "osmotic pull," is exerted by the sum of freely moving particles in solution exerting an effect on water in surrounding tissues (Johnson, 1998; Szlyk-Modrow et al., 1996). Because water tends to move down a gradient from an area of low concentration to an area of high concentration, an increase or decrease in plasma [Na^+] (osmolarity) has the capacity to cause large fluid shifts out of or into the cells. These fluid shifts can dramatically alter normal cellular function. Since a change in osmolarity reflects expansion or contraction of the extracellular fluid compartment, cardiovascular function may be affected as well (Johnson, 1998; Szlyk-Modrow et al., 1996). Extracellular fluid volume serves as one of the first lines of defense in the regulation of cardiac filling volume and mean arterial pressure. A negative change in osmolarity can negatively affect these hemodynamics, thereby ultimately affecting the ability to perfuse the tissues (Nose et al., 1988; McKeever, 2008; McKeever and Hinchcliff, 1995).

Potassium is the primary cation found within the cells; however, calcium and magnesium are additionally important to intracellular functions (Carlson, 1987; Greenleaf and Morimoto, 1996). Calcium within the tubular sarcoplasmic reticulum of muscle cells plays a vital role in excitation contraction coupling (muscle contraction). Magnesium is an important cofactor in many of the reactions involved in cellular metabolism (Carlson, 1987; Kingston and Bayly, 1993; McKeever, 1997). Major extracellular anions in the body include chloride, bicarbonate, and the phosphates (Carlson, 1987; Kingston and Bayly, 1993; McKeever, 1997; McKeever, 2008). Normal values for resting concentrations of the major electrolytes found in plasma, interstitial fluid, intracellular fluid, and sweat are listed in Table 6-1. These concentrations are tightly regulated to prevent cellular dehydration or swelling (edema).

Thermoregulation during exercise in the horse can result in a tremendous volume of fluid loss (hypertonic sweat). Thus, maintaining the fluid volume and solute composition of the intercellular and extracellular body compartments becomes a serious challenge (Carlson, 1983; Carlson 1987; McKeever and Hinchcliff, 1995). Compromised cellular function and cardiovascular stability (Wade and Freund, 1990) are serious considerations. Therefore, it is vitally important that plasma osmolarity be regulated within very narrow limits. The regulation of osmolarity is intertwined with the defense of ECF volume, PV, and cardiac filling pressure. Thus, its regulation involves an integrative response of multiple systems, including the cardiovascular, neural, endocrine, and renal systems (McKeever et al., 1993a); McKeever and Hinchcliff, 1995; Rowell, 1993; Wade and Freund, 1990).

Changes in plasma osmolarity are sensed by specialized cells within the supraoptic and paraventricular nuclei of the hypothalamus in the brain (McKeever and Hinchcliff, 1995; Wade et al., 1989; Wade and Freund, 1990). These osmoreceptors can detect changes in plasma osmolarity as small as 2 mOsm/L that, in turn, evoke a rapid change in the synthesis and secretion of the hormone arginine vasopressin (antidiuretic hormone [ADH]) by the posterior pituitary. Within minutes, an increase in vasopressin concentration causes renal reabsorption of water, thus correcting the volume deficits and alterations in electrolyte concentration suffered through the sweating process (Convertino et al., 1981; Wade and Freund, 1990). In addition, vasopressin stimulates thirst. The addition of water through

TABLE 6–1

Electrolyte Composition (mEq/L) of Plasma, Interstitial Fluid, Intracellular Fluid (Muscle), and Sweat

Electrolyte	Plasma Fluid	Interstitial Fluid	Skeletal Muscle	Sweat	Range
CATIONS					
Na^+	140	143	10	263	(132–593)
K^+	4	4.1	142	51	(32–78)
Ca^{++} (ionized)	2.5	2.4	4	22	(3–58)
Mg^{++} (ionized)	1.1	1.1	34	5	
ANIONS					
Cl^-	100	113	4	268	(174–432)
HCO_3^-	25	28	12		
$H_2PO_4^-$, HPO_4^{-2}	2	2	40		
Protein	14	0	50		
Other	7		7	84	

(Adapted from Schott HC, Hinchcliff KW: *Fluids, electrolytes, and bicarbonate,* Vet Clin North Am: Equine Pract, 9(3):577-604, 1993.)

drinking also has a positive effect on fluid balance and osmolarity (Wade and Freund, 1990).

PLASMA CONCENTRATION VERSUS PLASMA CONTENT

When considering the effects of acute exercise on key electrolytes, changes in concentration must be distinguished from changes in total *content* (McKeever et al., 1993c; Van Beaumont et al., 1972). By definition, the concentration of a substance is the amount of solute in a given volume of solvent. *Content*, in contrast, is the total amount of that solute in the fluid compartment or body, depending on the focus of analysis. For example, if the normal plasma concentration of sodium is 140 milliequivalents per liter (mEq/L), then the plasma *content* of sodium would be obtained by multiplying its concentration by the total plasma volume (McKeever et al., 1993c; Van Beaumont et al., 1972).

Calculating *content* change for an electrolyte will determine if the corresponding change in its concentration is caused by the addition or loss of the electrolyte itself or is the result of changes in plasma volume. When measured for the whole body, changes in *content* provide insight into how electrolyte concentrations are affected through routes of intake and loss. This is of particular interest when the changes are acute because of the stress imposed by exercise. Studies with human athletes have long used key formulas to calculate the total amount of specific electrolytes lost from the vascular compartment during exercise (Box 6-2) (Dill and Costill, 1974; Harrison, 1985; Van Beaumont et al., 1972). An example of their use would be in the examination of sodium losses during short-term exercise versus long-term exercise. Plasma sodium concentration is held within very narrow limits. During short-term exercise, these concentrations undergo minimal changes. However, the plasma *content* of sodium does show a decrease. In exercise of long duration, changes in plasma sodium concentration may still be minimal, but the changes in *content* are dramatic (Figure 6-3). These results suggest the fluid shifts that occur during exercise are, in part, caused by isotonic shifts (McKeever et al., 1993c; Schott and Hinchcliff, 1993).

Chloride concentrations, like sodium, are held within very narrow limits. However, in the case of this ion, due to large amounts lost in the sweat, there is a dramatic disproportional decrease both in its plasma concentration and its *content* (McKeever et al., 1993c; Schott and Hinchcliff, 1993). When considering potassium, the picture is just the reverse. Both the plasma concentration and *content* of potassium increase during high-intensity exercise. The measured increase in *content* allows for an accurate interpretation of the increase in concentration, being that it is caused by both loss of plasma fluid and addition of potassium to plasma when it "leaks" out of the contracting muscle cells. One of the benefits in measuring the plasma *content* of electrolytes is the knowledge it provides toward the dietary supplementation of electrolytes to replace their exercise-related losses.

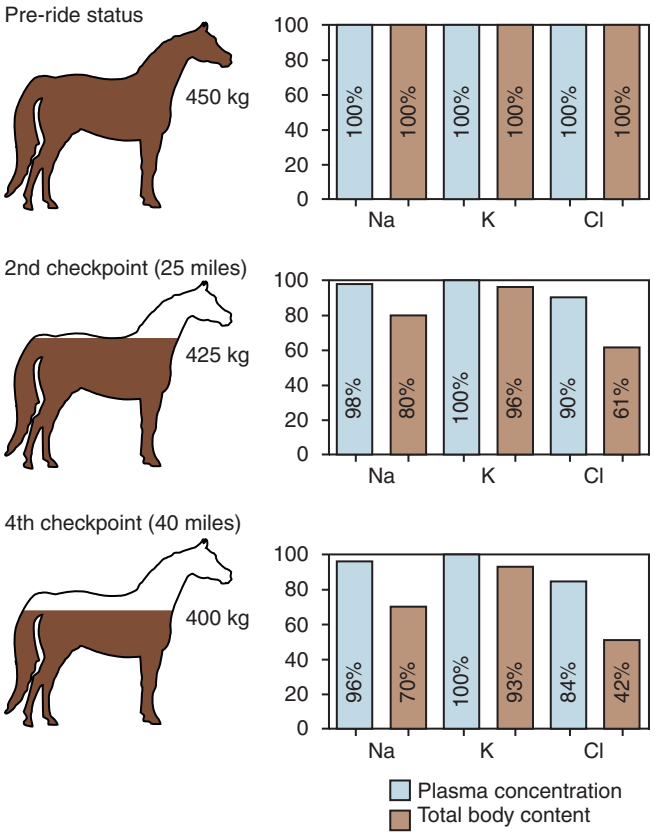

FIGURE 6-3 Changes in plasma volume and total body water with steady state exercise at 25 (*A*) and 40 (*B*) miles of endurance. Bar graphs demonstrate that the changes in the plasma concentration of key electrolytes do not reflect the magnitude of the decrease in the whole body content of those electrolytes due to sweating. *(Adapted from Schott HC, Hinchcliff KW:* Fluids, electrolytes, and bicarbonate, *Vet Clin North Am: Equine Pract, 9(3):577-604, 1993.)*

BOX 6–2

Calculation of Percent Change in the Content of a Plasma Constituent using Corrected Hematocrit

HCT_b = Resting Hematocrit

HCT_a = Corrected Hematocrit

Cn_b = Resting Concentration

Cn_a = Post–Concentration

Co = Content of Solute

$$\%\Delta Co = \frac{Cn_a - [HCT_a\,(100 - HCT_b) \times (Cn_b)] / HCT_b\,(100 - HCT_a)}{HCT_a\,(100 - HCT_b) \times (Cn_b) / HCT_b\,(100 - HCT_a)} \times 100$$

From McKeever KH, Hinchcliff KW, Reed SM, et al: Splenectomy alters the hemodynamic response to incremental exercise in the horse, *Am J Physiol* 265: R409, 1993b.

ELECTROLYTES AND ACID–BASE STATUS

When considering the effects of exercise on fluid balance and the internal environment of the body, changes in acid–base status must be considered as well. Blood pH is tightly regulated with small changes creating dramatic physiologic outcomes. In the horse, short-duration, high-intensity exercise creates a metabolic acidosis resulting in a decrease in pH from resting values of 7.4 to levels as low as 6.8. In contrast, there is an increase in pH during submaximal endurance exercise, rising up to 7.8. Among those variables that are closely linked to changes in pH are the dynamic

alterations in ICF and ECF and electrolyte homeostasis that occur with work. In the case of either high-intensity or submaximal exercise in the horse, changes in pH have been linked to changes in the major strong ions that are altered by increases (from rest) in workload. The major cations and anions are independent variables that contribute to the disturbances, and then the rapid restoration, of pH during and following the challenge of exercise (Lindinger and Waller, 2008).

There have been many papers published on the effect of acute exercise on whole body acid–base status in the horse (Kingston and Bayly, 1998; Lindinger and Waller, 2008). A large number of them have reported data obtained using the Henderson-Hasselbach equation, where pH = pKa + log $[HCO_3^-]/CO_2$ (Kingston and Bayly, 1998; Lindinger and Waller, 2008). The independent variables ($PaCO_2$ and $[HCO_3^-]$) are easily measured by using a blood gas analyzer. This method can determine if there is alkalosis or acidosis in the body and whether the change in pH is metabolic or respiratory in origin.

A change in the $[HCO_3^-]$ in the numerator of the Henderson-Hasselbach equation indicates that the change in pH is caused by metabolic factors such as the rapid rate of adenosine triphosphate (ATP) hydrolysis that occurs with high-intensity exercise or the excessive chloride losses associated with a lengthy submaximal session of work. If the change in pH is due to a change in the $PaCO_2$, then the mechanism for the alteration is considered respiratory in origin (Lindinger and Waller, 2008). The major disadvantage to the Henderson-Hasselbach method is the qualitative nature of the information it derives with regard to exercise-related acid–base status. This limitation has led researchers to seek more quantitative methods for determining the physiochemical mechanism behind exercise-induced changes in pH.

A quantitative method developed by Stewart (1981) simultaneously takes into account the variables measured in the Henderson-Hasselbach equation, along with the interplay needed to achieve equilibrium in the carbon dioxide system (Figure 6-4), the weak acids, and the strong ions (Box 6-3). The regulated dependent variables are [H+] and $[HCO_3^-]$. The independent variables measured for their effect on [H+] and $[HCO_3^-]$ include the $PaCO_2$, the A_{tot} (weak volatile acids, primarily plasma protein), and the strong ion difference (SID). There are many strong ions that contribute to the SID; however, most exercise-related studies focus on sodium, potassium, chloride, and lactate. Thus, SID = $[(Na^+ + K^+) - (Cl^- + LA^-)]$ (Constable, 1997; Kingston and Bayly, 1998; Lindinger and Waller, 2008). In the Stewart approach, pH decreases when the SID decreases. The opposite occurs when the SID increases with the horse becoming alkalotic. Some consider the Stewart approach cumbersome because it requires simultaneous measurement of several independent variables. However, the quantitative data gained through this method lend much greater insight into the relative contribution of multiple factors in the regulation of acid–base status.

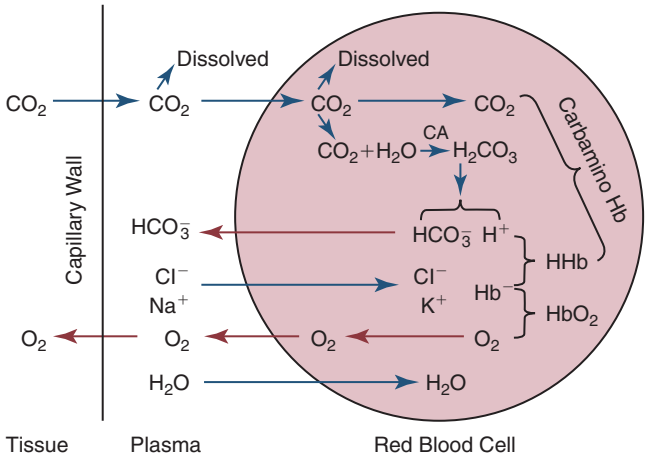

FIGURE 6-4 Red blood cell and its role in the transport of carbon dioxide and the regulation of acid–base status.

BOX 6–3

Equations for Stewart Model of Plasma Acid–Base Chemistry

Water Dissociation

$[H^+] \times [OH^-] = K^I_w$ (1)

Weak acid dissociation:

$[H^+] \times [A^-] = K_A \times [HA]$ (2)

Weak acid conservation:

$[HA] + [A^-] = [A_{TOT}]$ (3)

Bicarbonate formation:

$[H^+] \times [HCO_3^-] = K_C \times PCO_2$ (4)

Carbonate formation:

$[H^+] \times [CO_3^-] = K_3 \times [HCO_3^-]$ (5)

Electrical Neutrality

$[SID] + [H^+] - [HCO_3^-] - [A^-] - [CO_3^-] - [OH^-] = 0$ (6)

Kw^I is the ion product for water. $[A_{TOT}]$ is the sum of both undissociated protein concentration, [HA], and ionized protein concentration, $[A^-]$. K_A is protein dissociation constant; K_C is the combined equilibrium and solubility constants linking the partial pressure of carbon dioxide, PCO_2, and bicarbonate concentration, $[HCO_3^-]$. K_3 is the dissociation constant for the formation of carbonate, $[CO_3^-]$. [SID] is the difference in strong acid and strong base concentrations.

Used with permission: Watson PD, Using ACIDBASICS to teach Acid Base Equilibrium to Life Science Students, University of South Carolina

MAXIMAL EXERCISE

High-intensity, short-duration exercise results in a dramatic metabolic acidosis in the horse (see Table 6-1 and Table 6-2). Blood pH can drop from 7.4 (resting) to 6.8, and bicarbonate or total carbon dioxide (tCO_2) concentrations can show an increase of up to 10 mmol/liter from their normal resting concentrations (Kingston and Bayly, 1998; Lindinger and Waller, 2008). Following exercise, there is a rapid return to resting values in pH, bicarbonate, and tCO_2 concentrations, usually within 90 minutes. The respiratory compensation for metabolic acidosis is mechanistically different in the horse compared with humans. In humans, the hyperventilation observed at the ventilatory threshold is part of the mechanism for regulating pH through the elimination of CO_2 (Lindinger and Waller, 2008). However, while VCO_2 increases in the horse during exercise, the rate of elimination is inadequate because the horse does not hyperventilate during exercise. This is a species related difference that results in the well-recognized hypercapnea associated with high-intensity work in the horse. Coincident with the retention of CO_2 in the horse are the dramatic decreases in pH (Lindinger and Waller, 2008).

A portion of the drop in pH can be attributed to a dramatic change in SID during high-intensity exercise in the horse

TABLE 6–2

Summary of Selected Studies Showing the Effects of Maximal Exercise on Acid–Base Parameters

	VENOUS			ARTERIAL		
Source	pH	$[HCO_3^-]$	P_{CO2}	pH	$[HCO_3^-]$	P_{CO2}
CARLSON*						
Pre-ex	7.36 ± 0.01	27.6 ± 1.4	49.8 ± 2.3	7.38 ± 0.01	25.7 ± 1.4	43.7 ± 2.5
Max-ex	6.95 ± 0.02	30.1 ± 0.51	145.1 ± 5.0	7.11 ± 0.01	18.3 ± 1.2	59.8 ± 3.0
BAYLY et al*						
Pre-ex	7.33 ± 0.01	24.4 ± 1.5	48.9 ± 1.7	7.41 ± 0.01	27.9 ± 0.09	45.1 ± 0.5
Max-ex	6.87 ± 1.00	20.0 ± 1.3	128.6 ± 2.3	7.04 ± 0.04	10.7 ± 0.08	47 ± 1.9
HODGSON et al*						
Pre-ex	7.41 ± 0.02	—	48.5 ± 2.2	7.44 ± 0.01	—	49.2 ± 1.2
Max-ex	6.96 ± 0.02	—	115 ± 3.1	7.1 ± 0.04	—	53.1 ± 2.1
PARKS AND MANOHAR†						
Pre-ex	7.33 ± 0.01	—	42.3 ± 1.5	7.37 ± 0.01	21.2 ± 0.08	38.0 ± 1.2
Max-ex	6.99 ± 0.01	—	56.0 ± 2.3	7.23 ± 0.01	11.0 ± 0.5	27.0 ± 1.2

HCO_3^-, milliequivalent measurement; *Max-ex*, end of maximal exercise; P_{CO2}, millimeters of mercury measurement; *pre-ex*, pre-exercise;.

*Corrected to pulmonary artery temperature.

†Corrected to rectal temperature.

Adapted from Kingston JK, Bayly WM: Effect of exercise on acid–base status of horses. In Hinchcliff KW, editors: *Vet Clinics of North America: equine practice: fluids, electrolytes and thermoregulation in horses,* Philadelphia, 1998, WB Saunders, p 61.

TABLE 6–3

Summary of Selected Studies Showing the Effects of High–Intensity Exercise on Plasma Strong Ion Concentrations*

	CARLSON		TAYLOR et al	
Source Time	Pre-ex	Max-ex	Pre-ex	Max-ex
VENOUS				
$[Na^+]$	137.2 ± 1.5	150.3 ± 2.4	136.5	141
$[K^+]$	4.1 ± 0.3	7.9 ± 1.1	3.86 ± 0.01	5.75 ± 0.1
$[Cl^-]$	100.4 ± 0.8	99.8 ± 0.7	92.4 ± 0.5	90.3 ± 0.3
$[Lac^-]$	0.6 ± 0.1	20.0 ± 2.0	0.41 ± 0.02	5.31 ± 0.1
[SID]	40.3	38.4	46.55 ± 0.5	50.93 ± 0.4
ARTERIAL				
$[Na^+]$	137.1 ± 1.7	149.6 ± 1.2	136.8	138.3
$[K^+]$	4.0 ± 0.3	7.4 ± 1.0	3.84 ± 0.01	5.36 ± 0.02
$[Cl^-]$	101.2 ± 0.9	107.6 ± 1.2	95.7 ± 0.5	94.8 ± 0.3
$[Lac^-]$	0.5 ± 0.1	18.8 ± 1.9	0.41 ± 0.01	5.55 ± 0.1
[SID]	39.4	30.6	44.24 ± 0.5	40.89 ± 0.4

Lac, lactate; *max-ex*, maximal exercise; *pre-ex*, pre-exercise; *SID*, strong ion difference.

*All values are expressed as milliequivalents.

(Adapted from Schott HC, Hinchcliff KW: *Fluids, electrolytes, and bicarbonate,* Vet Clin North Am: Equine Pract, 9(3):577-604, 1993.)

(Constable, 1997; Kingston and Bayly, 1998; Lindinger and Waller, 2008). This change in SID is caused by transient changes in plasma electrolyte concentrations (Table 6-3). In races covering distances between 1200 m and 2400 m, changes in electrolyte and acid–base status are associated with increases in the concentrations of Na^+, K^+, Cl^-, and LA^-, all of which affect SID. High-intensity exercise also results in an increase in plasma protein concentration by two routes: (1) the loss of plasma water, and (2) the addition of plasma protein due to splenic reserve mobilization.

During high-intensity exercise, plasma sodium concentration increases because of movement of water out of the vascular compartment and the extracellular space. Despite this increase, and the concomitant increase in plasma protein, there is no change in chloride concentration (Constable, 1997; Kingston and Bayly, 1998; Lindinger and Waller, 2008). This

may reflect the effect of the chloride shift (see Figure 6-4) that is part of the bicarbonate system in the RBCs. It may also be a reflection of the large increases in the other strong anion, lactate. This anion is exchanged along with chloride across the muscle cell membrane. Similar to sodium, plasma potassium concentration increases during high-intensity exercise, reaching values greater than 10 mmol/L (Constable, 1997; Kingston and Bayly, 1998; Lindinger and Waller, 2008). However, the increased plasma potassium is transient, returning to the normal resting range within minutes after maximal exercise. Mechanistically, an accumulation of H+ in active muscle impairs the sodium-potassium-adenosine triphosphatase (Na^+- K^+ -ATPase) pump, which, in turn, decreases the reuptake of K^+ by the fibers. The functional significance of this flux of K^+ is a decrease in muscle membrane potential, which may contribute to fatigue during high-intensity exercise.

LOW–INTENSITY EXERCISE

During endurance exercise, changes in acid–base status are related, in part, to the tremendous amounts of sweat lost by the horse during prolonged bouts of work. In hot conditions, a horse competing in an endurance ride may lose up to 10% of its body weight. Even more critical is the fact that the sweat of the horse is *hyper*tonic compared with the plasma. This differs from humans, in whom the composition of sweat is hypotonic to the plasma (Kingston and Bayly, 1998). The functional significance of this species related difference may be related to thermoregulation. In the horse, the surface area to body mass ratio is less optimal than that of humans. Thus, the extra salt in the sweat enhances solvent drag and alters the vapor point of the solution as well. Some have suggested that this means the horse can get sweat onto the skin for faster evaporation, thus increasing the rate of cooling. However, the magnitude of the accompanying electrolyte losses can be substantial, having a significant effect on whole-body SID and, consequently, acid–base status (see Figures 6-3A and 6-3B; Table 6-4).

TABLE 6–4

The Effects of Prolonged Low–Intensity Exercise (40% $\dot{V}_{O2max}$) on Plasma pH; Lactate, Na^+, K^+, and Cl^- Concentrations; and Strong Ion Difference in Six Thoroughbred Horses Exercised over 45 km*

Parameter	Pre-ex	45 km
MIXED VENOUS		
pH	7.39 ± 0.01	7.48 ± 0.01
[HCO_3^-]	32.6 ± 0.43	36.9 ± 0.84
[Na^+]	138.3 ± 0.6	139.3 ± 0.5
[K^+]	4.24 ± 0.09	3.88 ± 0.14
[Cl^-]	99.9 ± 0.06	94.1 ± 1.1
[Lac^-]	1.04 ± 0.11	1.79 ± 0.19
[SID]	41.2 ± 0.87	48.5 ± 1.34

45 km, at the end of 45 km; *Lac*, lactate; *pre-ex*, pre-exercise; *SID*, strong ion difference.

*All values are expressed as milliequivalents.

(Adapted from Schott HC, Hinchcliff KW: *Fluids, electrolytes, and bicarbonate,* Vet Clin North Am: Equine Pract, 9(3):577-604, 1993.)

The flux, as well as the losses, of major anions and cations varies between individual animals. Plasma sodium concentration varies with the duration of exercise and the environmental conditions. Different studies have reported an increase in concentration, no change, or a decrease in concentration (Kingston and Bayly, 1998). In many cases, there is an initial isotonic shift of fluid from the vascular compartment to the interstitial space, followed by losses of sodium and water as exercise progresses for an extended time. Potassium concentration increases because of a loss of plasma water and leakage of potassium from the ICF compartment to the ECF compartment.

However, when it comes to electrolyte losses during endurance rides, the horse encounters a disproportional loss of chloride. Although changes in sodium and potassium concentrations can contribute to changes in acid–base status through their collective alterations in SID, the chloride loss seen in the horse has the most pronounced effect. Chloride losses during endurance exercise exceed sodium losses, making it the principal anion lost in the sweat. During endurance exercise, the observed decreases in plasma chloride concentration can exceed 15 mmol/L (Kingston and Bayly, 1998). This loss of chloride ions can lead to the renal retention of bicarbonate, which results in metabolic alkalosis. If the chloride losses are substantial, this renal effect on acid–base status can increase bicarbonate concentrations by up to 5 mmol/L (Kingston and Bayly, 1998). These documented changes during long-duration exercise in the horse have led to the conclusion that administration of bicarbonate, either as a treatment or as a feed supplement, is contraindicated for this group of equine athletes.

EFFECTS OF ACUTE EXERCISE ON FLUID AND ELECTROLYTE BALANCE

To defend the internal environment of the cells (cellular homeostasis), the horse must regulate blood volume, blood pressure, and the osmotic composition of the ICF and ECF compartments (Convertino, 1987; McKeever, 1998; McKeever and Hinchcliff, 1995; Nose et al., 1988; Rowell, 1983). Therefore, acute fluid and electrolyte shifts have differing functional significance related to their timing during exercise. Shifts occurring early during exercise appear primarily related to a systemwide redistribution of blood volume (Figure 6-5). Fluid shifts from capacitance vessels and the interstitial space to active circulation in the vascular tree for the purpose of increasing venous return and cardiac output. Later responses provide fluid and electrolytes for the production of sweat and thermoregulation. Finally, during prolonged exercise, decreases in fluid stores lead to dehydration, which, in turn, leads to thermoregulatory and cardiovascular instability. If repeated, over time, this latter challenge stimulates an expansion of plasma volume and the *content* of various electrolytes. This is a beneficial adaptive response known as *training-induced hypervolemia* (McKeever et al., 1987; McKeever et al., 2002).

INTERCOMPARTMENTAL FLUID SHIFTS AT THE ONSET OF EXERCISE

Senay (1978) demonstrated, in humans, that in the first seconds at the onset of exercise, there is a rapid net movement of protein and fluid from the interstitial space and lymphatics into the vascular compartment. Coupled with a redistribution

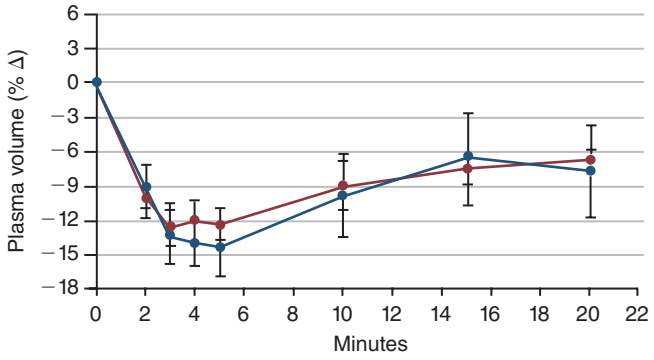

FIGURE 6-5 Changes in plasma volume in the horse during submaximal steady state exercise *(Adapted from McKeever KH: Body fluids and electrolytes: responses to exercise and training. In Hinchliff K, Kaneps A, Geor R, editors:* Equine exercise physiology, *Philadelphia, 2008, pp 328–349.)*

of blood from the venous capacitance side of the vascular system to the arterial side, this inward flux of protein and water causes a transient, short-lived increase in PV. This increase occurs for the provision of an adequate venous return to maintain cardiac filling pressure at a time when there is rapid vasodilation in the working muscles (Delgado et al., 1975; McKeever, 1998; Musch et al., 1997; Rowell, 1983; Rowell, 1987). A similar response that has been demonstrated in dogs probably occurs in horses as well (Coyne et al., 1990; Delgado et al., 1975; Musch et al., 1997).

At rest, albumin is the primary protein found in the vascular space and globulin represents the most prevalent protein found in the lymphatics (Coyne et al., 1990). With the onset of exercise in the horse, there is a dramatic shift in the albumin-to-globulin ratio. This shift is consistent with an inward flux of fluid from the interstitial space, which suggests that the horse's experience with the onset of exercise is similar to that of humans (Coyne et al., 1990; Senay, 1978). In the horse, plasma is also added to the central circulation from the splenic reserve (McKeever et al., 1993a; Persson, 1967). Splenic blood volume in the horse ranges from 6 L to 12 L with an HCT value between 70% and 80% (McKeever et al., 1993a; Persson, 1967). This means that with splenic contraction at the onset of exercise, the horse adds 1.6 L to 3.6 L of plasma to the central circulation. This volume is in addition to the volume added by intercompartmental fluid shifts described earlier (McKeever et al., 1993a; Persson, 1967). In addition to supporting the hemodynamic demands of exercise, this extra volume may also support metabolic demands by increasing circulating protein that may add extra buffering capacity to the central circulation.

Following this initial intercompartmental redistribution of water and electrolytes, plasma volume decreases rapidly (Harrison, 1985; McKeever, 1998; McKeever et al., 1993a; Nose et al., 1988). This secondary fluid shift is caused by a significant increase in mean arterial pressure and consequentially an increase in capillary hydrostatic pressure. These pressure increases cause water, electrolytes, and a small amount of protein to be extruded from the vascular compartment (Harrison, 1985; McKeever, 1998). Studies of horses performing moderate incremental exercise have demonstrated that this decrease in plasma volume is rapid and intensity dependent, with a 15% to 20% decrease in PV observed after only four 1-minute steps of incremental work (see Figure 6-1). This movement of water and salts bathes the interstitial space, where it is taken up by the working muscles, used to form sweat, or returned to the vascular compartment (McKeever et al., 1993a).

In mammals, the dynamic flux of fluid into and out of the vascular compartment is governed by Starling forces (see Figure 6-2). Net filtration and reabsorption across a vascular bed is the sum of forces affecting the movement of fluid and osmotically active substances (Carlson, 1987; Convertino, 1987; McKeever, 1998; Rowell, 1983; Rowell, 1993). These forces include capillary and interstitial hydrostatic pressures and capillary and interstitial oncotic pressures. On the arterial side of the resting capillary bed, hydrostatic pressure and interstitial oncotic pressure outweigh interstitial pressure and intravascular oncotic pressure. This favors a net movement of fluid out of the vascular compartment. However, during rest, venous oncotic forces outweigh the other forces, thus favoring a movement of fluid back into the vascular space.

During exercise, the balance of Starling forces is greatly affected by a larger increase in arterial hydrostatic pressure (Carlson, 1987; Convertino, 1987; McKeever, 1998; Rowell, 1983; Rowell, 1993). At the arteriole level this amounts to an increase of about 20 mm Hg in hydrostatic pressure enhancing the net force of fluid outward (Convertino, 1987; McKeever, 2008). On the venous side of the capillary beds, hydrostatic pressure is also elevated with a tendency for a net positive outward force. This means that during exercise, more fluid is shifted into the interstitial space than what is seen at rest. This outward flux of fluid has a functional significance. It can either be excreted as sweat (thermoregulation) or returned to the vascular compartment via the lymphatics (hemodynamic) (McKeever, 2008). The key here is that the decrease in PV seen at the onset of exercise (McKeever, et al., 1993a; McKeever et al., 1993b; McKeever, 2008) is dynamic and intensity dependent (see Figure 6-1) and occurs before the onset of sweat losses (see Figure 6-5) (McKeever et al., 1993a; McKeever et al., 1993b). However, PV decreases seen after these initial fluid shifts are the result of reductions in TBW caused by sweating (Carlson, 1983; Carlson, 1987).

FLUID AND ELECTROLYTE LOSSES ASSOCIATED WITH LONGER ACUTE EXERCISE

For the horse, evaporative cooling via sweating is the most efficient way to transition a large amount of heat from the core to the periphery (Carlson, 1987; Geor and McCutcheon, 1998a; Geor and McCutcheon, 1998b; McConaghy, 1994). During exercise, the removal of heat requires a significant redistribution of blood flow to skin. This is blood that could be used to supply the working muscles with nutrients and oxygen. However, the optimal perfusion of the working muscles is highly dependent on mean arterial pressure that must be kept within narrow limits (McKeever, 2008; Rowell, 1983; Rowell, 1993). Transient alterations in mean arterial pressure, as well as cardiac filling volume, are sensed respectively by high-pressure baroreceptors and cardiopulmonary volume receptors (McKeever, 2008; Rowell, 1983; Rowell, 1993). Rowell (1993) suggested that a feed-forward mechanism allows blood pressure to increase during exercise via an integrated response of these two layers of defense. This response includes an upward change in the set point of the receptors

during exercise, which removes the check on the system that would tend to limit an increase in cardiac output.

The shift in blood flow for thermoregulation, and its possible negative impact on mean arterial pressure, must be countered by other shifts in flow. Therefore, control of mean arterial pressure during increasing exercise intensity necessitates shunting blood away from nonobligate tissues. These "nonobligate tissues" include the splanchnic and renal vascular beds (Delgado et al., 1975; Rowell, 1993; Zambraski et al., 1984; Zambraski, 1990). As the intensity of exercise increases, changes in vascular tone in these regions become increasingly pronounced and are facilitated by both nervous system (catecholamines) and endocrine (plasma renin activity, vasopressin, etc.) effector signals (Degado et al., 1975; Rowell, 1993; Zambraski et al., 1984; Zambraski, 1990). In most cases, these factors influence vascular tone prior to any substantial loss of sweat (fluid) (Jimenez et al., 1998; Lindinger and Ecker, 1995; McKeever et al., 1991a; McKeever et al., 1992). Thus, adjustments in cardiovascular function and renal blood flow appear to be a response to the "stress of exercise" itself rather than to fluid and electrolyte imbalance.

As exercise progresses, fluid shifts from the vascular compartment to the interstitial space provide water for sweat production (McKeever, 1998; Rowell, 1993). Sweat loss causes a net reduction in TBW and a decrease in PV (see Figure 6-5). If plasma volume is not replaced, it eventually results in a decreased venous return and cardiac filling pressure (Convertino, 1987; McKeever, 1998). During exercise, the horse is able to maintain PV by utilizing fluid from the large colon and the cecum (McKeever, 1998). There are, however, limits to these fluid reserves, and during prolonged exercise, the large volume of hypertonic sweat produced by the horse will eventually lead to a compromised vascular volume (Geor and McCutcheon, 1998b). Cardiopulmonary volume receptors sense a drop in PV, the corresponding drop in cardiac filling pressure, and the resultant drop in stroke volume. To maintain cardiac output, this decrease in stroke volume is countered by an increase in heart rate. This increase in heart rate can occur even during steady state exercise and is referred to as "cardiac drift" (Rowell, 1993). If sweat production remains high, not only is the cardiovascular system compromised, there will also be progressive cellular dehydration, which will lead to decreased cell function, fatigue, and failure to thermoregulate (Lindinger and Ecker, 1995; McCutcheon and Geor, 1998; Sawka and Pandolf, 1990; Sejersted, 1992).

Along with the fluid loss experienced with severe sweat production, there is an associated loss of electrolytes stimulating a variety of endocrine responses. Human studies have documented that severe electrolyte loss can lead to weakness, muscle cramps, acid–base imbalance, and decreased performance (Sawka and Pandolf, 1990; Sejersted, 1990). Interestingly, training adaptations in humans include sweating earlier during exercise and sweating in greater amounts (Convertino, 1991). Similar adaptations have been seen in horses with training (McCutcheon and Geor, 1996; McCutcheon et al., 1999; McCutcheon and Geor, 2000) and heat acclimation (Geor et al., 1996). Trained human athletes also appear to have even more hypotonic sweat than their untrained counterparts as a result of aldosterone's action on the sweat glands (Convertino, 1991). To date, similar training-induced adaptations have not been demonstrated in the horse, partly because the equine sweat gland is not responsive to aldosterone and, thus, cannot conserve sodium. Compared with humans, the horse's sweat gland functions very simply, acting almost like a funnel, to allow a hypertonic solution of electrolytes to move from the interstitial space to the surface (McCutcheon and Geor, 1998). Teleologically, the solvent drag associated with a hypertonic sweat is beneficial for thermoregulation, as it facilitates the movement of a greater amount of water (and heat) outward. The extra salt in the sweat, as well as the protein latheren, also alters the evaporation point of sweat, thus possibly enhancing evaporative cooling. In the exercising horse, this is functionally significant because of the horse's less favorable (compared with human) ratio of surface area to fluid volume. The downside to this sweating mechanism is the potential for large fluid *and* electrolyte losses.

ENVIRONMENT, EXERCISE, AND SWEAT LOSS

Even under ambient conditions of mild temperature and low humidity, the exercising horse is presented with a significant thermoregulatory challenge. The dramatic increase in core temperature experienced by the horse during exercise requires an integrated response to transfer this heat to the environment. This transfer process can be exacerbated with increases in ambient heat and humidity. Recent studies of submaximal exercise in the horse have reported sweat rates exceeding 12 L/hr under conditions of high heat and humidity (see Chapter 8). This large volume of sweat is the result of diminished evaporative efficiency and leads to proportional decreases in body weight, TBW, and PV. This, in turn, leads to a diminished ability to thermoregulate as well as the compromise in hemodynamics discussed earlier in this chapter.

It is well understood that hot and humid conditions will increase the rate of sweating in the horse and speed up the progress of dehydration. As the state of dehydration worsens, the physiologic systems must prioritize their defense against this potentially life-threatening condition. The continued loss of total fluid volume is not only a threat to core temperature, but mean arterial pressure is at risk as well (Rowell, 1993). As dehydration progresses, maintaining cardiac output becomes the priority over thermoregulation. Thus, mean arterial pressure is defended at the expense of skin blood flow (Rowell, 1993). Sweat rate decreases, and the increase in core temperature begins to become exponential. The resulting hyperthermia can cause fatigue, cramps, heat stroke, and other thermal injuries. In a hot and humid environment, even a well-hydrated horse can encounter potentially life-threatening situations within a shorter period.

In addition to the loss of fluid volume, the production of large amounts of sweat presents another challenge for the equine athlete, the loss of electrolytes. Historically, exercise-related studies in the horse have demonstrated the loss of large amounts of key electrolytes (Carlson, 1983; Carlson, 1987). In older studies, the magnitude of this loss may have been overestimated because of sampling techniques (i.e., sweat scrapings) (Geor et al., 1996; Kingston et al., 1997). Nevertheless, more recent studies using more refined methods adapted from human research have documented significant losses in sodium and chloride (Kingston et al., 1999). The loss of sodium can become a problem particularly during recovery if a horse drinks too much water. This can lead to hyponatremia, which, if untreated, can lead to collapse and death (Geor and McCutcheon, 1998a;

McCutcheon and Geor, 1998b; Sosa-Leon, 1998). However, of greatest concern with a large volume of sweat loss in the horse is the associated loss of chloride. A disproportionate loss of this ion can potentially lead to a serious metabolic alkalosis and cell death (McCutcheon and Geor, 1998; Sosa-Leon, 1998).

Although sodium and chloride are the primary electrolytes lost in equine sweat, other elements such as magnesium and calcium have also been reported to be lost in measurable quantities (Carlson, 1987; Kingston, et al., 1997). In total, the high concentration of electrolytes in equine sweat make it hypertonic to plasma. Without electrolyte replacement through the diet, equine athletes can suffer significant deficits in these key elements. This is particularly true of horses competing in endurance activities or under hot and humid conditions. Thus, adequate watering, electrolyte supplementation, and the advanced planning of training sessions are essential to the health of the equine athlete.

THIRST, DRINKING, AND ELECTROLYTE INTAKE

In most cases, fluid and electrolyte losses following exercise can be compensated for through the provision of adequate water, a normal diet, and a salt and mineral supplement. With these ingredients in place, an equine athlete can usually recover from moderate, exercise-induced fluid and electrolyte losses. However, certain circumstances surrounding competition may put the horse at greater risk for dehydration and electrolyte deficiencies. These include hot and humid ambient conditions, long trailer rides immediately prior to an event, or limited recovery time between events (Gisolfi et al., 1990; Sosa-Leon, 1998). But even in these situations, fluid loss–related injuries can typically be avoided through the proper ingestion, and *timing*, of adequate amounts of water and electrolytes. To understand the impact of timing on the effectiveness of water and electrolyte intake, it is helpful to understand the horse's thirst mechanism itself.

As mentioned above, thirst and drinking are stimulated by the hormonal response to changes in osmolarity that occur with a decrease in total body fluid volume. Changes in osmolality are sensed by the paraventricular and supraoptic nuclei of the hypothalamus. With even a small increase in plasma osmolality, the hypothalamus directs the posterior pituitary to release arginine vasopressin (AVP). This hormone targets the kidneys and effects the conservation of water in the plasma (Freund et al., 1991; Nadel et al., 1993; Wade et al., 1989; Wade et al., 1990; see Chapter 7). Simultaneously, the kidneys themselves sense the increase in plasma osmolality and release renin into circulation. Renin is used by the liver to produce angiotensin, which, in turn, promotes the retention of sodium (and thus water) by the kidneys (Zambraski et al., 1984). Mechanistically, thirst can be stimulated by increases in circulating concentrations of AVP and angiotensin, as well as by changes in the concentration of calcium and other electrolytes in the cerebral spinal fluid (CSF). At rest, these drives for thirst and drinking are finely tuned in the horse.

With exercise, owners and veterinarians monitoring endurance events have long reported a suppression of drinking in horses, particularly at the end of endurance events (Butudom et al., 2002; Sosa-Leon, 1998). In many cases, clinicians have reported this suppression of thirst and drinking behavior lasting several hours following an event. Past research has documented this same phenomena in humans and dogs (Geelen et al., 1984; Greenleaf, 1994; Hubbard et al., 1990; Hubbard et al., 1994; Thrasher et al., 1981). Recent studies with the horse have found that there is an apparent suppression of AVP early in exercise (McKeever et al., 1991a). The suppression of this key hormone is associated with the cardiopulmonary baroreceptor response and mobilization of the splenic reserve at the onset of exercise. Again, the priority in the horse's "fight or flight" response (exercise) is the maintenance of mean arterial pressure at the expense of all else (McKeever, 1995; McKeever 2011). In the wild, thirst falls by the wayside when immediate survival is a matter of foot speed. This innate response remains in place in domestic equine athletes as well.

The volume of water that must be consumed by the horse to ward off, or alleviate, dehydration will obviously vary with the individual animal and conditions. National Research Council guidelines for feeding horses recommend 2 L to 4 L of water per kilogram of dry matter feed intake. In warm environments, this volume can increase by 15% to 20%. With prolonged exercise, this volume may increase by 300% or more. Some studies have documented that an active horse, under warm conditions, can consume 100 L (~ 26 gallons) of water per day (McKeever, 1997). Following exercise, when rehydration is imperative, is unfortunately exactly the time the horse's thirst mechanism can be suppressed. Comparative studies in humans and dogs have shown that this suppression can be exacerbated when cold, hypotonic water passes by the nerves in the mouth and throat (Greenleaf, 1994; Hubbard et al., 1990; Thrasher et al., 1981). Therefore, not only is the availability of water imperative after exercise, it should be warm and include electrolytes to help encourage drinking (Gisolfi et al., 1990; Sosa-Leon et al., 1995; Sosa-Leon et al., 1997). The addition of electrolytes helps restore osmolarity to a normal range. Interestingly, Australian research has demonstrated that endurance horses can be taught to drink warm water with electrolytes *during* competition (Sosa-Leon et al., 1995; Sosa-Leon et al., 1997; Sosa-Leon et al., 1998). At the very least, the old adage "water *ad libitum*" should be followed with every horse, particularly the equine athlete.

RENAL FUNCTION DURING EXERCISE

Acute and chronic renal responses to exercise are part of an integrative defense of blood volume, blood pressure, and osmolality (Convertino, 1991; McKeever and Hinchcliff, 1995). In general, exercise affects both renal blood flow and the mechanisms associated with the tubular reabsorption of water and electrolytes (Grimby, 1965; Hinchcliff et al., 1990; Hinchcliff et al., 1995a, Kachadorian and Johnson, 1970; McKeever, 1998; McKeever et al,, 1991a; Poortmans, 1984; Schott et al., 1991; Zambraski, 1990). These alterations in renal blood flow and renal function vary with both the duration and the intensity of exercise (Freund et al., 1987). Zambraski (1990) suggested that these alterations are both a response to the perceived stress of exercise as well as to the perturbations in fluid and electrolyte balance (Figures 6-6 and Figure 6-7). Conversely, postexercise changes in renal function are part of a long-term mechanism designed to restore lost water and electrolytes (Convertino, 1991; McKeever, et al., 1987; McKeever et al., 2002). In this manner, the kidney functions as a major effector organ in the *adaptive* expansion of PV and electrolyte

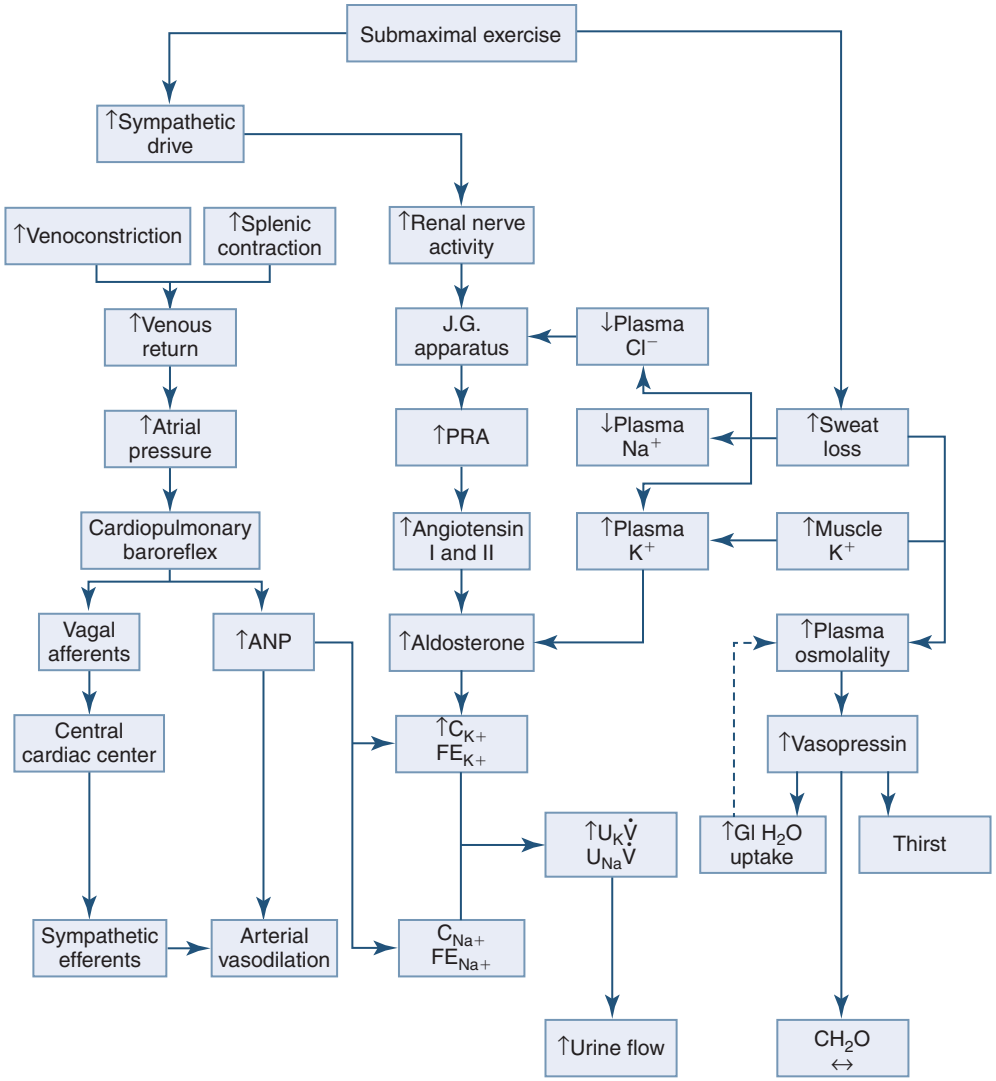

FIGURE 6-6 Renal response to low-intensity exercise *(Adapted from McKeever KH:* Effect of exercise on fluid balance and renal function in horses, *Vet Clin North Am: Equine Pract 14(1):23-44, 1998.)*

content that occurs with training. This expansion is referred to as *hypervolemia.*

EFFECTS OF EXERCISE ON RENAL BLOOD FLOW

Absolute renal blood flow (RBF) is not reduced in humans or horses during submaximal exercise. However, as a percentage of cardiac output, RBF does decrease (Hinchcliff et al., 1990; Zambraski, 1990). Hinchcliff et al. (1990) reported that renal blood flow averaged 15 mL/kg/min in the horse and that it did not change during low-intensity exercise. But because total cardiac output increased, RBF dropped from 23% of cardiac output at rest to 6% during low levels of work (Hinchcliff et al., 1990; Zambraski, 1990). Conversely, *high*-intensity exercise has been shown to cause substantial reductions in absolute renal blood flow in horses, humans, and swine but not in normal dogs (Hinchcliff et al., 1990; Zambraski, 1990). When work intensities exceed 50% to 60% of $\dot{V}O_{2max}$, renal vasoconstriction occurs coincident with detectable increases in renal nerve activity, circulating catecholamines, and plasma renin activity (Zambraski, 1990).

Schott, and coworkers were the first to demonstrate in the horse that high-intensity exertion causes a reduction both in absolute RBF and relative RBF (% of cardiac output) (Schott et al., 1991). When horses were exercised at a speed shown to produce an oxygen uptake of 100% $\dot{V}O_{2max}$, absolute RBF decreased from 9.0 L/min to 2.4 L/min (Schott et al., 1991). Surprisingly, *relative* RBF decreased from 22% of cardiac output at rest to 0.09% (Schott et al., 1991). This reduction in RBF resulted in a substantial drop in glomerular filtration rate (GFR) and a subsequent drop in urine flow. The excretion of solute-free water and various electrolytes dropped as well.

EFFECT OF EXERCISE ON GLOMERULAR FILTRATION RATE AND FILTRATION FRACTION

Blood flowing through the renal artery is filtered through millions of glomeruli in the kidney. The glomerulus is part of the nephron, the basic structural unit of the kidney. It is a complex structure made up of the afferent artery, Bowman's capsule, and the efferent artery (Zambraski, 1990). Algebraically, GFR is representative of the sum of the action of all the

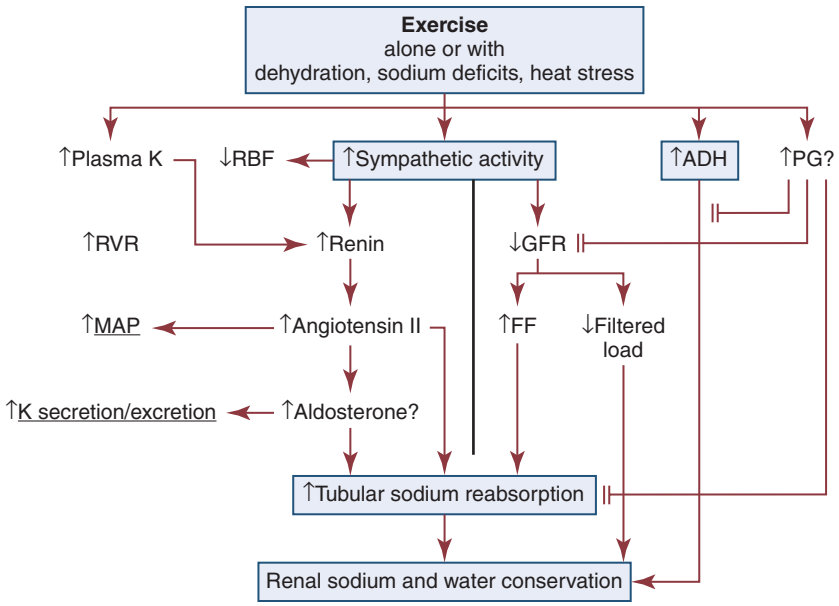

FIGURE 6-7 Renal response to high-intensity exercise. *(Adapted from Zambraski EJ: Renal regulation of fluid homeostasis during exercise. In Gisolfi CV, Lamb DR, editorsL* Perspectives in exercise science and sports medicine: fluid homeostasis during exercise, *vol 3, Carmel, IN, 1990, Benchmark, p 245.)*

nephrons and is autoregulated over a wide range of kidney blood flow. As with RBF, the effects of exercise on GFR in the horse varies with the intensity of exercise (Hinchcliff et al., 1990; Schott et al., 1991). GFR has been shown to increase or decrease during submaximal exercise, depending on hydration status. In studies where the subjects were hyperhydrated, GFR did not change during submaximal exertion (Zambraski, 1990). However, when individuals were euhydrated, or hypohydrated, changes in GFR were dependent on intensity, duration, or both (Zambraski, 1990; Zambraski et al., 1984). The greatest changes occurred when individuals performed high-intensity exercise (Freund et al., 1984; Zambraski, 1990). Interestingly, RBF can be dramatically reduced during exercise, although studies in humans showed that decreases in GFR do not necessarily parallel the decreases in RBF (Zambraski, 1990). Thus, glomerular filtration is somewhat protected in the face of exercise-induced reductions in RBF with a resultant increase in filtration fraction (FF; i.e., the GFR:RPF ratio).

Zambraski (1990) suggested possible mechanisms to explain the exercise-induced increase in FF. First, glomerular hydrostatic pressure is maintained and GFR is preserved through greater constriction of the efferent arteriole relative to the afferent arteriole. There are human data to support this hypothesis; however, data from comparable species are lacking. A second hypothesis postulates that changes in glomerular capillary filtration (Kf), simultaneously with decreases in glomerular capillary hydrostatic pressure, assist in maintaining GFR (Zambraski, 1990). Although not directly tested, this mechanism fits teleologically with data from human and animal studies that document exercise-induced increases in substances that affect Kf, such as vasopressin, prostaglandins, and angiotensin II (Zambraski, 1990).

The effects of exercise on GFR and FF appear to be similar in the exercising horse to those observed in exercising humans. Hinchcliff and coworkers (1990) reported that in horses, as in humans, there appear to be no alterations in GFR or FF during standing control or submaximal (50%-60% $\dot{V}O_{2max}$) exercise. In this study, creatinine clearance (i.e., GFR) ranged between 2.0 and 2.5 mL/min/kg, and FF averaged 23%. High-intensity exercise, however, produces a significant decrease in GFR and a significant increase in FF in the horse. Schott and coworkers (1991) demonstrated a 73% decrease in GFR during exercise performed at an intensity shown to produce $\dot{V}O_{2max}$. As with humans, horses performing high-intensity exercise had significant increases in filtration fraction from 16% at rest to 23.2% after running (Schott et al., 1991). Although drugs such as furosemide and phenylbutazone affect renal blood flow, they do not appear to alter GFR and FF in the horse during submaximal or maximal exercise (Hinchcliff et al., 1991; Schott et al., 1995b). These observations suggest that renal prostaglandins play a minimal role in mediating changes in GFR and RBF in the horse during exercise.

RENAL TUBULAR FUNCTION AND EXCRETION DURING EXERCISE

In simple terms, the kidneys filter the blood at the glomerulus and then selectively reabsorb or secrete essential and nonessential substances in the tubules. Normal fluid and electrolyte homeostasis requires the kidneys to eliminate excess water and electrolytes or, if there is a deficit, to reabsorb those vital components of the blood. Alterations in the GFR, in the tubular handling of water and solutes, or in both ultimately affect the volume of urine produced and the rate essential electrolytes are excreted over a given period. Studies of humans, dogs, and horses have demonstrated that changes in tubular handling of water and solutes vary with work intensity (McKeever et al., 1991a; Schott et al., 1991; Zambraski, 1990). These changes appear to be secondary to alterations in renal blood flow, GFR, and the filtered load of a given substance. Only a few studies have examined the effects of exertion on renal tubular function.

During submaximal exercise, performed at an intensity below 60% $\dot{V}O_{2max}$, urine flow increases in humans and in horses. However, low-intensity exercise resulted in a significant 45% increase in urine production, whereas the total volume of extra water lost (~6 mL/min) was reported to be small compared with the increased volume lost as sweat. McKeever et al. (1991a) reported that the increase in urine flow was caused by an exercise-induced increase in osmotic clearance. This increase in clearance was induced primarily by natriuresis and kaliuresis. The increase in sodium excretion appeared to be mediated by a concomitant increase in plasma atrial natriuretic peptide (ANP). Even so, the total amount of sodium lost via renal excretion was minimal. The authors suggested that during exercise, ANP, being a potent vasodilator, functions primarily to facilitate decreased vascular resistance in the working muscles. In turn, this accommodates increased atrial pressure caused by exercise-induced increases in venous return (McKeever et al., 1991a; 1991b). The relatively small but significant increase in sodium excretion observed in horses and humans, which occurs early in a bout of endurance exercise, appears to be a minor secondary response to the potent cardiovascular action of this hormone (see Figure 6-6).

Results from this same study also demonstrated a significant kaliuresis as well as the previously mentioned natriuresis, which suggests that the increase in potassium excretion was caused primarily by a rise in plasma aldosterone concentration. The increase in plasma K^+ concentration seen in submaximally exercised horses coincided with a secondary increase in plasma aldosterone concentration (McKeever et al., 1991a). Since there were limited decreases in plasma sodium concentration, the increases in aldosterone release may have been primarily in response to the rise in plasma K^+ concentration. This phenomenon has also been demonstrated by other researchers, who have shown the most potent stimulus for aldosterone secretion to be an increase in circulating potassium. Functionally, this increase in aldosterone prevents excessive increases in plasma K^+ concentration that can alter electrophysiologic gradients in muscles and other tissues. For example, several studies have documented that excessive increases in plasma potassium concentration appear to be one of many factors contributing to the onset of muscle fatigue.

Another major problem associated with exercise in the horse is an excessive and disproportional loss of chloride through sweat. McKeever et al. (1991a) demonstrated that when plasma chloride concentrations fall, renal chloride losses decrease. Similar reductions in chloride excretion are also seen in exercising humans (Zambraski, 1990). Since Na^+ and K^+ excretion increase in submaximally exercised horses, the authors suggested that mechanisms affecting the conservation of those electrolytes are not responsible for the increase in Cl^- reabsorption (McKeever et al., 1991a). During an hour-long bout of submaximal exercise, horses became alkalotic (McKeever et al., 1991a). These and other data suggest that renal mechanisms affecting the reabsorption of Cl^- and the secretion of HCO_3^- by the antiporter in the apical membrane of the intercalculated cell of the cortical collecting duct may be responsible for the conservation of chloride (McKeever et al., 1991a; Zambraski, 1990).

Last, with regard to submaximal exercise, solute-free water clearance does not appear to be altered in the horse, despite significant increases in plasma osmolality and plasma vasopressin concentrations (McKeever et al., 1991a). Similar observations have been made in exercising humans (Zambraski, 1990). Possible explanations for this paradox include the following: A decrease in renal blood flow could potentially decrease the filtered load of solute free water available for reabsorption by the action of AVP on the collecting ducts (Zambraski, 1990). However, this explanation would not be supported by the observation that submaximal exercise does not affect absolute RBF or GFR (Hinchcliff et al., 1990). It may, however, be possible that vasopressin's extrarenal functions are more important during exercise and actions on the kidneys are overridden. Such functions may include vasopressin's role in the vasoconstriction of the vascular beds in nonobligate tissues, its role in central mechanisms that stimulate thirst and drinking, and its action on the gut (Rowell, 1993). In the gut, vasopressin appears to act on the epithelium of the large intestine, enhancing the uptake of sodium and water (Bridges and Rummel, 1984). This protective effect would aid in the limitation of exercise-related fluid deficits more than potential reductions in free water clearance.

High-intensity exercise appears to have effects on renal function that are dramatically different from submaximal work (see Figure 6-7). Sodium excretion is dramatically reduced during high-intensity exercise in horses, pigs, and humans (McKeever, 2008; Zambraski, 1990). Several mechanisms could be responsible for the decrease in sodium excretion in the horse, including the following:

- A decrease in filtered load of sodium
- Activation of the renin-angiotensin cascade
- Elevation of plasma aldosterone concentration
- Direct neurogenic control

In the first instance, a change in filtered load sodium would mean that less solute would be presented to the tubules for reabsorption. This would require a reduction in GFR, which does not change in humans, pigs, or horses. Therefore, this does not appear to be a viable mechanism for the decrease in sodium excretion. Data have been published supporting the finding that the decrease in sodium excretion is not affected by pharmacologic blockade of the renin-angiotensin cascade (Zambraski, 1990). In the horse, aldosterone concentration increases with high-intensity exercise without a change in sodium excretion, and studies in other species have shown that sodium excretion returns rapidly to baseline following high-intensity work. These observations suggest that aldosterone is not the mediator of the antinatriuresis seen during exercise (McKeever et al., 1991a; Schott et al., 1991; Zambraski 1990). The rapidity in the recovery of sodium excretion has been further interpreted to suggest that a neural mechanism is in play (Zambraski, 1990). This theory is consistent with a reported intensity-dependent increased renal sympathetic nerve activity during exercise (Zambraski, 1990). Zambraski and others have suggested that on the basis of all the current evidence, it can be concluded that the mechanism behind exercise-induced increases in sodium reabsorption is primarily a direct neurogenic control (Zambraski, 1990).

Schott et al. reported that urine flow almost stopped during supramaximal exercise in the horse and was still below pre-exercise levels in the period immediately after exercise. High-intensity exercise also caused a decrease in urine osmolarity and osmotic excretion (Schott et al., 1991). With these results, a significant reduction in electrolyte excretion would be expected. Interestingly, there were only nonsignificant

reductions in the bulk excretion of K^+, and Cl^- during exercise and no change in Na^+ excretion despite a reduction in urine flow (Schott et al., 1991). This response contrasts with changes observed in other species (Zambraski, 1990). One explanation for this aberrational finding may be the design of the experiment. The findings of this study were based on results that pooled data from both exercise and postexercise periods. Thus, the observed dramatic postexercise diuresis, natriuresis, and kaliuresis (see below) may have been included in the period covering exercise tests and may have offset any exercise-induced decreases in electrolyte excretion that should have occurred with a reduction in RBF and urine flow (Schott et al., 1991).

High-intensity exercise also appears to affect urinary pH, an observation of interest to racing chemists. Gerken and coworkers (1991) reported that high-intensity exercise caused a transient, and substantial, reduction in urine pH that lasted for up to 60 minutes after exercise. The authors suggested that the more acidic urine may affect the results of the battery of tests used to detect foreign substances in the equine athlete. Further, they suggested that alkalinizing agents such as sodium bicarbonate may alter postexercise pH, thus further complicating drug detection efforts.

POSTEXERCISE CHANGES IN RENAL FUNCTION

Although the high-intensity exercise studies of Schott et al. (1991) did not document a change in tubular function, the authors did report a substantial postexercise increase in urine flow. Data were consistent with diuresis, kaliuresis, and natriuresis (Schott et al., 1991). Excretion rates for these substances returned to baseline by 30 minutes of recovery. The authors suggested that the increase in sodium and potassium excretion was most likely caused by an increase in ANP, a hormone that has been shown to increase during exercise in the horse. In the long term, the adaptations to exercise training involve a reduction in 24-hour urine output and an expansion of PV. These changes occur concomitantly with an increase in the content of sodium in the vascular compartment (McKeever et al., 2002).

THE ADAPTIVE (TRAINING) RESPONSE TO REPEATED EXERCISE

HYPERVOLEMIA

Repeated exercise (training) usually evokes an adaptive response that better prepares the horse's various physiologic systems for subsequent bouts of acute exertion (Figure 6-8). Disturbances in fluid and electrolyte balance require a two-phase response, with the early phase resulting in the replenishment of acute fluid and electrolyte losses and a secondary, or adaptive, phase that results in an enhanced ability to cope with future systemic disturbances. This "hypervolemic" adaptation involves a beneficial increase in blood volume due to an increase in plasma volume (Convertino, 1991; Harrison, 1985). Hypervolemia enhances both cardiovascular and thermoregulatory stability during the challenge of acute exercise. The increase in TBW helps ensure cardiovascular stability by maintaining venous return and, thus, cardiac output. The thermoregulatory benefits of hypervolemia are twofold. First, it enhances the ability to increase skin blood flow, thus enhancing the transport of heat from the core to the surface. Second, it increases the amount of fluid available for sweat production and evaporative

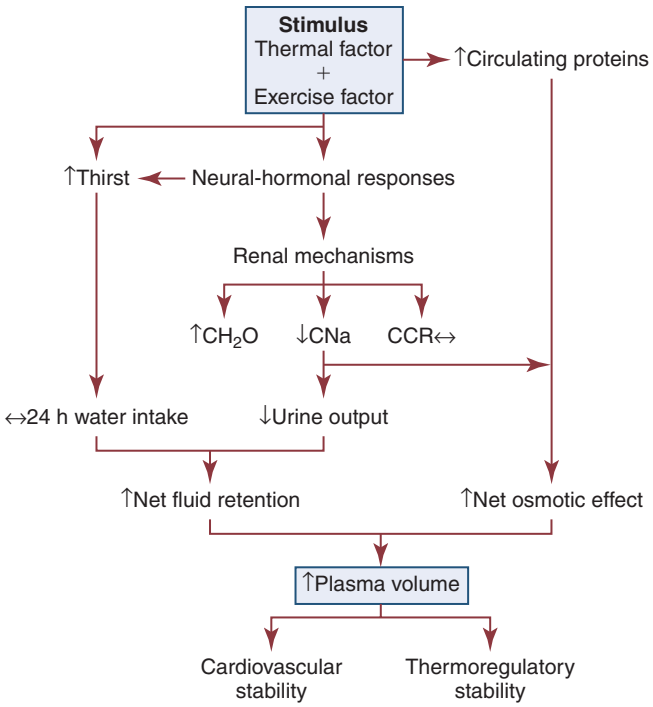

FIGURE 6-8 Suggested mechanisms for the exercise-induced hypervolemia seen in horses. *(Adapted from Convertino VA: Blood volume: its adaptation to endurance training,* Med Sci Sport Exerc *23:1338, 1991.)*

cooling. Both these adaptations make heat loss in the horse far more efficient.

Mechanistically, hypervolemia is an integrated effort of multiple systems to defend volume, plasma osmolality, and blood pressure. Comparative studies (Convertino et al., 1981; Convertino et al., 1983; Convertino, 1991; Costill et al., 1976; Freund et al., 1987; McKeever 1985) have demonstrated that approximately 60% of the mechanism behind the hypervolemic response is related to stimuli associated with the demands of thermoregulation. The remaining 40% of the response appears to be related to mechanisms directly associated with exertion (Convertino, 1991). These mechanisms counter acute fluid and electrolyte losses by stimulating the intake of water and by reducing renal removal of water and electrolytes.

Humans and horses lose a large amount of electrolytes in their sweat. Therefore, they have a physiologic drive to replenish fluid and electrolytes to defend both volume and tonicity. In horses and humans, drinking during and immediately after exercise only slows or partially counters the development of a fluid deficit at best and does nothing to counter any electrolyte deficit (Kirby et al., 1986; McKeever et al., 1987; McKeever et al., 2002). Studies of horses and humans show mixed results as to the role of water and electrolyte *intake* in the long-term response to exercise training. In humans, drinking does not account for all of the net water retention (Kirby et al., 1984). In fact, most of the actual expansion of TBW comes through renal mechanisms. In the horse, a recent study reported that water intake increased with training. However, the authors did not measure renal losses or conduct a balance study that would determine if the amount ingested actually contributed to an expansion of PV. Conversely, other studies have shown that training does not alter water intake in the horse. Instead,

they have reported that the horse appears to rely on renal mechanisms and an overall reduction in urine water loss to retain the sodium and water needed to expand PV and BV (McKeever et al., 1987; McKeever et al., 2002; Lindinger et al., 2000).

In both humans and horses, the decrease in urine output seen with training is caused by changes in postglomerular mechanisms rather than by a change in filtration rate (McKeever et al., 1987; McKeever et al., 2002). These renal adjustments are an adaptation, rather than a countermeasure, to the perturbations of acute exercise. In humans, the mechanism appears to be an aldosterone-mediated retention of sodium and water, which causes a net retention of water (Leutkemeier et al., 1994). Until recently, the mechanism behind the hypervolemic response was not as clear in the horse. An early study did not demonstrate any change in renal mechanisms affecting the retention of sodium and water. Instead, it was suggested that urea, rather than sodium, may be the solute responsible for the renal retention of water that leads to hypervolemia in the horse (McKeever et al., 1987; McKeever et al., 2002). However, the authors paradoxically demonstrated that there was a highly significant increase in plasma sodium content paralleling the increase in plasma volume. Interestingly, this net increase in retained sodium and water occurred despite increased losses through sweating. Since the rate of sodium intake was held constant, the only other route for its retention must have been either a more efficient uptake of electrolytes and water from the gut, a net retention by the kidneys early on in training, or both (Bridges and Rummel, 1984; McKeever, 2002). In humans, most of the response occurs in the first days of training. More recent work with the horse by McKeever et al. (2002) focused on the first days of training and demonstrated that there are dramatic reductions in urine output and excretion of sodium during this period (McKeever et al., 2002). Thus, like humans, the horse appears to undergo a similar adaptation in the aldosterone-mediated retention of sodium and water by the kidneys.

However, this research also found that renal retention of sodium and water did not fully counter the losses through sweat during the first days of training. The authors suggested that an enhanced aldosterone-mediated uptake of sodium and water from the large intestine may also contribute to the retention of electrolytes and water. Since the horse's large intestine serves as a fluid reservoir, this may be a warranted species-specific adaptation in response to the relatively larger electrolyte deficits (than in humans) associated with the production of hypertonic sweat. Enhanced intestinal uptake of sodium and water is supported by other studies demonstrating that aldosterone may enhance the transport of electrolytes and water from the digestive tract of the horse (Jansson et al., 2002).

Concurrent with the above-mentioned retention of water, sodium, and other vital electrolytes (which keeps the retained fluid isotonic), there is an increase in the plasma protein content (Kirby et al., 1984; Convertino, 1991). This increase in protein functions to keep the plasma iso-oncotic, thus holding water within the vascular space. Human studies suggested that the early (in training) increase in plasma protein content comes about from inward shifts of protein from the lymphatics and the interstitial space. Later in training, plasma protein is further increased from an overall net increase in plasma protein synthesis.

One aspect of the effect of training yet to be studied in the horse is whether there are alterations in cardiopulmonary baroreceptors that allow for the retention of the extravascular volume. Human studies have shown that training results in a downregulation of the ANP and neuroendocrine response to exercise, quite possibly to accommodate the hypervolemia associated with exercise training (Convertino, 1991; Rowell, 1993). Future research should determine if downregulation of these important control mechanisms also occurs in horses.

It is important to note that short-term horizontal studies of humans, dogs, and horses have demonstrated that the increase in PV occurs early in training and is followed by a subsequent slow increase in RBC volume (Figure 6-9). Therefore, when measuring HCT early in training, the greater PV on its own can give the appearance of anemia caused by the resulting lower concentration of RBCs. As training progresses, RBC volume slowly increases, and the early increases in PV level off and may even decrease. Thus, months into training, there is both a greater PV and RBC volume contributing to the increase in total blood volume. Interestingly, at this point, RBC concentration remains the same as its pretraining level, most likely to optimize both blood viscosity and oxygen-carrying capacity (Birchird, 2002; Sawka et al., 2000).

RED BLOOD CELL HYPERVOLEMIA AND OVERTRAINING SYNDROME

Overtraining syndrome is a phenomenon affecting both human and equine athletes (Bruin et al., 1994; Golland et al., 2003; Mackinnon and Hooper 2000; Persson Kreider et al., 1997). Broadly defined, overtraining syndrome is "an accumulation of training and/or nontraining stress resulting in long-term decrement in performance capacity with or without related physiological and psychological signs and symptoms" (Kreider et al., 1997; Mackinnon and Hooper, 2000). In short, in spite of training, athletic performance worsens because of insufficient

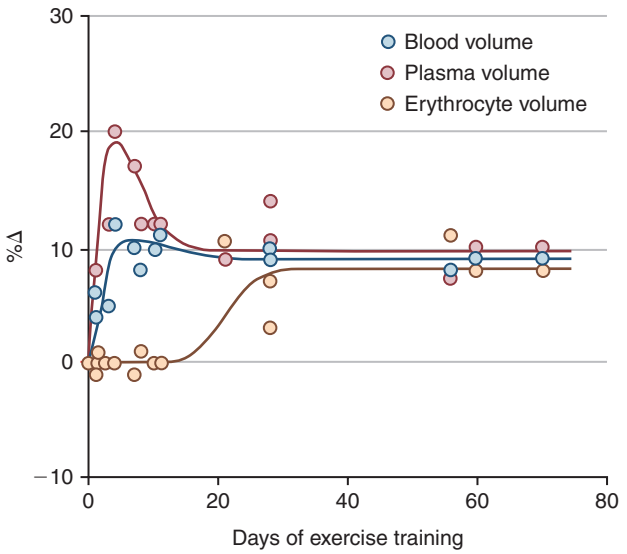

FIGURE 6-9 Estimated time course of relative (%) changes in blood volume (*middle line*), plasma volume (*upper line*), and erythrocyte volume (*bottom line*). Each point represents the average change reported in a group of (human) subjects from one investigation. Data were extracted from 18 investigations in which all three vascular volumes were reported. *(Adapted from Sawka MN, Convertino VA, Eichner ER, et al: Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness,* Med Sci Sports Exerc *32:332, 2000.)*

recovery between exercise bouts. This condition can last for months, even when training frequency and intensity are reduced (Mackinnon and Hooper, 2000). The etiology of overtraining syndrome is multifaceted, and clinical manifestations in humans include both the physiologic as well as psychological state of the individual (Kreider et al., 1997; Mackinnon and Hooper, 2000). Older studies of overtraining in the horse focused on a phenomenon referred to as *red cell hypervolemia*. This condition is characterized by an excess of RBCs that hypothetically limit performance (Funkquist et al., 2001; Persson, 1967; Persson et al., 1996; Persson and Osterberg, 1999). This condition should not be confused with the above-mentioned training-induced hypervolemia that involves the beneficial expansion of PV and HCT. For the most part, reports of RBC hypervolemia were based on cross-sectional studies of clinically normal horses versus "hypervolemic" horses within a regional (Sweden) population of (primarily) Standardbred horses. There have been no published data suggesting a widespread incidence of red cell hypervolemia in horses around the world. Moreover, excessive production of RBCs has not been observed as a consequence of overtraining syndrome in other athletic species. Mechanistically, it could be hypothesized that chronic exercise could disrupt the regulation of erythropoiesis through a chronic increase in the hormone erythropoietin (EPO) (McKeever, 1996; McKeever et al., 2006; McKeever et al., 2011). Unfortunately, none of the published red cell hypervolemia cross-sectional studies measured plasma EPO concentration; nor did they attempt to give some mechanistic insight into why such a highly regulated system would be disrupted by the stress of training. A more recent horizontal study of overtraining and the potential role of red cell hypervolemia convincingly demonstrated that there were no differences between normal and overtrained horses (Golland et al., 2003). In fact, the authors reported that RBC volume appeared to decrease slightly with long-term training. The data from this study strongly suggest that red cell hypervolemia is not a mechanism for the loss of performance in Standardbred horses. In light of these more recent findings, it is clear that more research is needed to determine the mechanism(s) associated with overtraining syndrome in performance horses.

EFFECTS OF AGING ON THE ACUTE AND CHRONIC RESPONSE TO EXERCISE

In humans, limited data have been reported comparing the thermoregulatory response of older and younger men and women to exercising in hot and humid environments. Some of these experiments have determined that the ability to dissipate heat during exercise declines with increasing age (Armstrong and Kenney, 1993; Kenney, 1995; McKeever, 2002). Suggested reasons for this age-related decline include lower cardiovascular capacity caused by the age-related decrease in cardiac output, alterations in mechanisms associated with the control of skin blood flow, and a possible state of hypohydration in older adults. Even though the data are mixed on the role of each of these factors, individually or in combination, it has been concluded that age influences thermoregulatory function during exercise in humans.

Although numerous studies have examined thermoregulation in young horses, data addressing the effects of age on the thermoregulatory response in this species are limited. McKeever and coworkers (2010) exercised young and old horses at the same submaximal absolute work intensity (1625 watts) until they reached a core body temperature of 40°C. The old horses reached this temperature in almost half the time of their younger counterparts. Heart rates of the older mares at 40°C were also substantially higher than those of the younger mares; however, both groups had similar core temperatures and heart rates 10 minutes after exercise (McKeever et al., 2010). Unfortunately, this study was only descriptive and was not able to address specific mechanisms involved in this apparent age-related difference in exercise-related thermoregulation.

However, data from human studies may shed light on reasons for the apparent impairment of thermoregulation in the older horse. Increasing cardiac output during exercise helps meet the dual demand for increased blood flow to the working muscle and skin (Armstrong and Kenney, 1993; Betros et al., 2002; Kenney, 1995; McKeever, 2002). In humans, the inability to adequately increase cardiac output during exercise will often compromise an older person's ability to dissipate heat and defend against hyperthermia (Armstrong and Kenney, 1993; Kenney, 1995). It could be suggested that this same inability occurs in the aging horse. The shorter time taken by older horses to reach 40°C during exercise and an almost 30% greater mean heart rate at this core temperature suggest that the older horses may have an age-related decrease in maximal cardiac output that leads to compromised thermoregulation during exercise. This speculation is supported by the decline in maximal heart rate and stroke volume reported by Betros et al. (2002).

The ability to thermoregulate is also related to hydration status. In humans, it is common for older individuals to have lower TBW (Armstrong and Kenney, 1993; Kenney, 1995). Data are mixed, however, as to whether older humans are chronically hypohydrated. Interestingly, McKeever and coworkers (2010) have suggested that older horses have a substantially lower pre-exercise PV compared with that in younger animals. Thus, older horses may start off with a significantly lower absolute amount of vascular fluid volume compared with younger horses, so cardiovascular and thermoregulatory stability is compromised during exercise.

An additional observation in the exercising older horse was a greater sweat rate compared with younger horses (McKeever et al., 2010). This could have been caused by alterations in skin blood flow. Skin blood flow was not measured in the study mentioned above, but human studies have demonstrated an impaired skin blood flow response to exercise in older individuals (Armstrong and Kenney, 1993; Kenney, 1995). The inability to keep cool, despite an increase in sweat rate in the older horse, would be consistent with the impairment of skin blood flow observed in humans. The mechanism for an age-related decline in skin blood flow during exercise may involve alterations in the sensitivity of neuroendocrine mechanisms affecting vascular tone. This is an area currently being investigated in humans and would be supported by age-related differences in the endocrine response to exercise in the horse as well (McKeever and Malinowski, 1999). Regardless of the mechanism, the older horse's decreased ability to thermoregulate during exercise warrants the extra attention of trainers, riders, and vets. Hydration status becomes even more important in the aging equine athlete.

CONCLUSION

Exercise places large demands on the cardiovascular system of the horse. These demands can be further magnified by environmental factors. End performance is limited in many respects by fluid and electrolyte stores as well as by the ability to maintain cardiovascular and thermoregulatory stability in the face of severe sweat loss. Studies of the exercising horse have been primarily descriptive, associative, or both, with only a limited number seeking to identify specific physiologic mechanisms associated with the control of fluid and electrolyte balance. More mechanistic studies are needed to fully understand the integration of the cardiovascular, endocrine, and renal systems in the defense of blood volume, plasma osmolarity, and blood pressure. Deeper understanding of the integration of these systems, and their response to the stress of exercise, can only lead to more informed and, thus, healthier methods for training the equine athlete.

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CHAPTER

7 Endocrine and Immune Responses to Exercise and Training

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Most horses spend the majority of their day eating, standing, and occasionally exercising. Physical activity can range from running up and down the fence line (in anticipation of the feed truck) to athletic training for a variety of competitive endeavors. Under resting conditions, the horse has a relatively easy job of maintaining the internal environment. However, work or exercise has the potential to be a major physiologic and homeostatic challenge that invokes an integrative response from multiple organ systems (McKeever, 2002). The response to exercise requires the transport of oxygen from the atmosphere to the cells in the working muscles, where it is utilized in metabolic pathways generating adenosine triphosphate (ATP) for fuel utilization (see Chapter 3). In reality, though, the adjustments to acute exercise require the coordination of several systems, including the respiratory, cardiovascular, muscular, integumentary, renal, hepatic, and digestive systems. Each tissue or organ called upon to facilitate activity must function in coordination with others in a variety of classic feedforward and feedback loops. To accomplish this, multiple layers of control exist in the body. The first muscle contractions associated with work will alter the mechanisms of autoregulation, causing changes in local control that are sensed peripherally. Longer or progressively more intense work causes systemwide alterations, which require integrated whole body responses that involve neural and endocrine mediation (Dickson, 1970; McKeever, 2002; McKeever and Hinchcliff, 1995; Rowell, 1993; Willmore and Costill, 1994).

The most rapid mechanisms used to facilitate a coordinated response to exercise involve an integration of signals in the periphery that are communicated via the nervous system to the central command centers, where adjustments are made to the respiratory and cardiovascular systems (McKeever and Hinchcliff, 1995; Rowell, 1993; Willmore and Costill, 1994). Initial rapid adjustments in cardiopulmonary function at the onset of exercise can be accomplished primarily via a shift in the autonomic tone, with an initial withdrawal of parasympathetic tone followed by an increased sympathetic drive with increasing intensity of activity. However, as exercise progresses beyond a few seconds, more sophisticated mechanisms are called upon to finetune the initial response to the disturbance of exercise.

Fine–tuning of the response to exercise that lasts longer than a few seconds is reliant on the regulation of several key variables governing the cardiopulmonary, vascular, and metabolic adjustments. Regulation allows the internal environment to be maintained within relatively narrow limits so as to maintain optimal function. This type of classic regulation involves a multiple system response where some variables are controlled so that those that are the most vital to the defense of the internal environment can be regulated. There is also a certain degree of redundancy in the systems which provides for more effective adjustments and tighter control. This type of integrative response is slower than a neural response because it requires communication between systems that relies on the secretion of substances by one tissue or organ that are transported remotely to other tissues or organs to evoke a response to adjust to the disturbance (McKeever and Hinchcliff, 1995; Rowell, 1993; Willmore and Costill, 1994).

ENDOCRINE SYSTEM AND HORMONES

By definition, a *hormone* is a signaling molecule that regulates and coordinates physiologic and metabolic functions by acting on receptors located on or in target tissues (Dickson, 1970; Willmore and Costill, 1994). These chemical messengers can be *endocrine*, if the target tissues are remote from the secreting organ; *paracrine*, if the hormone is acting locally; or *autocrine*, if the hormone is acting on the tissue or cell it was secreted from.

Most hormones fall into two major categories: (1) the steroid hormones and (2) the amine or peptide hormones. Steroid hormones include cortisol, aldosterone, and the reproductive hormones, testosterone, estrogen, progesterone. Steroid hormones have a classic ring structure and are lipid soluble, a characteristic that allows them to diffuse across cell membranes. Mechanistically, steroid hormones exert their effect through direct gene activation that occurs after diffusion into the cell (Figure 7-1). Once across the cell membrane, they bind to receptors in either the cytoplasm or in the nucleus. The complex formed by the steroid hormone and receptor induces the deoxyribonucleic acid (DNA) in the cell to produce messenger ribonucleic acid (mRNA), which, when it enters the cytoplasm, is transcribed, resulting in the production of proteins. The resultant protein (e.g., enzymes) leads to the physiologic action of the hormone on cellular function (Dickson, 1970; Willmore and Costill, 1994).

The amine/peptide hormones are hydrophilic rather than lipophilic and, thus, cannot pass through the cell membrane. Instead, these hormones bind in a lock and key fashion to very specific receptors on the surface of the cell membrane

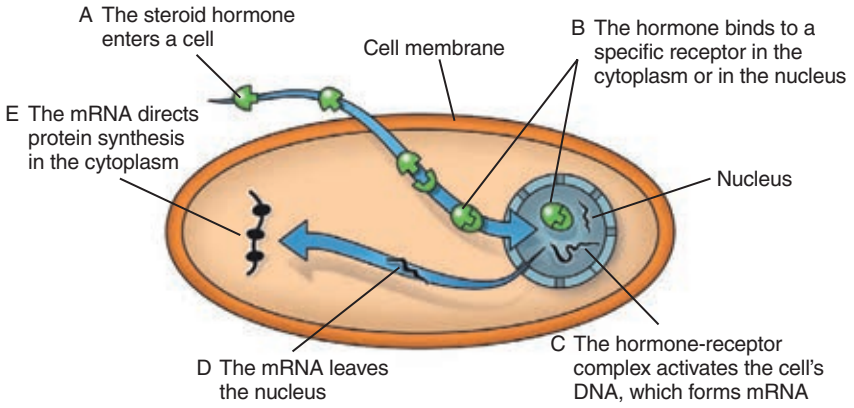

FIGURE 7-1 Gene activation mechanism associated with the action of a steroidal hormone. *(Adapted from Willmore JH, Costill DL: Hormonal regulation of exercise. In Willmore, JH, Costill DL, editors:* Physiology of sport and exercise: human kinetics, *Champaign, IL, 1994, p 122.)*

and serve as first messengers (Figure 7-2). The hormone–receptor complex remains in the membrane but is still able to activate the enzyme adenylate cyclase within the cytoplasm of the cell. Activation starts a cascade that results in the active formation of the enzyme cyclic 3', 5' adenosine monophosphate (cAMP) through the combination of adenylate cyclase and ATP (Dickson, 1970; Willmore and Costill, 1994). The enzyme cAMP serves as a second messenger and activates specific inactive protein kinases, which cause the conversion of inactive substrates into active substrates that have the capability to induce changes in cellular form or cellular function (Dickson, 1970; Willmore and Costill, 1994).

With the exception of the feedforward type of secretion of some hormones in immediate response to an emergency or stressor (i.e., onset of intense exercise), release of most hormones is part of a controlled negative feedback system that keeps a specific variable within narrow limits (Figure 7-3). The typical negative feedback system is composed of a variable that has an input or set point that is read or sensed by an integrator. For the system to be in a state of balance or homeostasis, the input and output signals must be the same. If the input variable is changed by a disturbance such as exercise, the integrator senses the mismatch and sends an error signal, usually via nerves, to the controller. The controller then alters the system via an effector. In many cases, the effector is a nervous signal. However, in some cases, the effector is another hormone. The effector causes a change in the controlled system, at which point a feedback sensor sends a signal to the integrator, which then determines if the input matches the output of the system. The more rapid responses used to maintain homeostasis are usually mediated by a neural control mechanism, with the less rapid responses usually mediated by endocrine mechanisms.

As an example of the complexity of systemic control, maintenance of mean arterial pressure (MAP) around a narrow set point is critical to cardiovascular performance (Rowell, 1993). Multiple systems are controlled to ensure that MAP is sufficient to allow perfusion of all the working muscles as well as obligate tissues during exercise. At the onset of exercise, there is a decrease in total peripheral resistance (TPR) that results from the opening of blood vessels in the working muscles, which allows for the increase in blood flow to those vascular beds. However, there is an almost simultaneous increase in cardiac output (McKeever, 2002; Rowell, 1993). This rapid increase in central cardiac function comes about through the matching of the input and output signals sensed by volume

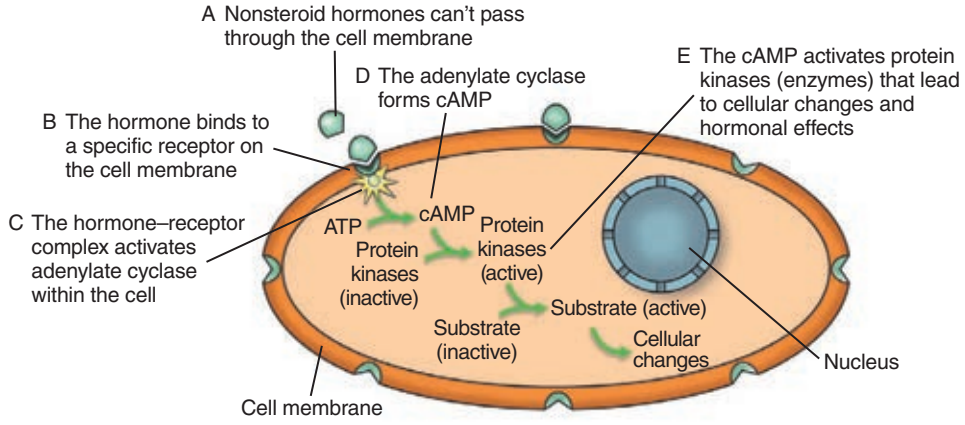

FIGURE 7-2 Second messenger mechanism associated with the action of nonsteroidal hormones. *(Adapted from Willmore JH, Costill DL: Hormonal regulation of exercise. In Willmore, JH, Costill DL, editors:* Physiology of sport and exercise: human kinetics, *Champaign, IL, 1994, p 122.)*

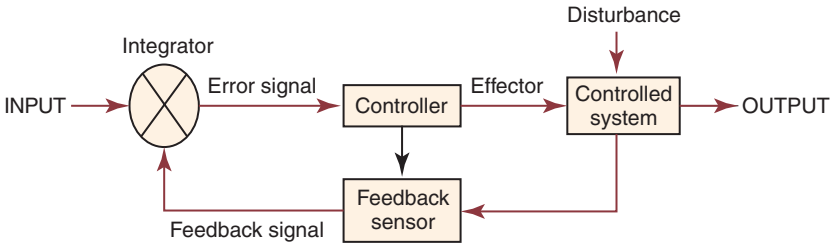

FIGURE 7-3 Components of a controlled negative feedback system.

and baroreceptors placed at strategic points in the cardiovascular system. Matching of the input and output signals (see Figure 7–3) by the vasomotor center of the medulla (the integrator) results in an error signal via the autonomic nervous system (the controller). A simultaneous withdrawal of vagal tone and increase in sympathetic drive results in the local release of norepinephrine (effector), which causes a rapid increase in heart rate and force of contraction that increase cardiac output (the controlled system) enough to maintain MAP. Higher-intensity exercise usually requires more dramatic responses, including a decrease in blood flow to nonobligate tissues such as the splanchnic vascular beds. The initial part of this response is mediated by neural mechanisms affecting the arterioles in those vascular beds. However, higher–intensity exercise also requires the added influence of endocrine effectors such as vasopressin and angiotensin II to cause sufficient vasoconstriction in nonobligate tissues to facilitate the rise in MAP needed for optimal cardiovascular function. Longer-term control of MAP can be affected by the duration of exercise which results in multiple strategies to control blood volume, defend cardiac filling pressure, cardiac output, and MAP (McKeever, 2002; Rowell, 1993).

MAJOR ENDOCRINE GLANDS AND HORMONES

PITUITARY GLAND

The pituitary, often referred to as the "master gland," is found at the base of the brain and is divided into three lobes: (1) the anterior, (2) the intermediate, and (3) the posterior. Hormones of importance during exercise are produced and released by the anterior and posterior lobes. The pituitary is linked to the hypothalamus, an area of the brain with many very specific neural tracts that act as feedback sensors. The hypothalamus also acts as the integrator in the system exerting control over the pituitary through neural and endocrine mechanisms. The endocrine mechanisms include various releasing hormones, inhibitory hormones, and other biochemical substances. The central location of this hypothalamic–pituitary axis makes it ideal for centrally mediating the control of a wide variety of functions. For example, the hypothalamic–pituitary–adrenal axis (HPAA) is intricately involved in the stress response, fuel mobilization, and adaptation to exercise. The hypothalamic–pituitary–gonadal axis (HPGA) directly controls hormones in males and females linked to hypertrophy, macronutrient storage and mobilization, and reproductive status (Dickson, 1970; Willmore and Costill, 1994).

Anterior Pituitary Hormones

Hormones produced by the anterior lobe of the pituitary include growth hormone or somatotropin (GH, ST), thyrotropin (thyroid–stimulating hormone, or TSH), adrenocorticotropin (ACTH), endorphins (EN), enkephalins, dynorphins, follicle–stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (Dickson, 1970; Willmore and Costill, 1994). The first three play an important role in growth and development in the young animal and in metabolism in the adult animal. The endorphins, enkephalins, and dynorphins are opiate-like peptide hormones that modulate analgesia and immune function and interact with the neural pathways of the hypothalamic–pituitary axis to influence releasing and inhibiting hormones (McLaren et al., 1989; Mehl et al., 2000). FSH, LH, and prolactin are considered hormones essential for normal reproduction and lactation. Research in species other than horses has demonstrated that severe or prolonged exertion can alter their release and, thus, normal reproductive cycles (Dickson, 1970; Willmore and Costill, 1994). Furthermore, prolactin appears to play an important role in the response to severe stress, interacting with many of the metabolic hormones. Though FSH and LH are technically anterior pituitary hormones, their function will be discussed later in the chapter in relation to the gonadal hormones which they influence.

Growth Hormone

GH affects all the cells in the body stimulating development and growth in younger animals. In mature animals, GH plays a vital role in muscle metabolism through its effects on protein synthesis and its role in fat and carbohydrate utilization (Dickson, 1970; Willmore and Costill, 1994). The importance of GH in maintenance of normal physiologic function and its possible role in slowing, or possibly even reversing, the effects of aging can be seen in some younger adult humans, where GH deficiency results in changes in appearance, decreased lean body mass, impaired immune function, and other "sequelae" of aging. Treatment of these individuals with recombinant human GH (hGH) results in increased lean body mass, decreased body fat, and increased muscle mass (Murray et al., 2001; Yarasheski, 1994). Chronic hGH administration also appears to increase strength and the ability to perform a battery of weight-lifting exercises in older male humans. These effects have led to increased doping with hGH in human athletes, partly because of improved repair and recovery as well as lack of valid tests for exogenous hGH until recently. Although there have been no reported effects of GH on aerobic capacity, the increase in muscle mass and strength have been shown to benefit the quality of life in humans by increasing the ability to do daily tasks such as maintaining balance while walking and climbing stairs. Those human experiments served as part of the justification for several recent studies of the efficacy of recombinant GH treatment in preventing or retarding functional decline in geriatric humans (Murray et al., 2001; Yarasheski, 1994) and in

geriatric horses (Horohov et al., 1999; Malinowski et al., 1997; McKeever et al., 1997).

Researchers conducting equine studies have asked "quality of life" questions similar to those posed in experiments using older humans. Those studies demonstrated that GH therapy increases nitrogen retention and improves appearance in geriatric horses, but they did not demonstrate any effect of chronic GH administration on body weight or the dimensions of several key muscles measured using ultrasonography (Malinowski et al., 1997). Functionally, chronic GH administration did not alter aerobic capacity or several commonly used indices of exercise performance, at least not in unfit old mares (McKeever et al., 1997). Furthermore, data from unfit geriatric horses indicated that exogenous GH did not alter lactate tolerance or cause an increase in maximal power that one would associate with an increase in muscular strength, which is a logical observation, since there was no evidence of an increase in muscle mass (McKeever et al., 1997). It is possible that the lack of significant findings in the horse may be due to insufficient dosage of GH, as was common in many of the early studies on GH and anabolic steroids in humans. Interestingly, studies of geriatric male humans have shown that recombinant GH therapy results in increased muscle mass and, in some cases, increased strength as measured in standardized tests performed with weightlifting equipment. However, although GH therapy does appear to increase strength in humans, there are no data to show that GH therapy alters maximal aerobic capacity or enhances endurance performance (Murray et al., 2001; Yarasheski, 1994).

Some studies of younger horses have demonstrated that administration of GH prevents some of the bone demineralization that occurs in the first months of intense training. Other studies found that there was minimal or no therapeutic benefit of administering GH to prevent tendon or cartilage injuries or to promote wound healing (Gerard et al., 2001; Gerard et al., 2002). It has also been demonstrated that GH does not alter aerobic capacity or markers of performance in young (~2 yr) Thoroughbreds (Gerard et al., 2001; Gerard et al., 2002). However, as with the studies of older horses, the experiments performed on younger animals had no way to evaluate the effects of GH administration on muscular strength. Data are clearly needed to determine if GH alters strength and power, with particular attention also paid to dose–response issues.

Thyrotropin

Release of TSH is stimulated by thyroid-releasing hormone (TRH), which is produced in the hypothalamus (Dickson, 1970; Willmore and Costill, 1994). Studies of humans and other species have demonstrated that acute exercise elicits mixed effects on TSH release. The release of TSH appears to be linked to exercise intensity, as mild and moderate exertions do not appear to have an effect on TSH release. However, there appears to be a threshold for stimulating TSH release, as plasma concentrations of this hormone increase with work intensity only when exercise intensity exceeds 50% of maximal oxygen uptake ($\dot{V}O_{2max}$) in humans. This breakpoint is common to several hormonal systems, including the observed increases in catecholamines, adrenocorticotropic hormone (ACTH), cortisol, plasma renin activity (PRA), etc. (Freund et al., 1991; Jimenez et al., 1993; Nagata et al., 1999). Interestingly, exercise duration beyond 40 minutes appears to also cause an increase in TSH. This observed increase during longer steady-state exertion is similar to the response of other metabolic hormones and may be related to substrate mobilization and attempts to prevent the onset of central fatigue mechanisms. Data on the effects of acute exercise and training on TSH in equine athletes are lacking.

Adrenocorticotropic Hormone

The release of ACTH is stimulated by corticotropin–releasing hormone (CRH), which is secreted by the hypothalamus (Dickson, 1970; Willmore and Costill, 1994). A number of published papers have demonstrated that exercise causes an increase in ACTH in the horse; however, most of those only report postexercise, rather than during-exercise, values (Elsaesser et al., 2001; McCarthy et al., 1991). The major conclusion of most exercise studies has been that both high-intensity and long-duration endurance exercises cause an increase in ACTH and subsequent increase in cortisol. If one looks at the role of ACTH and glucocorticoids in substrate mobilization and utilization during exertion, it is apparent that the ACTH or cortisol response is an appropriate and adaptive response to physical exertion, which itself is a stressor (Elsaesser et al., 2001; McCarthy et al., 1991; Caloni et al., 1999). In fact, mobilization of the "stress response" provides for remarkable regulation of homeostasis. As the intensity of exercise increases (and, hence, the threat to homeostasis increases), the activation of the HPAA exponentially increases to handle the stress of exercise (Nagata et al., 1999).

The ACTH response appears to be highly correlated to the catecholamine and lactate responses to incremental exercise, all of which also increased in a curvilinear fashion (Nagata et al., 1999). When horses were exercised at 80% or 110% $\dot{V}O_{2max}$, the ACTH response was rapid, and concentrations increased in a linear fashion until the end of the exercise test (Nagata et al., 1999). Postexercise concentrations fell rapidly, returning to baseline within 60 to 120 minutes (Nagata et al., 1999). These responses are similar to those reported for humans and other species (Nagata et al., 1999; Willmore and Costill, 1994). With chronic exposure to a stressor, as would happen during overtraining, regulation of the HPAA is challenging, as exhaustion may result from the increased allostatic load.

Endorphins, Enkephalins, and Dynorphins

These peptides are released from the pituitary in response to stress, pain, or pleasure. Although some classify these substances as hormones, others classify them as neurotransmitters (McLaren et al., 1989; Mehl et al., 2000). Nevertheless, these substances are naturally occurring opiat-like analgesics primarily derived from the pre–prohormone proopiomelanocortin (POMC) that may allow a horse to tolerate higher-intensity exercise (Art et al., 1994; Caloni et al., 1999; Li and Chen, 1987; McCarthy et al., 1991; McLaren et al., 1989; Mehl et al., 2000). The endorphins, enkephalins, and dynorphins play a role in the response to physiologic and psychological stress and appear to modulate pain perception as well as immune responses (McLaren et al., 1989; Mehl et al., 2000). Given that an overload of duration, intensity, or resistance is needed for exercise training to elicit an adaptive response, the endorphins may play an important role in allowing a horse to better tolerate the progressive increases in exercise intensities or longer durations needed to improve fitness and performance. Mehl

and coworkers (McLaren et al., 1989; Mehl et al., 2000) have demonstrated that a threshold exists for evoking an increase in plasma beta (β)-endorphin concentration. This threshold appears to correspond to approximately 60% $\dot{V}O_{2max}$. Interestingly, this is the same point where one sees a curvilinear increase in the catecholamines, plasma renin activity, plasma lactate concentration, and several other variables. This suggests a close interplay between these factors in the transition from low-intensity and primarily aerobic exercise to higher-intensity exercise with an increasing anaerobic component (Convertino et al., 1983; Freund et al., 1991; Jimenez et al., 1993; Nagata et al., 1999). Duration of exercise also appears to affect the magnitude of the endorphin response with greater plasma concentrations as horses approach fatigue (McLaren et al., 1989; Mehl et al., 2000). Training appears to alter the endorphin response to acute exertion with greater plasma concentrations observed in the postexertion peak occurring between 5 and 10 minutes after exertion (Malinowski et al., 2003).

Posterior Pituitary Hormones

Hormones released from the posterior lobe of the pituitary, which technically comprises neural tissue rather than glandular tissue, include arginine vasopressin (AVP) and oxytocin. These two protein hormones are actually produced in the hypothalamus by specialized bundles of nerves (Dickson, 1970; Willmore and Costill, 1994). Arginine vasopressin is synthesized in cells of supraoptic and paraventricular nuclei and stored in vesicles in the nerve endings located in the posterior pituitary. Oxytocin causes smooth muscle contraction in the epididymis of males and the uterus of females, and it also acts on mammary tissue causing milk let-down in lactating mares. However, oxytocin's role in the response to exercise is unclear.

Arginine Vasopressin

Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), is associated with acute as well as chronic defense of blood pressure, plasma volume, and fluid and electrolyte balance (Dickson, 1970; McKeever, 2002; McKeever and Hinchcliff, 1995; Wade, 1984; Zambraski, 1990). The physiologic actions of AVP include vasoconstriction and decreased free water clearance (McKeever and Hinchcliff, 1995; Wade, 1984; Willmore and Costill, 1994; Zambraski, 1990). AVP also plays a major role in the short-term and long-term control of cardiovascular function during and following exercise (Dickson, 1970; Willmore and Costill, 1994). Additionally, AVP is a critical stress hormone and serves as a secretagogue of ACTH. In fact, during acute exercise and in response to other noxious acute stressors (i.e., surgery, shock), AVP appears to be an even more potent stimulus for ACTH secretion than is CRH (Schmidtt et al., 1996).

The mechanism for the release of AVP primarily involves osmoreceptors in the supraoptic and paraventricular nuclei of the hypothalamus and cardiopulmonary baroreceptors in the atria of the heart (Dickson, 1970; McKeever and Hinchcliff, 1995; Wade, 1984; Zambraski, 1990). Data from rats and other species have shown that a very small 1% to 2% decrease in cell volume in the hypothalamus or change in extracellular osmolality of 2 to 4 milliosmole per kilogram (mOsm/kg) will stimulate secretion of AVP and intake of water (Wade, 1984). Exercise causes an increase in plasma AVP that is correlated with both duration and intensity (Convertino, 1991; Convertino et al., 1983; Wade, 1984). Comparative data show that AVP is secreted during exercise in concentrations well above the threshold level associated with its antidiuretic effects, suggesting that its extrarenal actions are more important during acute exercise (McKeever and Hinchcliff, 1995; McKeever et al., 1991a; McKeever et al., 1993; McKeever et al., 1991; McKeever et al., 1992b; Wade, 1984). Extrarenal actions include AVP's action as a powerful vasoconstrictor, an ACTH stimulus, and an important component in the control of blood pressure during exercise and its action on splenic blood vessels to prevent resequestration of the splenic reserve in the horse (Davies and Withrington, 1973; McKeever and Hinchcliff, 1995). Interestingly, some studies suggest that drinking water, especially cold hypotonic water, during exercise may suppress AVP and thirst, leading to dehydration (Zambraski, 1990). However, sustained elevations in AVP stimulate thirst and drinking water after exercise, cause a decrease in free water clearance by the kidneys, and may influence the uptake of sodium and water from the colon (McKeever and Hinchcliff, 1995; Zambraski, 1990).

In exercising horses, plasma AVP concentration was recently reported to increase from approximately 4.0 picogram per milliliter (pg/mL) at rest to about 95 pg/mL at a speed of 10 meters per second (m/s). It was also reported that the relationship between AVP concentration and exercise intensity was curvilinear and did not plateau at speeds producing maximal heart rate (McKeever et al., 1992b). Another paper reported that AVP increases during steady-state submaximal exercise in horses without a change in free water clearance (McKeever et al., 1991a). However, the increase does not become significant until between 20 and 40 minutes of exertion (McKeever et al., 1991a). Two possible explanations have been given for the delay in AVP secretion in submaximally exercised horses (McKeever et al., 1991a). First, there appears to be a suppression of AVP secretion due to the volume overload sensed by the neural pathways associated with the atrial baroreceptors and the hypothalamus (McKeever and Hinchcliff, 1995; McKeever et al., 1991a). Second, AVP release is inhibited by the increase in atrial natriuretic peptide (ANP) concentrations at the beginning of exercise (McKeever and Hinchcliff, 1995; McKeever et al., 1991). Nevertheless, an increase in AVP concentration has been seen with prolonged exercise that appears to be related to sweat losses and decreases in body water that altered plasma osmolality and blood pressure (McKeever and Hinchcliff, 1995; McKeever et al., 1991; McKeever et al., 1992b). Studies of humans have demonstrated that training alters the slope of the AVP response to acute exercise, suggesting a change in the sensitivity to the exercise challenge (Freund et al., 1991; Convertino et al., 1983; Convertino, 1991). No studies on the effect of training on the AVP response to acute exertion in the horse have been published.

THYROID

The thyroid is located in the neck close to the larynx region. It plays a major role in the control of the basal metabolic rate, which has led some to refer to it as the "body's thermostat" (Dickson, 1970; Willmore and Costill, 1994). The two iodine–containing hormones produced by the thyroid, triiodothyronine (T3) and thyroxine (T4), act on all cells in the body affecting metabolic rate and, subsequently, energy metabolism. The cells of the thyroid have three major actions when it comes to the synthesis and secretion of T3 and T4: (1) collection and transport of iodine, (2) synthesis and secretion of the glycoprotein

thyroglobulin into the intracellular colloid, and (3) removal of T3 and T4 from thyroglobulin and secretion into the bloodstream. Thyroid hormones circulate in plasma in both a free form and a protein-bound form, with the bound form accounting for 99.98% of the circulating hormone and the unbound form being the active hormone able to influence cellular metabolism. The thyroid also produces calcitonin, an important hormone in the control of calcium metabolism with potent effects on bone mineral density (Dickson, 1970; Willmore and Costill, 1994).

Triiodothyronine and Thyroxine

The release of these hormones is stimulated by TSH which, as previously mentioned, is released during exercise. As with TSH, the release of T3 and T4 is associated both with the intensity and duration of exercise in humans and horses (Gonzalez et al., 1998; Willmore and Costill, 1994). Irvine (1967) demonstrated that training increases both the secretion rate of T3 and T4 by approximately 65%. This would indirectly support the suggestion that TSH, which increases with training in humans and other species, is also increased with training in the horse. Training also increases the turnover rate of the thyroid hormones, though not necessarily in a clinically relevant manner that would suggest hyperthyroid function (Willmore and Costill, 1994).

Calcitonin

In addition to its control of metabolic rate, the thyroid is vital to calcium homeostasis. For this function, the thyroid synthesizes and produces calcitonin (Dickson, 1970; Willmore and Costill, 1994). Calcitonin plays a role in calcium homeostasis either by inhibiting osteoclast activity in bone or through its action on the kidney tubules to cause an increase in calcium loss by actively inhibiting tubular reabsorption. New bone is formed by osteoblasts and reabsorbed by osteoclasts. In young growing horses, both osteoclasts and osteoblasts are active; however, the activity of osteoblasts outpaces the activity of osteoclasts, allowing for bone growth and development (Dickson, 1970). To this end, calcitonin appears to be more important in the young growing animal through its inhibitory action on the osteoclasts. Calcitonin is also important in the healing of fractures (Dickson, 1970). Chiba and coworkers (Chiba et al., 2000) documented substantially elevated plasma calcitonin concentrations in racehorses with various fractures. Studies have also demonstrated that there is a period of bone demineralization in young Thoroughbreds in the first few months of training. Growing and adult humans who exercise regularly have increased bone density (Willmore and Costill, 1994). Although a great deal of work has examined markers of bone turnover, more data are needed to determine if acute and chronic exertion affect plasma calcitonin concentrations (Chiba et al., 2000; Geor et al., 1995; Murray et al., 2001).

PARATHYROID GLAND

The parathyroid glands, which are located close to the thyroid gland, regulate calcium homeostasis by synthesizing and secreting parathyroid hormone or parathormone (PTH) in response to a change in plasma calcium (Ca^{++}) concentration (Dickson, 1970; Willmore and Costill, 1994). PTH has receptors in the intestinal tract, in the osteoclasts in bones, and the tubules of the kidneys. The action of PTH to stimulate osteoclast activity is antagonistic to calcitonin's inhibitory action. The resultant effect is a net bone reabsorption and the release of calcium and phosphate into the bloodstream. Actions on bone are relatively slower compared with PTH's ability to alter both the uptake and excretion sides of the homeostatic balance equation by acting on the intestine and the kidney tubule (Dickson, 1970). PTH has a profound ability to enhance the enzymatic pathway that mediates increases in intestinal absorption of calcium and phosphate (Dickson, 1970; Willmore and Costill, 1994). At the same time, PTH can act on the kidney tubules where it enhances calcium reabsorption and phosphate excretion (Dickson, 1970; Willmore and Costill, 1994).

As with calcitonin, most equine research has focused on effects of repeated exercise on markers of bone turnover (Chiba et al., 2000; Geor et al., 1995; Murray et al., 2001). It is well recognized that nutritional influences can alter calcium and phosphate balance and bone metabolism. Thus, more work is needed to determine if exercise intensity, duration, or both are factors affecting PTH concentrations. Furthermore, data are needed to determine if exercise alters synthesis and secretion rates, receptor numbers and sensitivity, and general interplay of PTH and calcitonin in bone metabolism.

ADRENALS

The adrenal glands are multilayered organs that sit atop the kidneys. Functionally, the primary layers are the adrenal medulla and the adrenal cortex (Dickson, 1970). The medullary portion of the adrenal produces the catecholamines, epinephrine (E), and norepinephrine (NE), which have the potential to affect most cells in the body (Dickson, 1970; Willmore and Costill, 1994). In general, E potentiates the response to exercise causing profound effects on central cardiovascular and respiratory function. It can cause increases in muscle blood flow and can mobilize glycogen and free fatty acids to fuel exertion. The adrenal cortex contains three specialized zones: (1) the zona glomerulosa, (2) the zona fasciculata, and (3) the zona reticularis. The cortex produces a multitude of steroid hormones that fall into three major categories: (1) the mineralocorticoids (aldosterone), (2) the glucocorticoids (cortisol), and (3) the onadocorticoids (androgens and estrogens) (Dickson, 1970; Willmore and Costill, 1994).

Hormones Produced by the Adrenal Medulla (Catecholamines)

The release of the catecholamines has its origin in the fight-or-flight response. This "stress" response involves the local release of NE from the sympathetic nerve endings (acting as a neurotransmitter) and a systemic release of E and NE from the adrenal medulla. Receptors for the catecholamines are specialized and are divided into two primary categories, referred to as alpha (α)- and beta (β)-adrenergic receptors. These two major categories are divided into subcategories, namely, α_1, α_2, β_1, β_2, and β_3 receptors (McKeever, 1993).

Sympathetic nervous activity increases with intensity and duration of exercise, but notable changes in plasma catecholamine concentrations are not always apparent below 50% of maximal aerobic capacity (McKeever, 1993). Plasma catecholamine levels increase in a curvilinear fashion with increasing exercise intensity and are highly correlated with plasma lactate concentrations (Gonzalez et al., 1998; Jimenez et al., 1993; Nagata et al., 1999). The measurable increase appears to coincide with the intensity at which one would expect complete parasympathetic withdrawal (Freund et al., 1991; Jimenez et al.,

1993; Nagata et al., 1999). These increases in the catecholamines, particularly E, enhance the increase in heart rate (a chronotropic effect), force of cardiac contraction (an inotropic effect), and cardiac output (McKeever, 1993; McKeever and Hinchcliff, 1995). The catecholamines also play a role inducing splenic contraction and the delivery of 6 to 12 liters (L) of blood into the central circulation at the onset of exercise in horses (McKeever, 1993; Persson, 1967). Even with this mobilization of reserve blood volume, the demands of exercise may exceed central cardiovascular capacity in the horse. Thus, during high-intensity exercise or long-duration exercise, the catecholamines contribute to the vasoconstriction that decreases blood flow to nonobligate tissues (McKeever, 1993; McKeever and Hinchcliff, 1995; Rowell, 1993).

The catecholamines are also linked with the respiratory response to exercise (Jimenez et al., 1993; McKeever, 1993; McKeever and Hinchcliff, 1995; Plummer et al., 1991; Sexton and Erickson, 1986; Snow and Rose, 1981). At the onset of exercise the β_2-adrenergic receptor action relaxes tracheal and bronchial smooth muscle, increasing airway diameter, decreasing airway resistance, and, thus, facilitating movement of greater amounts of air into and out of the lungs. Although the sympathetic system is not directly responsible for the control of ventilation during exercise, the increase in ventilatory drive associated with activation of the motor cortex can be enhanced during exercise by catecholamine secretion and augmentation of the sensitivity of chemoreceptors in the carotid bodies. During high-intensity exercise, ventilation may be further affected by catecholamine release as part of the stress response (McKeever, 1993).

The catecholamines also have major effects on metabolic pathways associated with substrate utilization during exercise (McKeever, 1993; Willmore and Costill, 1994). Increases in sympathetic activity result in an increase in hormone-sensitive lipase and, subsequently, an increase in circulating free fatty acids. Exercise-induced and anticipatory secretion of catecholamines also cause an increase in glycogen breakdown resulting in increased glucose availability. It has been suggested that one way that warmup exercises benefit the athlete is through activation of these metabolic pathways, which allows for elevated blood concentrations of glucose and free fatty acids prior to race-induced increases in utilization, thus facilitating delivery of substrate to tissues without a significant lag time (McKeever, 1993; Willmore and Costill, 1994).

The above-mentioned responses have been well documented during acute exercise. Recent data also suggest that exercise training alters adrenergic receptor numbers and sensitivity in selected tissues. For example, β-adrenergic receptor numbers are unchanged in cardiac muscle with training, whereas α-adrenergic and muscarinic receptor numbers are reduced. Both β-adrenergic receptor numbers and sensitivity are increased in skeletal muscle and in vascular and bronchial smooth muscle (McKeever, 1993; Willmore and Costill, 1994). Changes in receptor number and sensitivity with training may be important with respect to adjustment of drug doses for the animal that has been trained extensively versus an animal that is at the beginning of a training program or one that is being reconditioned following removal from training (McKeever, 1993; Willmore and Costill, 1994).

Primary Hormones Produced by the Adrenal Cortex

Some sources suggest that more than 30 structurally distinct steroid hormones are secreted by the adrenal cortex. Secretion of the mineralocorticoid aldosterone and the glucocorticoid cortisol is, however, the most important to the physiologic response to exercise (Dickson, 1970; Willmore and Costill, 1994).

Aldosterone

Aldosterone plays an important role in electrolyte homeostasis, particularly sodium and potassium balance (Dickson, 1970; McKeever, 1998; McKeever and Hinchcliff, 1995; Willmore and Costill, 1994). It is well recognized that aldosterone acts on the kidneys to enhance sodium (and chloride) reabsorption and potassium excretion. Aldosterone also acts on the intestines to facilitate the uptake of electrolytes and water. Aldosterone release can be stimulated by decreases in plasma $[Na^+]$ or by increases in plasma $[H^+]$, plasma $[K^+]$, plasma ACTH, by increased renin (McKeever and Hinchcliff, 1995; McKeever, 1998), or by both. However, the most potent of these stimuli is an increase in plasma $[K^+]$ (McKeever, 1998; McKeever and Hinchcliff, 1995). Studies of horses have attempted to identify factors that may stimulate the release of aldosterone during exercise (Cooley et al., 1994; McKeever et al., 1987; McKeever, 1998; McKeever et al., 1991a; McKeever et al., 1992b; McKeever et al., 1997;). In one study, plasma $[Na^+]$ was not significantly affected by exercise and thus, a decrease in plasma $[Na^+]$ did not appear to have been the primary stimulus for aldosterone release (McKeever et al., 1991a). The primarily supported mechanism for the release of aldosterone appears to be a proportional increase in the plasma renin–angiotensin–aldosterone cascade, where an increase in renin results in the generation of angiotensin I and angiotensin II, with angiotensin II stimulating the production and release of aldosterone (Costill et al., 1976; Kosunen and Pakarinen, 1976; McKeever et al., 2000; Zambraski, 1990).

The relationship between plasma aldosterone concentration and exercise intensity has been reported in horses running on a treadmill. Aldosterone increased from concentrations around 20 to 50 pg/mL at rest to almost 200 pg/mL at a speed of 10 m/s (Kokkonen et al., 2002; McKeever et al., 1992b). A linear relationship was found between exercise intensity and aldosterone concentration. However, unlike renin, aldosterone concentration did not reach a plateau at maximal heart rate (HR_{MAX}). Another study found that during submaximal exercise, increases in plasma aldosterone concentration paralleled changes in renin but that the magnitude of the increase in renin (66%) was less than the relative increase (709%) in aldosterone concentration (McKeever et al., 1991a). The authors concluded that factors other than renin also affected the release of aldosterone in the horse (McKeever et al., 1991a). Of all the parameters reported, a significant increase in plasma $[K^+]$ may have served as the strongest stimulus for the release of aldosterone (McKeever and Hinchcliff, 1995). An increase in plasma $[K^+]$ as small as 0.3 milliequivalent per liter (mEq/L) can be sufficient to stimulate the secretion of aldosterone, independent of the renin–angiotensin cascade, through the conversion of cholesterol to pregnenolone or at a later step in the biosynthetic pathway (McKeever and Hinchcliff, 1995). This is consistent with the acute homeostatic requirements of the horse, since a major perturbation in electrolyte homeostasis observed during endurance exertion was an increase in plasma $[K^+]$ and not a drop in plasma $[Na^+]$ (McKeever et al., 1992b). As with humans, aldosterone has a minimal role in the acute response to exercise in horses. However, aldosterone concentration remains elevated for hours after exercise and may affect the long–term reabsorption

of sodium and water (Convertino, 1991; Hyyppa et al., 1996; McKeever, et al., 2002b) by the kidneys and by the intestinal tract (Bridges and Rummel, 1984; Kokkonen et al., 2002)

Cortisol

The major glucocorticoid secreted by the adrenal glands is cortisol. However, some cortisone, corticosterone, and deoxycorticosterone are also produced (Dickson, 1970; Willmore and Costill, 1994). Cortisol, cortisone, and corticosterone can be found in a ratio of 16:8:0.5 in the plasma of horses; therefore, most exercise studies have focused on cortisol (Dickson, 1970). Cortisol undergoes diurnal variation, with peak levels found in the early morning between 06:00 and 10:00 and lowest levels found in the late evening and overnight periods (Dickson, 1970; Willmore and Costill, 1994). Although glucocorticoids are typically, and correctly, referred to as "stress" hormones, they are also released under a multitude of normal situations not necessarily characterized as stressful. Thus, it is the appropriateness of their release and the magnitude of their release that would indicate whether a given physiologic or psychological disturbance (or stressor) can be classified as a mere perturbation or a true threat to homeostasis.

Release of cortisol allows an individual to tolerate and adapt to challenges to homeostasis that occur in everyday life. To this end, the functional effects of cortisol fall into two major categories: (1) substrate mobilization and (2) immune modulation (Dickson, 1970; Willmore and Costill, 1994). During acute exercise, itself a distinct challenge to homeostasis and a unique stressor, cortisol stimulates substrate mobilization by enhancing gluconeogenesis, mobilizing free fatty acids through lipolysis, and increasing the availability of amino acids through protein degradation (Willmore and Costill, 1994). At the same time, cortisol release decreases glucose utilization by nonessential tissues to spare it for use by the central nervous system (Willmore and Costill, 1994). One could speculate that such an action could delay the onset of central fatigue that occurs during endurance exertion when blood glucose concentrations drop (Farris et al., 1998; McKeever, et al., 2002b). The increase in protein catabolism results in amino acid availability as a source of energy when glucose levels begin to drop. They are available after exercise to repair tissues and for the synthesis of enzymes involved in many cellular pathways. Cortisol's modulation of immune function results from its actions as an antiinflammatory agent and suppressor of immune reactions (Dickson, 1970; Willmore and Costill, 1994).

Overall, these actions may be of significant benefit in the response to training. Overload through increased exercise intensity, resistance, or duration is necessary for training to stimulate an adaptive response to exercise. The minor disruption of function and structure in the cells of muscles results in protein accretion, substrate uptake and deposition, and other beneficial remodeling that increases the functional capacity of the cells. Cortisol's suppression of immune function and antiinflammatory effects may actually provide a permissive environment for tolerating the slight amount of "muscle damage" needed for training-induced remodeling. In this context, it is likely that cortisol serves as an important signal for cellular and muscular repair following intense training. Though cortisol is a catabolic hormone, it would be incorrect to interpret its acute secretion during exercise as an undesirable event if optimal adaptations to training are desired. Instead, cortisol *clearance* and recovery from the stressor may be the more important physiologic occurrences.

Many studies have demonstrated that cortisol is increased in the horse during a wide variety of exercise activities, from racing, to polo, to endurance rides (Caloni et al., 1999; Crandell et al., 1999; Horohov et al., 1999; Hyyppa, 2001; Snow and MacKenzie, 1977; Snow and Rose, 1981). The release of cortisol in the horse appears to be affected both by intensity and duration of exercise (Snow and MacKenzie, 1977; Snow and Rose, 1981). However, excessive increases in cortisol concentrations following exertion can be a marker of too much exercise. Prolonged cortisol recovery times as well as either inappropriately high or low plasma concentrations of cortisol may be a marker of overtraining in the horse. As mentioned above, postexertion effects of cortisol may elicit a permissive effect that is beneficial for training adaptation by preventing the immune system from eliciting an inflammatory and immune reaction to acute exercise (Horohov et al., 1999; Toutain, et al., 1995). Several studies on horses have followed cortisol levels for extended periods following exercise. Those experiments demonstrated that exercise caused a sixfold increase in adrenal cortisol secretion and a two- to three-fold increase in plasma cortisol concentration. Urinary cortisol concentrations also increased threefold with a return to baseline levels by 10 hours after exertion (Toutain, et al., 1995). The authors also noted a substantial increase in liver clearance of cortisol.

Prior studies have suggested that peak postexercise cortisol concentrations are reached earlier in trained horses and that trained horses have a faster cortisol recovery time (Snow and MacKenzie, 1977; Snow and Rose, 1981). On average, peak cortisol levels were observed at about 30 minutes after exertion. Much of this enhanced recovery would be dependent on optimal training volume and rest to produce training adaptations. Still, improved training status may improve the potential to rapidly switch from a catabolic state to an anabolic state.

PANCREAS

The pancreas is a V–shaped organ that lies along the duodenum. Structurally, most of the pancreas is composed of acini, which function as exocrine cells secreting digestive enzymes and bicarbonate into the small intestine via the pancreatic duct. The endocrine function of the pancreas is mediated by the cells of the islets of Langerhans. These specialized cells are arranged as branching cords that are surrounded by a large network or plexus of capillaries. The cells of the islets are classified into three types: (1) the α-cells which produce glucagon, (2) the β-cells which produce insulin, and (3) the δ-cells which produce somatostatin (Dickson, 1970; Willmore and Costill, 1994). Of these hormones, the most important during exercise are insulin and glucagon because of their actions in the control of glucose metabolism.

Insulin

Insulin functions as part of the feedback system controlling blood glucose concentration (Dickson, 1970; Willmore and Costill, 1994). Insulin is primarily a glucose "storage" hormone because it facilitates glucose uptake by the cells, promotes glycogenesis, and inhibits gluconeogenesis (Dickson, 1970; Giraudet et al., 1994; Ralston, 1992; Willmore and Costill, 1994). As such, it is arguably the most potent anabolic hormone. At rest, insulin is the "key" that opens the cellular door to allow uptake of glucose. However, during

exercise, the working muscles take up glucose without insulin. Thus, insulin is very important during the recovery from exercise when glycogen repletion is most active (Dickson, 1970; Davie et al., 1994; Davie et al., 1999; De La Corte et al., 1999; Willmore and Costill, 1994).

The insulin response to acute exercise has been well documented with the horse which, like humans and other species, suppresses insulin during exercise (Duren et al., 1999; Geor et al., 2000; Snow and Rose, 1981). This suppression appears to have a threshold of 50% of $\dot{V}O_{2max}$, which coincides with the increase in catecholamines seen during exercise (McKeever, 2002). Mechanistic studies have demonstrated the link between exercise-induced increases in sympathetic drive, particularly due to epinephrine's inhibitory effects on insulin, and changes in insulin and glucagon secretion in the horse (Geor et al., 2000). Functionally, this allows the animal to increase gluconeogenesis to maintain blood glucose concentrations during exercise (Geor et al., 2000; Willmore and Costill, 1994). Suppression of insulin and maintenance of blood glucose concentration prevents the onset of central mechanisms of fatigue (Farris et al., 1998; McLaren et al., 1989). Much of the recent work on the insulin response to exertion has centered on the composition and timing of pre-exercise feeding (Crandell et al., 1999; Frape, 1988; Hyyppa et al., 1999; Pagan and Harris, 1999; Poso and Hyyppa, 1999). High-carbohydrate feeds are beneficial for optimal muscle glycogen synthesis to fuel exercise. However, the resultant increase in blood glucose seen after a horse eats a high-carbohydrate ration usually evokes an increase in insulin secretion. The goal of recent research has been to prevent this feed-induced spike in insulin that would tend to decrease blood glucose right before or during exercise (Williams et al., 2001a). This phenomenon has often been referred to as "rebound hypoglycemia" (Jentjens and Jeukendrup, 2002). Recent research in humans has suggested that rebound hypoglycemia may be influenced by the timing of carbohydrate intake and the intensity of the exercise but that any hypoglycemic effects may have little, if any, impact on performance (Moseley et al., 2003).

Glucagon

The functions of glucagon are in opposition to insulin in that it stimulates gluconeogenesis and inhibits glycogenesis (Dickson, 1970; Willmore and Costill, 1994). Glucagon is one of many hormones needed for substrate mobilization, and thus, it increases during exercise in the horse (Giraudet et al., 1994). As such, glucagon is important for maintaining glucose concentrations during exercise, a role that is especially important during endurance activities, during which a drop in blood glucose concentrations leads to the onset of central fatigue. Published data from several studies have demonstrated that endurance horses have an increase in glucagon. This increase in glucagon is also altered by exercise intensity, and its release appears to be under the influence of the increases in sympathetic drive and the catecholamines (Geor et al., 2000). Training appears to alter the sensitivity of the glucagon response to exercise, enhancing the ability to mobilize glucose during exertion (Willmore and Costill, 1994).

Other Pancreatic Hormones

Other hormones produced by the pancreas and associated with both endocrine and exocrine pancreatic function may play an important role in modulating substrate disposition during and after exercise. These hormones include pancreatic polypeptide and somatostatin of pancreatic origin (Farrell, 1992). Though amylin and galanin are also produced in the pancreas and can affect pancreatic function and influence pathways involved in insulin regulation (Farrell, 1992) their contributions and responses to exercise in the horse remain relatively unknown.

Pancreatic Polypeptide

This hormone does not appear to affect insulin or glucagon concentrations. However, comparative studies have suggested that pancreatic polypeptide affects digestion (Farrell, 1992). Hall and coworkers point out that pancreatic polypeptide inhibits pancreatic exocrine function and bile secretion, an observation that they considered appropriate for a horse during long-term exercise when food intake would tend to be minimal. Information regarding the effect of exercise on pancreatic polypeptide is minimal. However, Hall and coworkers demonstrated that pancreatic polypeptide increases in the horse with endurance exercise from concentrations averaging 20 picomoles per liter (pmol/L) at rest to levels as high as 102 pmol/L after an 80-km ride. Lower concentrations seen after a 42-km race would suggest a dependence on duration, which may be related to the degree of hypoglycemia seen in the horses after exertion. These results are similar to those seen in other species (Farrell, 1992). We are unaware of any published studies that have examined the effect of exercise intensity on pancreatic polypeptide in the horse.

Somatostatin

Though produced in the hypothalamus, the gastrointestinal tract (see below), and the pancreas, there appear to be different functions for this hormone, depending on the origin. For example, it is well recognized that the somatostatin produced in the hypothalamic region of the brain inhibits somatotropin (i.e., GH) as well as thyrotropin release. However, somatostatin of pancreatic origin is produced by the delta cells of the pancreas and functions locally to alter pancreatic function as well as regionally to possibly alter blood flow and also restrict nutrient absorption. Hall and coworkers have suggested that this fits with the well-documented reduction in splanchnic blood flow that occurs during exercise. A small but significant increase in somatostatin concentration has been demonstrated during endurance exercise with no differences due to duration (42 km versus 80 km). This is consistent with studies of humans and other species (Farrell, 1992). Published studies of the horse have not examined the effect of exercise intensity on concentrations of somatostatin.

CIRCULATING GASTROINTESTINAL ("GUT") HORMONES

Several other substances with endocrine and paracrine function are secreted by the digestive tract (Farrell, 1992). These substances alter digestive function and, thus, may influence digestion and the absorption of substrates during exercise (Farrell, 1992).

Gastric Inhibitory Peptide

GIP is a 42–amino acid peptide that has a dual action: (1) inhibiting gastric acid production and (2) stimulating insulin secretion. Hall and coworkers reported that plasma GIP concentrations were not altered during endurance exercise

(42 km and 80 km). However, those results should be interpreted with caution as the authors had relatively low animal numbers. The longer endurance competition resulted in a nonsignificant decline in plasma GIP concentration from approximately 75 pmol/L before exercise to about 50 pmol/L after 80 km. The decline in GIP and its insulin secretagogue action would be consistent with the substantial and significant suppression of insulin concentrations reported in those same horses, though there were likely other mechanisms also influencing this effect.

Vasoactive Intestinal Polypeptide

VIP is a 28–amino acid peptide that serves as a neurotransmitter and is secreted by nerve fibers in the gastrointestinal tract. Multiple actions of VIP include vasodilation, stimulation of glucagon secretion, and enhancement of the stimulation of substrate release through lipolysis and hepatic glycogenolysis. Endurance exercise has a pronounced effect on VIP secretion, with increases in plasma concentrations that appear to be affected by exercise duration. This coincides with the energy substrate needs associated with prolonged exercise.

Gastrin

A 10–amino acid peptide, gastrin stimulates gastric acid secretion and is affected by prior ingestion of food. Limited data are available on the effects of exercise on the plasma concentration of this hormone in the horse. However, Hall and coworkers found that plasma concentrations of gastrin were not altered by a 42-km endurance ride and that they were substantially increased following a longer 80-km ride. One would presume that the horses in the longer ride went for a longer time without food intake; therefore, this increase in gastrin secretion would seem unwarranted. However, one wonders if this paradoxical increase contributes to excessive acid production and gastric ulcer formation when food is withheld from a horse for a long period.

Other Gastrointestinal Peptides with Endocrine or Paracrine Actions

Most of the gastrointestinal peptides with endocrine or paracrine actions have not been studied in the horse. However, comparative studies have shown that they have localized action within the gastrointestinal tract. Many of these substances have been characterized as neurotransmitters that act on local tissues by altering membrane transport, stimulating motility, and, in some cases, stimulating acid production (Farrell, 1992). For example, gastrin-releasing peptide (also referred to as *bombesin*) stimulates gastrointestinal motility and the release of gastrin. Secretin and enteroglucagon both inhibit acid secretion in the stomach (Farrell, 1992). Comparative studies have shown that an increase in both hormones is seen in peripheral blood collected after exercise in humans and dogs (Farrell, 1992). Motilin, a peptide that stimulates motility of the gastrointestinal tract, has also been shown to increase with exercise in humans. Comparatively, enkephalins appear to have the opposite effect by decreasing motility. Other peptides such as substance P, neuropeptide Y, and peptide YY appear to alter gastrointestinal tract motility, but little is known about changes during exercise. Interestingly, the functional link between all the gastrointestinal tract hormones appears to be their actions on motility and, possibly, transport (Farrell, 1992). These actions may make them important in the uptake of water, electrolytes, and energy substrates during and after exercise.

HORMONES RELATED TO APPETITE AND ENERGY BALANCE

Maintaining energy balance is crucial for the optimal health and performance of exercising horses. The energy expended during exercise directly affects energy homeostasis because the horse has to increase energy intake to compensate both for the energy lost during exercise and for the energy required for the recovery and repair of tissues. Although the neuroendocrine control of energy balance has been studied extensively in humans and rodents, it is really just beginning to be examined in horses. Examples of some of the endocrine mediators measured to better understand the control of energy balance are the hormones leptin, adiponectin, ghrelin, and cholecystokinin.

Leptin

Leptin is an adipocyte-derived hormone and a 16-kilodalton (kD) protein product of the *ob* gene that acts as an indicator of energy balance and a signal for satiety (Halaas et al., 1995; Zhang et al., 1994; Zhang et al., 2002). Sensed levels of leptin influence neural transmission in brain pathways, affecting food intake and energy utilization (Schwartz et al., 2000). High levels of leptin appear to favor increases in energy expenditure while decreasing food intake, and vice versa (Schwartz et al., 2000). Food intake is altered by leptin through its influence on responsive neurons in the brain that either activate a feeding or satiety system (Schwartz et al., 2000). The feeding or orexigenic system contains neuropeptide Y, agouti-related protein, and other hormones and neurons that signal an animal to increase food intake. The satiety system, however, involves neurons containing proopiomelanocortin (POMC) and α-melanocyte stimulating hormone which decrease food intake (Saper et al., 2002). Leptin increases energy expenditure by stimulating the sympathetic nervous system's effects on brown adipose tissue, directly increasing the expression of uncoupling protein 1, and by possibly increasing the expression of uncoupling protein 3 in muscle (Giacobino, 2002). Furthermore, leptin strongly stimulates triglyceride and fatty acid cycling by increasing lipolysis and fatty acid oxidation (Reidy and Weber, 2002). Leptin is secreted in proportion to fat mass (Reidy and Weber, 2002), although humans who are obese seem to be "resistant" to its weight-loss properties (Zhang et al., 1994; Hamilton et al., 1995).

In horses, plasma leptin is correlated with percent fat mass and body condition score (Buff et al., 2002; Kearns et al., 2002). Leptin has also been found to have a seasonal variation in both young and old mares, with plasma leptin levels increasing in the summer and decreasing in the winter, in correlation to body weight and percent fat mass (Fitzgerald and McManus, 2000). Furthermore, 24-hour fasting decreases plasma leptin levels in young and mature mares (McManus and Fitzgerald, 2000). Interestingly, one study showed that serum concentrations of leptin were higher in geldings and stallions versus mares, which is incongruent with human literature, in which females have been reported to have higher leptin levels than do males, with differences not completely explained by the greater percent fat mass in females (Buff et al., 2002; Saad et al., 1997). In rats, research has demonstrated that male rats have higher leptin concentrations in blood compared with female rats

(Mulet et al., 2003). The reason for the discrepancy between species is unclear.

With regard to exercise, there have been some interesting findings in humans on how the type and duration of exercise effect energy balance and leptin concentrations in blood. To date, no such studies in horses have been published. In humans, however, studies involving short-duration exercise (<60 min) with varying intensities have generally shown no change in leptin concentrations caused by exercise (Dirlewanger et al., 1999; Weltman et al., 2000). Studies that have reported changes in leptin concentration with short-duration exercise have generally attributed the changes to hemoconcentration or circadian rhythm (Fisher et al., 2001; Kraemer et al., 1999a). It is possible that short-duration acute exercise does not cause a sufficient energy deficit to cause a disruption in long-term energy balance. Also, the interaction between other hormones (e.g., cortisol, insulin, glucose, epinephrine, and norepinephrine) that fluctuate during exercise and have been found to either stimulate or inhibit leptin secretion remains to be seen (Essig et al., 2000; Fisher et al., 2001; McKeever, 2002).

Longer duration exercise (≥60 min) of varying intensities has been shown to decrease or cause no change in leptin concentrations (Leal-Cerro et al., 1998; Racette et al., 1997; Torjman et al., 1999). Interestingly, studies showing reductions in leptin increased sampling times for up to 48 hours after exercise, with a reduction in the 24-hour mean and amplitude of the circadian rhythm of leptin (Essig et al., 2000; Olive and Miller, 2001). It appears that long-duration exercise provides enough of an energy deficit to decrease leptin levels, which, in turn, will increase food intake to help maintain energy balance (Karamouzis et al., 2002).

Training is another aspect of exercise that is of interest to scientists studying leptin concentrations and energy balance. Training regimens have had different effects, depending on duration and intensity of exercise and subjects used. Overall, training for less than 12 weeks generally causes no change in leptin levels, although individuals with type II diabetes did show a reduction in leptin concentrations after 6 weeks of low-intensity walking and cycling, independent of body composition changes (Halle et al., 1999; Houmard et al., 2000). Training regimens for longer than 12 weeks can cause a reduction in fat mass, which lowers leptin levels, and yet some studies reported a reduction in leptin independent of fat mass changes (Reseland et al., 2001). It is possible that these changes may be dictated by alterations in leptin receptor sensitivity. Additionally, it appears that females are more sensitive to the training effect on leptin levels, with several studies showing that only female subjects demonstrated lower leptin concentrations in response to training (Hickey et al., 1997). This may be one of the reasons that leptin has been associated with athletic amenorrhea in humans (Laughling and Yen, 1997).

Leptin has several potential roles in terms of the health of exercising horses. As a signal of energy homeostasis, leptin concentrations in horses can help scientists to determine if a horse is in positive or negative energy balance and can provide supportive data regarding a horse's body condition and percent fat mass. Negative energy balance is detrimental to exercise performance, reproductive status, and overall health (Saris, 2001). Furthermore, determining how exercise and training affects leptin concentrations in horses will allow scientists to better understand how horses regulate their energy balance so that training regimens and diets can be adjusted accordingly to optimize the health of the athlete.

Adiponectin

Adiponectin is another hormone secreted from adipocytes, with its role in metabolism related to the regulation of glucose, insulin, and adipocyte metabolism (Berg et al., 2002). In contrast with leptin, adiponectin levels are decreased in obese and insulin resistant humans and animals (Arita et al., 1999; Hotta et al., 2000). With regard to exercise, adiponectin may have a role in the increased insulin sensitivity seen as a result of training in both humans and horses (Hayashi et al., 1997; Malinowski et al., 2002). However, this is not always a consistent finding, as at least one study of humans undergoing exercise training found that plasma adiponectin concentrations did not change relative to the increased insulin sensitivity caused by training (Hulver et al., 2002).

In horses, adiponectin is negatively correlated with percent fat mass in yearling fillies and mature mares (Kearns et al., 2002). The study of adiponectin in horses is of importance as it is likely related to the insulin resistance commonly seen in older horses and horses with pituitary adenomas. Studies have shown that older mares with impaired glucose tolerance were able to improve their insulin sensitivity with 12 weeks of training (Malinowski et al., 2002). It would be of interest to determine if adiponectin has a role in this insulin-sensitizing phenomenon.

Ghrelin

Another hormone involved in the control of appetite and energy balance is ghrelin, a protein hormone secreted from the stomach, which was first discovered as a potent growth hormone secretagogue (Kojima et al., 1999). Ghrelin has received most of its attention, though, due to its role in initiating food intake in humans and rodents (Wren et al., 2001). In meal fed animals, including humans, rodents, and sheep, ghrelin increases before meal feeding in anticipation of the meal and will also increase during times of fasting (Cummings et al., 2001; Murakami et al., 2002; Sugino et al., 2002). In rats, ghrelin stimulates gastric acid secretion in the stomach (Date et al., 2001; Masuda et al., 2000). Few studies that examine the role of ghrelin in relation to exercise in any species have been conducted to date. One study in humans, however, demonstrated that ghrelin levels did not change during submaximal aerobic exercise in healthy adults (Dall et al., 2002).

Ghrelin is a valuable hormone to attempt to study in horses, as it may have a significant role in helping horses to maintain energy balance. In addition, high-performing equine athletes often have problems with inappetance and gastric ulcers that may be related to abnormal ghrelin concentrations and ghrelin's stimulation of gastric acid (Murray et al., 1989). Humans with anorexia exhibit higher ghrelin concentrations compared with their normal counterparts, with a presumed "ghrelin" resistance contributing to the cachexia of this eating disorder (Otto et al., 2001; Rigamonti et al., 2002).

Cholecystokinin

The peptide hormone cholecystokinin (CCK) is secreted from the small intestine and is involved in energy balance by signaling fullness and decreasing food intake in humans, rodents, and ruminants (Ballinger et al., 1995; Grovum, 1981). To date, there has been little published data on CCK in horses. However,

theoretically, this hormone may play a role in the inappetance commonly seen in heavily exercised horses. In a study conducted in humans, exercise increased plasma CCK concentrations fourfold. Although CCK values returned to normal at the end of exercise, equine researchers have speculated that CCK concentration may increase in response to exercise in horses and remain elevated, contributing to a lack of interest in feed seen in some equine athletes (Bailey et al., 2001).

Finally, it must be recognized that there is also an interaction among many of the above-mentioned hormones in relation to energy balance. For example, CCK enhances the effect of leptin administration on weight loss, and the pair may directly decrease food intake (Emond et al., 1999; Matson et al., 2002). Leptin and adiponectin, although expressed in opposite concentrations to one another, may be regulated similarly for short-term alterations but differently for long-term regulation (Zhang et al., 2002). Ghrelin, however, is upregulated during leptin therapy, though these increases in ghrelin are not able to overcome the decreased food intake caused by leptin (Bagnasco et al., 2002; Beretta et al., 2002). Hence, it is clear that these endocrine mediators should be studied collectively to determine how they interact to regulate various systems.

In conclusion, the regulation of energy balance in horses and how it is affected by exercise is a field that has yet to be investigated in depth. However, data published in humans and other species demonstrate the importance of such research, especially with regard to gastric ulcer syndrome and inappetance commonly seen in heavily exercised horses, as well as obesity and insulin resistance seen in many older horses. Hopefully, future research in this field will elucidate the mechanisms of energy balance in horses, how this balance is maintained in response to exercise, and ways in which management practices can be changed to help horses remain in energy balance and achieve optimal performance and health.

KIDNEYS

Filtration of the blood and conservation of vital substances are the most commonly acknowledged functions of the kidneys (McKeever, 1998; Zambraski, 1990). However, their close link to cardiovascular function is more complex and multifaceted. The basic filtration unit is the kidney glomerulus. Each of these glomeruli has a specialized group of cells that form what is referred to as the *juxtaglomerular apparatus (JGA)*. These regions have specialized cells that act as feedback sensors monitoring flow (and pressure), sodium and chloride concentration, and arterial oxygen partial pressure (pO_2). One of the major hormones produced by the kidney is renin, the activating substance in the renin–angiotensin–aldosterone cascade, which has the potential to alter blood pressure, blood volume, and blood tonicity. The kidney also produces erythropoietin, which acts on precursor cells in the bone marrow to stimulate red blood cell production. Both of these hormone systems play an important role the defense of normal cardiovascular function (McKeever, 1998; Zambraski, 1990).

Renin

Renin facilitates the conversion of angiotensinogen to angiotensin I, which is ultimately converted to angiotensin II, which then stimulates the production and release of aldosterone. During exercise, renin activity is a measure of the rate of the generation of angiotensin I (McKeever et al., 1992b; Zambraski, 1990). Angiotensin I and angiotensin II are powerful vasoconstrictors involved in the control of MAP and blood flow during exercise. After exercise, renin can directly affect renal function, and angiotensin stimulates thirst and drinking, thus altering postexercise fluid balance (Convertino, 1991; McKeever et al., 1992b). Three major mechanisms that may account for the increase in renin during exercise are (1) renal nerve stimulation via increased sympathetic drive, (2) changes in renal blood flow and pressure associated with JGA function, and (3) changes in electrolyte (sodium and chloride) concentrations at the JGA in the kidney (McKeever et al., 1992b; Zambraski et al., 1984; Zambraski, 1990).

Previous studies have measured renin in horses at rest, after exercise training, during steady-state exercise, or after brief maximal exercise (Cooley et al., 1994; McKeever et al., 1987; McKeever et al., 1991a; McKeever et al., 1992b; McKeever, 1998; McKeever et al., 2000). Strong linear correlations between work intensity (and duration), increases in renin, and heart rate were reported up to treadmill speeds of approximately 9 m/s (McKeever et al., 1992b). Above 9 m/s, heart rate and renin reached a plateau and did not increase when the speed was increased from 9 to 10 m/s (McKeever et al., 1992b). In previously published studies of horses, renin increased from 1.9 ± 1.0 nanogram per milliliter per hour (ng/mL/hr) at rest to a peak of 5.2 ± 1.0 ng/mL/hr at 9 m/s (McKeever et al., 1992b). The observed concurrent plateau in renin and heart rate supports the suggestion that the increase in renin during exercise in the horse is linked to sympathetic drive. Such is also the case in other species in which mechanistic studies have demonstrated a correlation between renal sympathetic nerve activity and renin (Zambraski et al., 1984; Zambraski, 1990). During steady-state submaximal exercise, the major factor stimulating an increase in renin early in exercise was an increase in sympathetic drive (McKeever, 1998; Zambraski, 1990). However, a secondary increase in renin was seen in horses after 40 minutes of exercise and was most likely caused by a decrease in plasma [Cl^-] concentration, which fell significantly during this period, and not plasma [Na^+], which remained constant (McKeever et al., 1991a).

Functionally, an increase in renin during exertion has been shown to result in an increased plasma angiotensin II concentration (McKeever, 1998; Zambraski, 1990). Interestingly, horses given Enalapril, the angiotensin-converting enzyme (ACE) inhibitor, had significantly lower plasma angiotensin II and aldosterone concentrations and pulmonary artery pressures during exercise compared with horses given a placebo (McKeever et al., 2000). These observations demonstrate that the renin–angiotensin cascade plays a role in the control of blood pressure during exercise in the horse.

Erythropoietin

Erythropoietin (EPO) is a peptide hormone that is produced by the kidneys in response to hypoxia sensed by pericellular cells positioned in the vasculature of the renal matrix (Giger, 1992; Kearns et al., 2000; McKeever, 1996). Recent papers have documented the effects of a variety of perturbations, including the effects of blood loss, acute exercise, and altitude, on EPO production in humans (Giger, 1992; McKeever et al., 1999). If cardiopulmonary adjustments are insufficient to prevent hypoxemia and if the above-mentioned perturbations are large enough to cause a decrease in renal arterial pO_2, then plasma EPO concentrations increase, and there is a subsequent stimulation of erythropoiesis in humans. The resultant increase in red blood cell (RBC) volume would be expected to couple

with other compensatory mechanisms to return arterial pO_2 to normal levels.

McKeever et al. (2002c) recently reported that acute exercise does not appear to stimulate an increase in plasma EPO concentration in normal horses. This is similar to observations made in several studies on humans, which demonstrated that neither the intensity nor the duration of acute exercise alters plasma EPO concentrations in humans (Bodary et al., 1999; Schmidt et al., 1991). This makes intuitive sense because one would speculate that if acute exercise caused substantial increases in EPO production and release, then repeated exercise (i.e., training) would cause a sustained increase in EPO. Any such hypothetical repeated increase in circulating EPO concentration would be expected to cause a sustained stimulation of erythropoiesis. Taken a step further, this would eventually cause a red cell hypervolemia or polycythemia and potentially detrimental increases in blood viscosity. Mechanistically, the acidosis of exercise appears to inhibit EPO production (Ekhardt et al., 1990).

Altitude appears to cause a transient increase in plasma EPO production in humans and horses (McKeever et al., 2002c; Boning et al., 1997; Milledge and Cotes, 1985; Willmore and Costill, 1994). However, in studies on horses, plasma EPO concentrations were seen to increase only during the first 3 hours of the first day at 3800 meters (McKeever et al., 2002c). This is similar to findings in studies of humans, which reported a "temporary rise" in EPO concentrations in mountaineers climbing both at 4900 and 7600 m (Boning et al., 1997). One explanation for this rapid return to baseline concentrations is a rapid compensation of cardiorespiratory mechanism to the challenge of altitude that would tend to limit changes in arterial pO_2 at the kidney (Boning et al., 1997). Interestingly, exercise performed at altitude did not induce a secondary increase in plasma EPO concentration (McKeever et al., 2002c). The authors concluded that hypoxia positively affects EPO production in the horse rapidly on the first day at altitude, with a rapid return to pre-altitude concentrations (McKeever et al., 2002c). Their data suggest that horses have an innate ability to tolerate the acute challenges induced by exercise and altitude.

Administration of recombinant human erythropoietin (rhEPO) has been shown to increase hemoglobin concentration and exercise capacity in humans with chronic renal failure (Adamson and Vapnek, 1999; Berglund and Ekblom, 1991; Cowart, 1989). Even relatively small doses of rhEPO have been found to increase hemoglobin concentration by 30% and increase endurance performance anywhere from 10% to 19% in healthy human subjects (Adamson and Vapnek, 1999; Berglund and Ekblom, 1991; Cowart, 1989). Purportedly, human athletes, their trainers, or both have decided to use higher-than-recommended doses of erythropoietin, with the rationale that the more rhEPO injected, the greater the increase in aerobic capacity (Adamson and Vapnek, 1999; Berglund and Ekblom, 1991; Cowart, 1989). Injections of rhEPO have been shown to elevate resting hematocrit to levels greater than 55% in humans, which increases blood viscosity and clotting and worsens the risk of heart attack or stroke (Adamson and Vapnek, 1999; Cowart, 1989). These increases in hematocrit and blood viscosity may have caused excessive increases in blood pressure and clotting problems related to the deaths of human athletes in Europe (Adamson and Vapnek, 1999; Cowart, 1989). Unfortunately, these practices have entered equine sports medicine, with clinicians, horse trainers, and racing commission personnel reporting that this drug is being misused in racehorses to improve racing performance through an increase in blood volume and oxygen-carrying capacity (Jaussaud et al., 1994; McKeever, 1996; Souillard et al., 1996). However, two major problems can develop in horses (McKeever, 1996; Piercy et al., 1998). First, while the horse can tolerate hematocrits of 50% to 60% during exercise, no one knows what happens to a horse's cardiovascular system if the normal resting hematocrit is artificially elevated to values of 70% to 80%. A large increase in resting hematocrit coupled with splenic reserve mobilization may produce dangerously viscous blood that may lead to sudden death during or after exercise (McKeever, 1996). If this increase in resting RBC volume were to be coupled with furosemide-induced fluid losses, the results could be devastating (McKeever, 1996). A second major potential problem associated with rhEPO misuse in the athletic horse is its reactivity with the horse's immune system. Some horses have purportedly developed life-threatening anemia associated with an immune reaction to both the exogenous rhEPO and the animal's own EPO (McKeever, 1996; Piercy et al., 1998). Clinically, resting hematocrit has been seen to drop below 20% in horses reacting to rhEPO administration, with some horses requiring life-saving blood transfusions (Piercy et al., 1998).

Recently, studies of splenectomized and intact horses have demonstrated that rhEPO administration in low doses causes substantial increases in resting hematocrit, RBC volume, $\dot{V}O_{2max}$, blood viscosity, and selected hemodynamic variables during incremental exercise performed on a treadmill (McKeever et al., 1993b; McKeever et al., 1999). In splenectomized horses, administration of rhEPO in low doses (15 international units per kilogram [IU/kg]) three times a week for 3 weeks increases in resting hematocrit from 37% up to 46% (McKeever et al., 1993b). The resulting 13% increase in RBC volume was associated with a 19% increase in $\dot{V}O_{2max}$ and substantial increases in blood viscosity (McKeever et al., 1993b). Another study also demonstrated that administration of low-dose rhEPO (50 IU/kg, three times per week for 3 weeks increases RBC volume, $\dot{V}O_{2max}$, and the velocity at $\dot{V}O_{2max}$) (McKeever et al., 1999). Though viscosity was not measured in that study, postexercise hematocrits were in the low 70s and considered dangerously high (McKeever et al., 1999). Horses in that study also developed antibodies to rhEPO (McKeever, unpublished data).

HEART AND BLOOD VESSELS

The heart and blood vessels play both paracrine and endocrine roles in the control of cardiovascular function. Although there are several mechanisms worthy of discussion, two hormones appear to play a major role during exercise: (1) atrial natriuretic peptide and (2) the endothelins (McKeever and Hinchcliff, 1995).

Atrial Natriuretic Peptide

ANP is produced by the heart and is important in the regulation of blood flow and blood pressure during exercise (Freund et al., 1988b; McKeever and Hinchcliff, 1995). Granules of ANP are stored within the walls of the atria and are released during atrial stretch (McKeever and Hinchcliff, 1995; Richter et al., 1998). Receptor sites for ANP have been identified in the posterior pituitary, the kidneys, vascular smooth muscle, the adrenal cortex, the heart, and the lung. This hormone causes a rapid and profound vasodilation and a pronounced natriuresis. ANP inhibits vasopressin, renin, and aldosterone secretion and also inhibits the binding of aldosterone at the kidney tubule

(McKeever and Hinchcliff, 1995; McKeever et al., 1991b). On a practical level, ANP may be involved in accommodating the exercise related shifts of blood volume in the horse (McKeever and Hinchcliff, 1995; McKeever et al., 1991). Evidence for this was provided by two recent studies that demonstrated that plasma ANP increases in a linear fashion with increasing work intensity, rising from 5 to 10 pg/mL at rest to concentrations exceeding 60 pg/mL at speeds eliciting $\dot{V}O_{2max}$ (McKeever and Hinchcliff, 1995; McKeever et al., 1991b; McKeever and Malinowski, 1999; Nyman et al., 1998). Mean ANP concentration was also highly correlated with heart rate (McKeever et al., 1991b). Furthermore, ANP increased from about 10 pg/mL at rest to a peak of 40 pg/mL after 40 minutes of steady-state submaximal exercise (Kokkonen et al., 1999; McKeever and Hinchcliff, 1995; McKeever et al., 1991a). Nyman and coworkers (Nyman et al., 1998) found similar peak plasma ANP concentration during steady-state exertion and reported that ANP concentrations were altered by hydration status. Horses that were overhydrated had the highest ANP concentrations during exercise compared with control and dehydrated horses (Nyman et al., 1998). Another study found no differences between arterial and mixed venous ANP concentrations, suggesting that ANP is either not metabolized by the lung or is released from the left atrium at a rate matching pulmonary metabolism (McKeever et al., 1992a). Even more recent work has examined the effects of exercise on ANP, with a special focus on its interaction between fluid and electrolyte status and other endocrine responses (Kokkonen et al., 1995; Kokkonen et al., 2002). The authors concluded that ANP remains elevated after exercise and that this was a response to the exercise-induced increase in circulating blood volume rather than an interaction with vasopressin and the catecholamines (Kokkonen et al., 2002).

Endothelin

The endothelins are peptide hormones that have pronounced effects on neuroendocrine control of cardiovascular function (Maeda et al., 1996; McKeever et al., 2002; Rossi, 1993; Rubany and Shepherd, 1992). The endothelins ET-1, ET-2, and ET-3 are isoforms of a 21-amino-acid polypeptide with pronounced effects on both central and peripheral control of cardiovascular function. The three sequences of endothelin are structurally and pharmacologically distinct, arising from what has been called "big endothelin," a 39-amino-acid precursor molecule. The half life of ET-1 is very short (only a few minutes), which is consistent with its role in control of vascular tone (Rossi, 1993; Rubany and Shepherd, 1992). Factors affecting the release and metabolism of endothelin include increased blood flow, vasopressin, angiotensin, shear stress, and thrombin (Rossi, 1993; Rubany and Shepherd, 1992).

Many studies have shown that ET-2 and ET-3 are limited in their vascular effects and that ET-1 has the most pronounced effect on peripheral vascular tone (Rossi, 1993; Rubany and Shepherd, 1992). Endothelial cells produce ET-1 exclusively (Rossi, 1993), and circulating levels of this hormone may play a role in certain forms of hypertension (Rossi, 1993; Rubany and Shepherd, 1992). ET-1 and ET-2 (to a lesser degree) are potent vasoconstrictors that can increase systemic and pulmonary arterial blood pressure and cause alterations in cardiac output and the distribution of blood flow in the peripheral circulation (Rossi, 1993; Rubany and Shepherd, 1992). Thus, ET-1 may play a role in the redistribution of blood flow and control of blood pressure during exercise. ET-3 appears to play a role in modulating the release of vasopressin from the hypothalamus, and Rossi (1993) showed that it amplifies free water excretion independent of renal and systemic hemodynamic, osmotic clearance, and circulating vasopressin concentrations, or all of these.

Resting plasma concentrations of immunoreactive ET-1 measured in horses (Benamou et al., 1998; Benamou et al., 1999; Benamou et al., 2001a; Benamou et al., 2001b; McKeever and Malinowski, 1999; McKeever et al., 2002) appear to be similar to the relatively low concentrations reported for other mammalian species such as the rat, dog, pig, cow, and man (Richter et al., 1994; Rossi, 1993; Rubany and Shepherd, 1992). Studies of ET-1 in horses have focused primarily on either understanding the role in respiratory disease (Benamou et al., 1998; Benamou et al., 1999; Benamou et al., 2001a; Benamou et al., 2001b) or aging (McKeever and Malinowski, 1999; McKeever et al., 2002). In some of those studies, ET-1 was found to be elevated in blood and bronchiolar alveolar lavage fluid in resting horses with respiratory disease (Benamou et al., 1998; Benamou et al., 1999). Many recent experiments have only reported on samples obtained either before and after exertion (Benamou et al., 1998; Benamou et al., 1999) or at rest and at the speed eliciting $\dot{V}O_{2max}$ (McKeever et al., 2002). However, one study examined plasma ET-1 concentrations in the horse during exercise, rather than just collecting blood samples before and after exercise, and no changes were revealed in plasma ET-1 concentration during exercise (McKeever et al., 2002). Interestingly, although plasma ET-1 concentration did not change with increases in exercise intensity, it did increase substantially in samples collected immediately after the exercise stimulus was withdrawn and in blood collected 2 minutes following cessation of exercise (McKeever et al., 2002). However, plasma ET-1 concentrations were back to normal by 10 minutes (McKeever et al., 2002). This rapid response may be physiologically significant, as it coincides with the rapid recovery and transient decreases in cardiovascular function reported in other studies of horses that have involved protocols with a quick stop of the treadmill (McKeever and Hinchcliff, 1995; McKeever et al., 2000). Similar postexertion increases in plasma ET-1 concentration have also been reported in studies of normal humans and in studies where the subjects had various diseases affecting the vasculature. In one of those experiments, even greater increases in plasma ET-1 concentrations were seen in dehydrated humans (Richter et al., 1994). The greater increase in plasma ET-1 concentration caused by volume depletion supports the suggestion that ET-1 plays a role in the modulation of vascular tone in the defense of MAP (Rubany and Shepherd, 1992). Thus, the postexertion increase in plasma ET-1 concentration observed in horses (McKeever et al., 2002) is consistent with previously published reports on the hemodynamic and endocrine responses to exercise in horses (Cooley et al., 1994; McKeever and Hinchcliff, 1995). An increase in plasma ET-1 concentration following rapid cessation of prolonged exercise, at a time when postexertion blood pressure would be expected to fall, fits with the neuroendocrine response to other perturbations affecting vascular fluid volume, cardiac filling pressure, and MAP. This would be an appropriate response in the regulation of cardiovascular function during the transition from exertion to recovery when heart rate, cardiac output, and

blood pressure are declining rapidly in the face of exercise-induced vasodilation of vascular beds supplying muscles.

Elevated resting plasma ET-1 concentrations in rats and humans with diseases, particularly in humans with chronic obstructive pulmonary disease (COPD) and pulmonary hypertension, has been well documented (Maeda et al., 1996; Manohar et al., 1993; Rubany and Shepherd, 1992). Benamou et al. (1999) demonstrated that postexercise ET-1 concentrations were substantially elevated in the bronchoalveolar lavage fluid of horses with exercise-induced pulmonary hemorrhage (EIPH). In another study, Benamou et al. (2001b) demonstrated that ET-1 type A receptors mediate the vasoconstrictor action of ET-1 in the pulmonary and systemic circulations of the horse. However, data from another study (Benamou et al., 2001a) suggest that ET-1 is not a mediator of the acute hypoxic pulmonary hypertension response to exercise, but it may serve as a modulator of the acute response or slower phase of hypoxic pulmonary hypertension response to exercise. More work is needed to determine if ET-1 plays a role in the pathogenesis of EIPH, especially since some feel increases in pulmonary artery pressure during exercise may contribute to this phenomenon (Manohar et al., 1993; Pascoe, 1996).

GONADS AND REPRODUCTIVE HORMONES

Reproductive hormones are essential for the health and well-being of a mare or stallion. However, exercise performance is not affected, per se, by reproductive hormones. Nevertheless, recent human research has focused a great deal of attention on the effects of exercise on the female reproductive cycle and the interaction of prolactin, LH, FSH, estrogen, and B-endorphin (Willmore and Costill, 1994). Human studies have also examined the effects of acute exercise on the health of pregnant women. More work is needed in the horse to determine if exertion affects these hormones, especially in endurance horses and in pleasure horses that are ridden while they are in foal. The following evidence is based primarily on studies in humans. Although FSH and LH are technically anterior pituitary hormones, their effects will be discussed here in relation to estradiol and testosterone responses.

Follicle Stimulating Hormone

The primary role of follicle stimulating hormone (FSH) involves the regulation of reproductive capacity in both men and women. Although intensity and duration determine the response of many hormones to the stimulus of exercise, it appears that neither of these factors affects FSH secretion. For example, Semple et al. (1985) recorded the hormonal response in 10 healthy males prior to and within 30 minutes of completing the Glasgow marathon. In agreement with the high degree of stress involved in running a marathon, plasma cortisol levels increased two- to fivefold. LH and testosterone significantly declined, but FSH remained unchanged. Marathon running is primarily an aerobic exercise, but Raastad and colleagues (2000) took similar measurements in healthy men during both moderate- and high-intensity resistance training. Despite the markedly different exercise intensity, FSH remined unchanged. Similar results have been seen in trained and untrained subjects (Di Luigi et al., 2002), and also in women regardless of contraceptives use or exercise intensity (Bonen et al., 1979). Thus, results from a wide range of exercise interventions, obtained from a broad group of subjects, all agree that exercise appears to have little impact on FSH secretion.

Luteinizing Hormone and Estradiol Interactions

LH has a primary role in the reproductive systems of both males and females. In the female, LH is largely responsible for ovulation and menstrual cycle regulation. Evidence suggests that in premenopausal women, consistent workouts above the lactate threshold can disrupt the luteal phase of the menstrual cycle (Rogol et al., 1992). Although the overall menstrual cycle length was not significantly impacted, a shift in the phases resulted in a significantly decreased luteal phase. This has been shown to decrease the fertility of women because of a decreased ability of the corpus luteum to secrete progesterone in sufficient doses or duration to support implantation and pregnancy (Balash et al., 1987; Jones et al., 1970). Baker et al. compared resting estradiol and LH concentrations in female runners and found that they were significantly lower in amenorrheic women compared with eumenorrheic women (Baker, 1981). This reduction in LH has also been seen in male runners (McColl et al., 1989; Semple et al., 1985). Decreases in LH appear to be caused by a disruption in the hypothalamic–pituitary–gonadal (HPG) axis. Blunting of the HPG response originates with a decrease of gonadotropin-releasing hormone (GnRH), which would decrease LH release, which leads to decreases in estradiol, progesterone, or testosterone. Most of the decreases in LH and estradiol seem to appear with long-duration, sustained exercise. However, not all exercise has reported a blunted estradiol response. Jurkowski et al. used varying exercise intensities (30%, 60%, or 85% $\dot{V}O_{2max}$) to compare the postexercise estradiol concentration in healthy premenopausal women (Jurkowski et al., 1978). Results indicated a dose-dependent increase in estradiol after exercise. Most studies that have shown increased estradiol concentrations have used relatively shorter-duration acute exercise bouts (Consitt et al., 2002). Keizer and coworkers found increased estradiol in subjects after an acute aerobic session using a bicycle ergometer. However, they attributed the increase in estradiol concentration following a 40-minute exercise bout (70% $\dot{V}O_{2max}$ for 10 min, 25% $\dot{V}O_{2max}$ for 30 min) to a decrease in the metabolic clearance rate of the hormone (Keizer et al., 1980). The increases in estradiol concentration immediately after acute exercise are consistent with increases in testosterone in men.

An increased estradiol concentration or the maintenance of normal estradiol concentrations is a major factor in the maintenance of a regulatory menstrual cycle and ovulation. Other health complications can arise from consistently lowered estradiol. When estradiol remains low, bone resorption can be greater than bone formation, leading to an increased risk for osteopenia and bone fracture (Matkovic et al., 1994). Winterer et al. (1984) found a significant correlation between decreased estradiol concentrations, decreased bone density, and causes of menstrual dysfunction (Winterer et al., 1984).

Conflicting research exists as to the "direct cause" of disturbances in the menstrual cycle and the HPG axis. Contrary to the belief that exercise itself is the direct factor in menstrual disturbances, emerging evidence suggests that energy balance, or inadequate caloric intake, is the major contributor (Loucks et al., 1998). Williams et al. used an amenorrheic female monkey model to monitor the impact of energy availability on reproductive hormones and dysfunction during a consistent exercise regimen. On hypercaloric refeeding, the amenorrheic monkeys began to re-establish their ovulatory cycles, with the time-frame for re-establishment based on caloric intake

(Williams, 2001). It appears that much of the reduction in serum estradiol and resultant menstrual dysfunction is related to insufficient energy and nutrient intake necessary to achieve energy balance consistent with the demands of the activity.

Luteinizing Hormone and Testosterone Interactions

The effects of LH in males reside at the site of Leydig cells. These cells, located in the testes, are responsible for the production of about 95% of circulating testosterone (Horton et al., 1978). Häkkinen et al. (1988) reported a decrease in LH secretion after a single bout of resistance exercise. Nindl et al. (2001) demonstrated an overnight blunted LH production rate after a resistance training (RT) protocol, with a concomitant decline in testosterone concentration. However, it should be noted that the RT protocol administered was 2 hours in length, whereas most studies that have shown elevated testosterone after exercise were of shorter duration (<90 min) (Kraemer, 1999a; Kraemer et al., 1990). The sustained RT protocol may have made the hormonal environment more closely resemble that seen during sustained aerobic exercise, considering that LH concentration declines during sustained aerobic exercise (Karkoulias et al., 2008; Semple et al., 1985). McColl et al. compared endurance-trained men with a control group and found significantly lowered resting testosterone and mean LH concentrations in the trained group.

Increased testosterone concentrations have been associated with increased muscle hypertrophy (Sinha-Hikim et al., 2002). Therefore, any exercise training that has the ability of increasing testosterone concentration is likely to contribute to increased muscle mass. Acute exercise-related increases in plasma testosterone appear to depend on intensity, volume, duration, and degree of muscular activation. Hakkinen et al. (1993) compared low-volume, high-load RT (20 sets of 1, 100%) to high-volume, moderate-load RT (10 sets of 10, 70%) and only found a significant increase in total and free serum testosterone in the high-volume group. Along with intensity and volume, a significant contributor to testosterone concentration increases appears to be the muscle mass recruited. The larger the muscle mass recruited, the greater the testosterone concentration increases, as demonstrated by 15% increases in testosterone following deadlifts versus 7% increases following bench press (Volek et al., 1997). Furthermore, the response of testosterone to training appears to exist in both old and young individuals, with a larger absolute increase in the young (Kraemer, 1999b; Metivier et al., 1980). Contrary to high-intensity RT exercise, long-duration endurance exercise appears to cause a significant decline in postexercise serum testosterone (Semple et al., 1985). This coincides with a decline in LH concentration, with similar results seen in both untrained and trained individuals (Karkoulias et al., 2008).

Further understanding of the mechanism behind the rise in testosterone concentration is warranted. Increases in testosterone from acute exercise training appear to be regulated by some mechanism other than just LH response. The acute increase in testosterone concentration after a high-intensity exercise bout may be partly due to decreased testosterone clearance or a shift in plasma volume, rather than an increase in testosterone production and secretion per se (Hudson, 1985, Sutton et al., 1978). Besides altered hepatic testosterone clearance or reduced plasma volume, increased testosterone release from the adrenal cortex may contribute to some of the increased testosterone but not to an appreciable amount in males. The anabolic effects of testosterone appear to be dependent on the concentration of testosterone in blood, not simply on a change in secretion. A dose-dependent increase in plasma testosterone had a positive linear correlation with muscle hypertrophy in man (Sinha-Hikim et al., 2002). This indicates that regardless of the cause of an increased testosterone concentration, the increase alone has potential anabolic effects. However, these effects appear to depend on the duration of the exercise. Although most RT sessions appear to cause an increase in testosterone concentration, RT of 2 hours resulted in decreased testosterone (Nindl, 2001b). This supports data from endurance-based activities indicating that sustained exercise will result in decreased testosterone.

The exercise itself is not necessarily the only part of a training program that can impact serum testosterone. For example, Volek et al. demonstrated increased testosterone after acute high-intensity resistance training using a 5-set, 10-repetition protocol of bench press and jump squats. However, they also noted a significant correlation between dietary nutrient consumption and resting testosterone serum content (Volek et al., 1997). A significant positive correlation was seen between fat intake and serum testosterone, particularly for monounsaturated fatty acid (MUFA) and saturated fats (SFA). They, therefore, concluded that not only does an acute bout of high-intensity RT increase postexercise testosterone levels, but dietary fat intake (MUFA and SFA) also appears to have a significant role in elevation of resting testosterone levels.

ENDOCRINE MEDIATION OF SHORT–TERM CONTROL OF CARDIOVASCULAR FUNCTION

The cardiovascular response to exercise is dependent on a multisystem defense of blood volume, MAP, and plasma tonicity (McKeever and Hinchcliff, 1995). These mechanisms ensure adequate blood flow to the working muscles and obligate tissues, along with the provision of adequate fluid volume for sweating and thermoregulation. The maintenance of cardiovascular homeostasis during exercise is mediated by neuroendocrine mechanisms that ensure that the system can meet the increased demand for blood flow to the working muscles during exercise (Convertino, 1991; McKeever, 2002; McKeever and Hinchcliff, 1995).

The anticipation of exercise in humans and horses can invoke a withdrawal of parasympathetic control and an increase in sympathetic nervous activity resulting in an increase in heart rate, force of contraction, stroke volume, and cardiac output. Rowell (1993) suggested that the "range of parasympathetic control of the heart by central command determines the level of exercise at which the activation of the sympathetic nervous system occurs." In horses, resting heart rate averages 30 to 40 beats per minute (beats/min). Initial increases in heart rate up to approximately 120 beats/min are associated with the withdrawal of parasympathetic tone. However, further increases in heart rate during exercise, up to a maximal heart rate between 200 and 240 beats/min, are associated with increases in sympathetic activity and catecholamine release. This increase in heart rate coupled with increased stroke volume from 0.7 L at rest to almost 2 L during strenuous exercise results in an increase in cardiac output from an average of 30 L/min at rest up to nearly 300 L/min during maximal exercise (Kraemer, 1999b; Metivier et al., 1980). The cardiovascular system responds to exercise with dramatic increases in heart rate, force of cardiac contraction, and subsequent increases in stroke volume and

cardiac output (Rowell, 1993). These central cardiovascular responses are rapid and concurrent with venoconstriction and arterial vasodilation in the working muscles (Rowell, 1993).

Adjustments in peripheral vascular resistance that are mediated by the cardiopulmonary baroreflex cause a redistribution of blood volume from "storage" in highly compliant venous capacitance vessels into the arterial side of the cardiovascular system, enhancing venous return (Rowell, 1993). In the horse, there is the added component of splenic reserve mobilization which further enhances venous return and circulating RBC volume (McKeever and Hinchcliff, 1995; Persson, 1967). Splenic contraction is mediated by direct stimulation from the sympathetic nervous system through the action of norepinephrine and epinephrine on α–adrenergic receptors (Persson, 1967). Between 6 L and 12 L of blood can be delivered into the central circulation at the onset of exercise allowing the equine athlete to reach a maximal aerobic capacity (145–200 mL/kg/min in fit horses) that is almost three times greater than that of human athletes (McKeever and Hinchcliff, 1995; Persson, 1967). This extra volume is rapidly accommodated through arterial vasodilation, which is mediated by increases in sympathetic neural outflow, through increases in ANP, and through local chemoreceptor mechanisms (McKeever and Hinchcliff, 1995; McKeever et al., 1991). Resequestration of the splenic reserve is prevented by the action of vasopressin and the catecholamines on splenic arterioles (Davies and Withrington, 1973).

MAP increases with exercise intensity are essential for increasing cardiac output in the face of decreases in resistance in the vascular beds of the working muscles. Rowell (1993) suggests that in addition to input from the cardiopulmonary baroreceptors, a functional arterial baroreflex and muscle chemoreflexes are essential for the regulation of heart rate, cardiac output, and arterial pressure during exercise. Rowell (1993) further suggests that the operating point of the arterial baroreflex is "reset during dynamic exercise with adjustments in autonomic tone to compensate for the mismatch between cardiac output and vascular conductance."

Modulation of the blood flow and blood pressure response to exercise involves input from both high-pressure and low-pressure baroreceptors. Low-pressure (cardiopulmonary) baroreceptors are volume receptors located primarily within the atria and the pulmonary circulation. At the start of exercise, increased venous return results in atrial stretch, eliciting a reflex response by the cardiopulmonary baroreceptors. Nerves within the atria serve as stretch receptors, sensing volume overload or underload. The output from these nerves is conducted centrally via vagal afferents and integrated into the central control of peripheral vascular tone (McKeever and Hinchcliff, 1995; Rowell, 1993). The endocrine component of this baroreflex involves the release of ANP, which has potent vasodilatory properties and causes a reflexive decrease in vasopressin release (Freund et al., 1988b; McKeever and Hinchcliff, 1995).

Even with the mobilization of reserve blood volume, the demands of high-intensity or long-duration exercise may exceed central cardiovascular capacity. Baroreceptor control of arterial tone becomes vital to the maintenance of cardiac output and MAP, whereas sympathetic-induced vasoconstriction decreases blood flow to nonobligate tissues during high-intensity exercise (McKeever and Hinchcliff, 1995; Rowell, 1993). This response is even more pronounced during long-term exercise, when fluid losses associated with sweating compromise vascular fluid volume and venous return. Without replacement or compensation, decreases in venous return associated with fluid losses can cause a decrease in cardiac output and decreased blood flow to the working muscles and to the vascular beds associated with thermoregulation (Convertino, 1991; McKeever and Hinchcliff, 1995; Rowell, 1993). To maintain cardiac output under these conditions, the body compensates by increasing heart rate because of the concomitant decrease in stroke volume, a phenomenon termed cardiovascular "drift," which is associated with an increase in sympathetic activity and circulating catecholamines (McKeever, 1993).

Exercise training produces chronic adaptations in the cardiovascular system that are mediated through changes in neuroendocrine control (Convertino, 1991; McKeever and Hinchcliff, 1995; Wade, 1984). Work from several species has shown that exercise training produces an expansion of plasma volume (Convertino, 1991; McKeever et al., 1985; McKeever et al., 1987; McKeever, et al., 2002b). Trained horses have significantly greater blood volumes compared with untrained horses (Convertino, 1991; McKeever et al., 1987; McKeever, et al., 2002b; Persson, 1967), with increases in plasma volume of 30% observed after only 1 week of exercise training. Humans show significant alterations in sodium and water excretion, which are attributable to repeated exercise-induced increases in plasma aldosterone concentration (Convertino, 1991). In the one study of the hypervolemic response in horses, the authors reported no change in resting plasma aldosterone concentration or sodium excretion. However, their measurements were taken at 1-week intervals and may have missed changes in sodium excretion that is reported to occur in the first days of training in humans (McKeever et al., 1987). A more recent study that focused on changes occurring during the first days of training demonstrates that plasma aldosterone concentration remains elevated for almost 24 hours (McKeever et al., 2002b). It appeared that as in humans, an aldosterone-mediated retention of sodium and water by the kidneys and digestive tract are a vital part of the hypervolemic response to training in the horse (Convertino, 1991; McKeever, et al., 2002b).

ENDOCRINE CONTROL OF METABOLISM DURING ACUTE EXERCISE

Performance of exercise requires the transduction of potential or stored energy into kinetic energy and the endocrine system plays an integral role in the coordination of the mobilization and utilization of carbohydrates and free fatty acids (Rose and Sampson, 1982; Rose et al., 1983). The need for a rapid provision of metabolic substrates to fuel exercise and to prevent central fatigue is facilitated by a rapid increase in sympathetic drive and an increase in the rate of catecholamines released from the adrenal medulla. The degree of this response is correlated both with exercise intensity and exercise duration (Dickson, 1970; Willmore and Costill, 1994). At the onset of exercise, the catecholamines act on the liver and muscles to increase the rate of glycogenolysis. The impact on the liver results in an increase in circulating blood glucose concentrations. The catecholamines also stimulate the release of hormone-sensitive lipase, which acts on triglycerides to mobilize free fatty acids. Free fatty acids are important for endurance activities, in which the use of fat to fuel exercise spares glycogen by offsetting the amount of glucose needed to fuel the activity.

However, fat cannot be used alone as a fuel source as "fat burns in the flame of carbohydrates." An increase in circulating catecholamines also inhibits insulin and stimulates glucagon release. As with catecholamines, glucagon also stimulates gluconeogenesis and inhibits glycogenesis, thus playing an important role in maintaining blood glucose concentrations during exercise and delaying the onset of fatigue. Glucagon can also stimulate the breakdown of protein and the release of amino acids, which can be used as a fuel source by the liver. The effects of catecholamines and glucagon can be further augmented by the release of cortisol, which is affected by the intensity and duration of the activity. Cortisol is, thus, a metabolic hormone that stimulates gluconeogenesis, fatty acid mobilization, and protein breakdown. As a result of the proteolytic actions, amino acids not used to fuel exercise may provide resources for the synthesis of new proteins needed to repair muscle and replace enzymes used in the various metabolic pathways (Dickson, 1970; Willmore and Costill, 1994).

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CHAPTER

8 Thermoregulation

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Exercise requires derivation of energy for muscular contraction from the conversion of stored chemical energy to mechanical energy. This process is relatively inefficient, and about 80% of the energy released from energy stores is lost as heat. Effective dissipation of this heat load is required if life-threatening elevations in body temperature are to be avoided. The physiologic mechanisms that result in this heat dissipation, governed by the thermoregulatory system, are essential if the horse is to function as an athletic animal (Åstrand and Rodahl, 1979; Brody, 1945).

The primary means of heat dissipation in the horse is evaporation of sweat, particularly in warm ambient conditions. Evaporative cooling is an efficient mechanism enabling horses to perform a variety of athletic events, with only relatively minor elevations in body temperature. However, exercise-induced heat stress can occur when heat production during exercise exceeds heat dissipation and body temperature reaches critical levels. This is likely when animals are forced to exercise in adverse environmental conditions (i.e., high temperature and humidity), when they have been inadequately conditioned, or when they are suffering an impairment of the thermoregulatory system (anhidrosis) (Hafez, 1986; McCutcheon and Geor, 1998). Careful preparation for athletic events, monitoring during events, and early recognition of impending signs of heat stress will minimize the risk of development of life-threatening hyperthermia. Examples of the effectiveness in devising strategies for horses coping with a combination of adverse environmental conditions with large exercise-induced heat loads occurred prior to the Olympic Games in Atlanta. Equestrian events were held in the summer when heat and humidity were high. By utilizing acclimatization strategies, advanced cooling techniques, conducting events when ambient temperatures and solar heat gain was minimized, and modifications to the length of competition, heat illness was avoided in horses performing in those competitions (Jeffcott and Kohn, 1999).

Horses have coped with temperatures as variable as 58°C in Northern Australia to at least –40°C in western Canada, Scandinavia, and Russia. Despite large fluctuations in environmental temperature, horses are able to maintain their internal body temperature within a very narrow range by elaborate thermoregulatory mechanisms. The basis of this thermoregulatory control mechanism is via alterations in blood flow that allows regulation of heat flow between the animal and its environment (Cymbaluk and Christison, 1990).

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MECHANISMS OF HEAT TRANSFER

Heat will flow from one area to another by four basic mechanisms: (1) radiation, (2) convection, (3) conduction, and (4) evaporation (Monteith, 1973; Yousel, 1985). Homeothermy requires that heat produced or gained from the environment equals heat loss to the environment, as indicated by the following equation (Yousel, 1985):

$$\text{Gains} = \text{Losses}$$
$$M - W = \pm R \pm C \pm K + E$$

where:
M = metabolic heat production
W = mechanical work
R = heat exchange by radiation
C = heat exchange by convection
K = heat exchange by conduction
E = heat exchange by evaporation

RADIATION

Radiation involves the movement of heat between objects without direct physical contact via electromagnetic radiation of two distinct types: (1) *Short-wave,* or solar, *radiation* is received from the sun by any object exposed to sunlight. (2) *Long-wave radiation* is emitted and absorbed by the surfaces of all organisms and relates to heat interchanges between an animal and its surroundings (Åstrand and Rodahl, 1979).

The heat load from solar radiation, both direct and reflected, can be significant in hot environments, where animals are exposed to sunlight for prolonged periods. When an animal is standing in bright sunlight, the amount of solar radiation absorbed may substantially exceed its own metabolic heat production.

CONVECTION

Convection occurs within all fluids (including gasses such as air) due to the mixing of particles within that milieu. Temperature differences within the fluid result in a difference in the density of the fluid particles. Warm particles are less dense and will rise, whereas cold particles fall. Free convective heat transfer takes place at the surface of a solid body within a fluid medium, which is at a different temperature. This type of transfer takes place continuously between the surface of the body and the surrounding air. Forced convective heat transfer occurs if there are fluid movements induced by gross pressure differences, for example, caused by wind blowing across the body surface. Free convection at the skin surface can result in significant heat losses if ambient air temperatures are low

(Åstrand and Rodahl, 1979). A hair coat will entrap a layer of air close to the skin and resist convective heat transfer; this is the purpose of a hair coat. Wind will increase forced convective losses by disrupting this insulating layer. The hair coat of the horse is generally fine and sleek in summer to aid heat loss, whereas in winter a thick hair coat develops, in association with acclimatization to cold stress, resulting in increased insulation.

CONDUCTION

Direct transfer of heat between surfaces that are in contact occurs as a result of *conduction* (Åstrand and Rodahl, 1979). In the standing horse, most of this transfer is to air, which has poor thermal conductivity, and thus, conductive heat transfer plays a small role in the total heat balance. However, this route of heat transfer can become significant if the animal is lying on a cool or wet surface or, indeed, if the horse is repeatedly bathed in a cold fluid such as water.

A number of behavioral strategies are utilized by animals to affect conductive heat exchange. Changes in posture can alter the surface area available for heat exchange. For example, by lying down and drawing the limbs close to the body, the surface area can be reduced considerably. In contrast, morphologic adaptation by burros and mules (long ears, short legs, and lean body conformation) increases available surface area for convective heat loss and may help increase heat tolerance (Bligh, 1973).

The extremities of animals, the limbs and head, have a high surface area to mass ratio, and thus, maximal conductive heat exchange can occur at these sites. Changes in surface temperature at these sites can be affected by alterations of skin blood flow. The skin temperature of the horse, reflecting skin blood flow, varies in direct proportion to ambient temperature, with greatest temperature changes occurring at the extremities (Bligh, 1973).

At low ambient temperature, local vascular shunts direct blood away from the extremities to reduce the rate of convective heat loss, whereas at high temperatures, vasodilation occurs to promote heat loss. An example of how horses utilize this mechanism is seen when skin temperature falls from 26°C at an ambient temperature of 25°C to 17°C at 15°C (ambient) and even further vasoconstriction occurs at an ambient temperature of 5°C, with skin temperature decreasing to 10°C (McCutcheon and Geor, 1998; Rowell, 1986).

EVAPORATION

Evaporation is the principal means by which homeotherms such as horses lose heat in warm environments via the physiologic processes of panting, sweating, and insensible perspiration. The conversion of water from liquid to vapor is an endothermic process. Thus, evaporation of water at the surface of the body results in heat loss. The exact amount of energy involved in this process is dependent on the temperature and vapor pressure of the surrounding air. The latent heat of vaporization of 1 g of water is 598 calories (cal) (2501 joules [J]) at 0°C and 575 cal (2406 J) at 40°C. Evaporation of 1 liter (L) of sweat in a human being can remove 580 kcal (2428 kilojoules [kJ]) from the body (1 cal = 4.186 J) (Åstrand and Rodahl, 1979).

The skin–ambient vapor pressure difference is the driving force for vaporization. When the vapor pressure at the skin surface reaches a maximum value, corresponding to saturation at skin temperature, sweat rate exceeds evaporation rate, and sweat drips off the skin without resulting in cooling. This corresponds to the limit of the efficiency of sweating and is usually associated with rising body temperature. This limit will be reached faster if the air vapor pressure is high, as occurs in humid conditions.

During panting, inspired air is almost fully saturated with water at a temperature similar to the deep body temperature as it passes over the wet surfaces of the upper respiratory tract. The associated heat loss is governed by the ambient air humidity and respiratory ventilation rate. Although horses do not pant routinely, there is evidence of considerable evaporative heat loss from the respiratory tract during exercise in response to normal ventilation (Hodgson et al, 1993).

Insensible perspiration involves the passage of water through the skin by processes other than sweating. The skin is not impermeable to water, and thus, water can diffuse out as a result of the skin–ambient vapor pressure gradient. In humans, approximately 10 milliliter per square meter per hour (mL/m^2/hr) of water passes through skin in ambient conditions, increasing to 30 mL/m^2/hr in a warm environment. The average human has a body surface area of 1.5 to 2.0 m^2 (Åstrand and Rodahl, 1979).

REGULATION OF INTERNAL BODY TEMPERATURE

Thermoregulatory mechanisms maintain the body temperature of homeotherms within a narrow range by regulating heat production and heat loss. A complex neurophysiologic process regulates internal body temperature. The principal neuronal elements of the thermoregulatory system are peripheral thermoreceptors, the spinal cord, and the hypothalamus. Peripheral thermoreceptor organs are located in a number of locations, including skin, the buccal cavity, skeletal muscle, the abdomen, regions of the spinal cord, the medulla oblongata, and preoptic–anterior hypothalamic region of the midbrain. These temperature-sensitive structures are responsible for detection of a disturbance and produce proportional nerve impulses. For information from thermosensitive receptors in several parts of the body to be translated into appropriate instructions to the effector organs, there must be convergence of the neural pathways from these temperature sensors and transmission of this information to an interpretive center. A coordinating center in the central nervous system receives afferent nerve impulses and produces efferent impulses, initiating a correction that is transmitted to the effector organs. There is considerable evidence from experiments in a variety of animal species that the hypothalamus may represent this interpretive center. Thermal stimulation of peripheral temperature sensors, the spinal cord, and the hypothalamus results in appropriate thermoregulatory effector activity. However, destruction of the hypothalamus downregulates and even turns off thermoregulatory responses to local heating of all these structures. This may be interpreted to suggest that the integrity of particular hypothalamic areas is necessary for normal thermoregulation and that most of the regulation of internal temperature occurs as a function of the hypothalamus. Once the hypothalamus signals the effector organs, these end organs are responsible for correction of the initial disturbance, which, in turn, results in reduction of stimulation by the sensory organs as thermoregulation is effected (Rowell, 1986).

PHYSIOLOGIC THERMOREGULATORY MECHANISMS FOR HEAT LOSS

The thermoregulatory system utilizes various mechanisms of heat flow to effect heat loss from the body. Highly effective

mechanisms for dissipating heat include sweating and panting (or elevated respiratory volume), which exploit the significant heat loss associated with the evaporation of water, in addition to convective heat loss. The cardiovascular system has a critical role in thermoregulation, with blood flow being used as a means for heat transfer from sites of heat production within the body core to areas where dissipation of heat can occur, primarily skin and the respiratory tract (Hodgson et al., 1993).

MECHANISMS OF EVAPORATIVE HEAT LOSS

The principal physiologic thermoregulatory mechanisms that utilize the vaporization of water from the body surface are panting or increased respiratory volume and sweating. Hominoidae, including humans, and Equidae (horses, mules, donkeys, etc.) are the only species that depend on sweating as the primary mechanism for thermoregulation, whereas sheep, dog, and pig rely much more on respiratory heat loss (McLean, 1973).

Panting or increased respiratory volume has advantages over sweating in that its efficiency is not limited by the hair coat, which can insulate the animal against both radiant heat and the effects of cold, as well as reducing fluid and electrolyte losses in sweat. However, disadvantages of panting exist, particularly during exercise, when there is competition between the need for appropriate gaseous exchange and optimum evaporative heat loss (Ingram, 1975).

Humans versus Horses

Sweating is the most important means of heat loss in humans, with respiratory heat loss accounting for only 11% of total heat loss in a thermoneutral environment. This percentage increases with rising core temperature (Åstrand and Rodahl, 1979).

Similar to humans, horses rely primarily on sweating for heat loss, with the respiratory tract contributing to heat loss, particularly during exercise. Respiratory frequency has been reported to be dependent on environmental temperature during rest and exercise, increasing 1.9 breaths/min for every 1°C increase in ambient temperature. Investigations of the exact proportion of heat dissipated by the respiratory tract have reported different values depending on the type of horse, the ambient temperature, and the exercise intensity. Ponies at rest in ambient conditions (21 to 23°C) lose 14% to 22% of total heat production by pulmonary ventilation, whereas horses in a cooler environment of 16°C have respiratory heat loss of 38% at rest and 17% during maximal exercise. Although horses do not normally pant, if evaporation of sweat is limited by high humidity or anhidrosis, they may experience heat-induced tachypnea. Pulse–respiration inversion (respiratory rate in excess of heart rate), frequently shown by endurance horses during recovery from exercise in hot humid environments, is likely a form of panting in horses (Geor and McCutcheon, 1996).

The reliance of humans and horses on sweating may relate to their need to lose heat during sustained activity. This is supported by the greater sweat rate produced in horses resulting from epinephrine infusion compared with elevations of environmental temperature.

In horses, rectal temperature is reported to be significantly higher (~0.5°C greater) in hot environments than in thermoneutral environments. Such temperature variations may reflect an adaptation to desert environments, similar to that seen in camels. To limit the need for sweating, some species inhabiting hot, arid environments have relatively labile body temperatures allowing storage of heat. Body temperatures of the camel may rise to 41°C during the heat of the day and decrease to as low as 34°C during the night. Such thermolability obviates the need for evaporative cooling, thus resulting in a mechanism of conserving water (Bligh, 1973).

EVAPORATIVE HEAT LOSS FROM THE RESPIRATORY TRACT

Heat exchange occurs within the large surface area of the upper respiratory tract. Inspired air is heated to body temperature and saturated with water vapor by the time it reaches the alveoli. During expiration, some heat passes back to the mucosa, with resultant condensation of water. The difference between initial heat transfer to the inspired air and subsequent transfer back to the mucosa is the mechanism of respiratory heat loss. The quantity of heat loss depends on the environmental temperature and humidity; the warmer and more humid the air, the smaller is the heat loss. In some animals, panting increases ventilation rate, resulting in greater heat loss from the respiratory tract (Bligh, 1973).

EVAPORATIVE HEAT LOSS FROM SWEATING

Function of Sweat Glands

The glands of the general body surface of humans have a primary heat-regulatory function, whereas the glands on the palms and soles are concerned with emotional responses and are insensitive to heat. The sweat glands of the horse resemble the sweat glands of humans in their heat-regulatory function (Carlson, 1983).

Chemical Composition of Sweat: Humans

The ionic concentration of sweat in humans varies markedly between individuals and is strongly affected by the sweating rate and the state of heat acclimatization of the subject. Human thermogenic sweat is hypotonic relative to plasma. Sodium chloride is the main constituent, with the chloride concentration being 30 to 50 millimolars (mM) and sodium concentration 20 to 60 mM (Table 8-1). The concentration of sodium chloride (NaC1) increases as sweat rate increases initially. In fact, increased short-term high-intensity activity results in increases in the concentrations of these electrolytes in serum and sweat. Thus, during short-term strenuous exercise, the NaC1 concentration may increase by up to 50% above initial values. In contrast, prolonged exercise tends to result in decreases in the concentration of electrolytes in sweat (Amatruda and Welt, 1953).

Potassium concentration in sweat is only slightly higher than that of plasma at low sweat rates (10 to 35 mM) and decreases to 5 mM as sweat rate increases. Potassium secretion is not affected by acclimatization or dietary intake. Sweat calcium concentration similarly decreases with increasing sweat rate from 3 to 10 mM to 1 to 2 mM. Bicarbonate concentration is 2 to 10 mM and tends to increase with sweat rate. Traces of magnesium, iodide, phosphorus, sulfate, iron, zinc, copper, cobalt, lead, manganese, molybdenum, tin, and mercury are also present, as well as negligible amounts of vitamins (Robinson and Robinson, 1954).

Formation of Sweat in Humans

In humans, a highly sophisticated mechanism for electrolyte reabsorption is present in sweat glands. An ultrafiltrate of plasma-like isotonic precursor fluid (Na^+ = 150 mM, Cl^- = 124 mM) is

secreted by the secretory coil of the sweat gland. As the precursor fluid flows through the duct, much of the sodium chloride is reabsorbed in excess of water, resulting in sweat that is hyposmotic.

The absorption of NaC1 by the duct is due to active transport of the Na^+ by a sodium–potassium (Na^+–K^+)-sensitive adenotriphosphatase (ATPase). Na^+ diffuses passively from the lumen to the cell interior and is then actively pumped out from the cell to the interstitium at the peritubular cell membrane in exchange for K^+, with Cl^- passively following Na^+ (Gordon and Cage, 1966).

The principal factor resulting in the reduction of NaC1 in sweat that occurs with prolonged heat exposure appears to be an increase in activity of the pituitary–adrenal cortex mechanism elicited by a salt deficiency.

Equine Sweat Composition

The composition of horse sweat during exercise, heat stress, and epinephrine infusion has been measured by a number of investigators. The concentration of electrolytes reported in these studies is presented in Table 8-1. A number of methods of sweat collection have been utilized in these studies, including directly scraping sweat off the horse, collecting the drops that run off the horse, and collecting the sweat onto absorbent pads. Residual electrolytes from previous sweating and artificial elevation due to evaporation also will alter the sweat composition. These methodologic problems may explain the significantly higher electrolyte concentration reported by the early investigators when compared with later studies (McCutcheon and Geor, 1998; Smith, 1890).

Equine sweat, unlike that of humans, is hypertonic relative to plasma, with a Na^+ concentration similar or slightly higher than plasma, Cl^- significantly higher, and K^+ 10 to 20 times greater than serum concentrations. High-intensity exercise in horses produces more dilute sweat than low-intensity prolonged exercise, a process that may be caused by increased epinephrine concentrations that result during high-intensity exercise. Epinephrine infusion results in production of more dilute sweat than that occurring during exercise. Epinephrine concentrations are elevated during exercise in horses, and sweat in this species is produced in response to both sympathetic nervous activity and circulating epinephrine (McCutcheon and Geor, 1998).

Sweat produced by the horse has an unusually high concentration of a protein relative to many other species. This protein is referred to as *latherin* and is responsible for producing the lather seen on horses after exercise. Latherin has a surfactant-like action, promoting spreading and evaporation of sweat and possibly aiding evaporation and cooling (Eckersall et al., 1982).

TABLE 8–1

Electrolyte Composition of Equine and Human Sweat

	Na^+	K^+	Cl^-
HORSE			
Jirka and Kotas, 1959	382	48	432
Soliman and Nadim, 1967	593	48	–
Carlson and Ocen, 1979	132	53	174
Kerr et al., 1980	146	55	199
Rose et al., 1980	249	78	301
Kerr et al., 1983	147	57	200
Plasma composition	139	3.7	100
MAN			
Costill et al., 1977	50	4.7	40
Plasma composition	140	4	101

Innervation of the Sweat Glands

The primary sudomotor mechanism in humans is cholinergic, whereas in the majority of other species this mechanism is essentially adrenergic-sympathetic. Sweating appears to be under sympathetic nervous control in the cow, sheep, goat, pig, donkey, and horse. This innervation involves α-receptors in the cow, sheep, and goat, α- and β-receptors in the dog, and β-receptors in the horse (Evans, 1955).

The exact action of the nervous system on sweat glands has not been elucidated. The final stimulus to the glands must be humoral, either bloodborne or released from adjacent nerve endings. For example, sweat glands in humans and horses and the footpads of dogs and cats have a nerve supply closely associated with them. However, in the majority of other species, there must be a nonneural peripheral component in the sudomotor control mechanism. The sweat glands of humans, dogs, and horses respond to adrenergic and cholinergic drugs, whereas those of sheep and goats, donkeys, and pigs respond to adrenergic but not cholinergic substances (Snow, 1977).

Experimentally, sweating in horses can be stimulated by intravenous and local injection of epinephrine. However, norepinephrine, the usual adrenergic postganglionic neurotransmitter, results in a minimal sweating response. Certainly, during exercise, the circulating epinephrine concentration is sufficient to cause sweating (Snow, 1977).

CIRCULATORY ADJUSTMENTS FOR THERMOREGULATION

The flow of blood is a highly effective avenue for heat transfer via conduction. By altering blood flow between various organs, the cardiovascular system may act as a major thermoregulatory effector mechanism. Thermoregulation via adjustments in blood flow is so efficient that thermal stability under the range of thermoneutral conditions can be maintained by the balance between peripheral vasodilation and vasoconstriction.

The principal means for the role of the cardiovascular system as a thermoregulatory effector include (1) increasing the cardiac output ($\dot{Q}$°) and (2) redistributing the cardiac output, particularly blood flow to the skin (Rowell, 1986).

Skin Blood Flow

On exposure to heat, there is a resultant increase in blood flow to skin, inducing conduction of heat to the surface of the body and, thus, increasing skin temperature and facilitating convective heat loss from the body to the environment. In more severe heat stress, blood flow to skin also provides the latent heat for vaporization of sweat and supplies fluid for sweat production. In cold environments, reduced blood flow to skin decreases skin temperature, thereby limiting heat loss from the body (Rowell, 1986).

The anatomic arrangement of the skin vasculature is designed to facilitate heat transfer. Three plexuses of vessels

are present in skin such that a large volume of blood can be redistributed to skin to maximize heat loss. Specialized vessels, arteriovenous anastamoses (AVAs), are present in skin and contribute to this process. AVAs are short vessels that connect arteries and veins, and opening of these vessels results in bypass of capillary beds, allowing a greatly increased blood flow through skin.

Control of skin blood flow is primarily mediated by the sympathetic nervous system. Response to heat involves vasodilation of arterioles and AVAs, whereas vasoconstriction in response to cold involves both arterioles and veins (Rowell, 1986).

Cardiac Output

In response to heating, cardiac output ($\dot{Q}$°) rises to maintain central blood pressure in the face of increased skin blood flow. In humans, cardiac output commonly increases 50% to 70% in response to heat stress and may more than double if the increase in core temperature exceeds 2°C. The increase in sympathetic nervous activity associated with heat stress results in a rise in heart rate and an increase in myocardial contractile force and stroke volume (Rowell, 1986). The alteration in $\dot{Q}$° in the horse during heat stress is not well understood.

Redistribution of Cardiac Output

Redistribution of $\dot{Q}$° will differ among species, depending on the relative importance of the two major heat loss mechanisms: sweating and panting. In panting animals such as sheep and dogs, heat stress causes major increases in blood flow to respiratory muscles and the nasobuccal regions. However, in humans and horses, which rely on sweating for heat loss, there is an increased blood flow to the skin (Hales, 1973; Hodgson et al, 1993; Rowell, 1986).

Redistribution of $\dot{Q}$° in response to heat stress has been studied in ponies. Given the similarities between the thermoregulatory systems of horses and humans, similar responses to heat stress appear to occur. Exact measurements of redistribution of the $\dot{Q}$° are not possible in humans. However, all available estimates indicate that the entire increase in $\dot{Q}$° that occurs in heat stress is directed to the skin. In all the major organs in which flow has been measured (splanchnic, renal, and skeletal muscle), decreases in blood flow occur. The decrease in renal and splanchnic blood flows are in the order of 25% to 40% during significant heat stress. This matches the situation that occurs in ponies with the degree of reduction in cardiac output to the viscera directly relevant to the degree of heat stress, exercise intensity, and duration of exercise (McConaghy et al., 1996).

THERMOREGULATION DURING EXERCISE

In the 1930s, Nielsen demonstrated that a stable elevation of internal body temperature occurs during exercise in humans with this change proportional to the intensity of exercise and relatively independent of environmental changes. In 1966, Saltin and Hermansen further investigated this relationship in humans and discovered that body temperature elevation during exercise is related to the relative work load rather than the absolute work load. A similar relationship is reported to exist in horses. In 1949, Robinson first reported the linear relationship between sweating rate and internal temperature in humans and observed that the elevated body core temperature during exercise triggers a heat-dissipation response that is related to the magnitude of the elevation. These observations have been instrumental in the attempt to define temperature regulation during exercise (Nielsen, 1939; Saltin and Hermansen, 1966).

Under normal circumstances, during exercise, a metabolic heat load is produced that is proportional to the intensity of the exercise bout. This heat load is transferred from the working muscles to the body core, resulting in elevation of core temperature. Sweating is initiated at a certain core temperature, dictated also by skin temperature, and sweating continues at a rate proportional to the increase in core temperature. At some point, determined by environmental conditions, the evaporative rate will match the rate of energy production. At this point, heat production will equal heat loss, and the body temperature will become stable and constant at a higher than resting core temperature (Rowell, 1986).

ENERGY EXCHANGES DURING EXERCISE

Heat transfer in the horse during exercise is depicted in Figure 8-1. At the onset of exercise, the rate of heat production in muscle greatly exceeds the rate of heat dissipation, resulting in a rapid elevation of muscle temperature. Muscle temperature can increase at a rate of 1°C per minute at the beginning of strenuous exercise, and muscle temperatures of 45°C have been reported in exercising horses. Heat flows down the temperature gradient from the muscle to the surrounding tissue primarily via convection. An additional small amount of heat is transferred by direct conduction. The convective transfer occurs as a result of blood flow through the muscle. Muscle blood flow is greatly increased during exercise, which both increases oxygen supply and enhances removal of metabolic wastes and heat. Increases in blood flow to the working muscles of the pony of more than 10-fold from resting levels have been recorded (Hodgson et al., 1993; McConaghy, 1996; McConaghy et al., 1996).

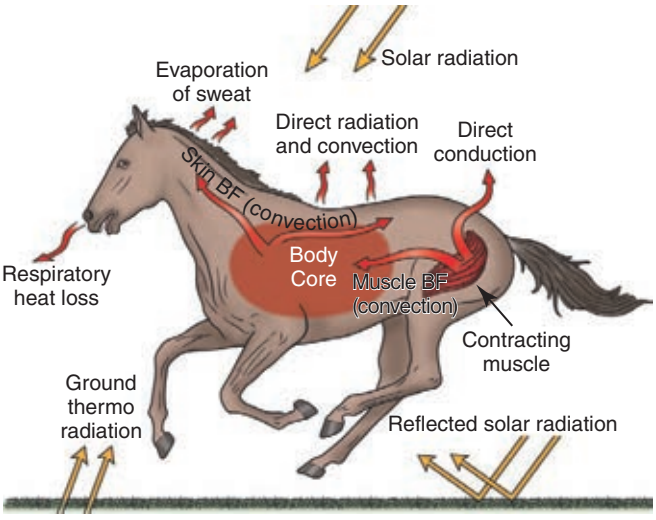

FIGURE 8-1 Mechanisms for the transfer of heat within the body of a horse during exercise. Heat is gained as a byproduct of muscular work and via radiation from the environment. Heat load is dissipated via evaporative, convective, and conductive mechanisms. BF = blood flow

During the early minutes of exercise, core blood is heated with heat storage exceeding heat dissipation, and core temperature rises in proportion to the exercise intensity. The rise in core temperature during exercise has a number of advantages. It allows storage of heat, which reduces the amount of heat that must be dissipated. In addition, a moderate elevation of muscle temperature results in an improvement of muscular performance, facilitates oxygen release from the RBCs, and augments an increase in maximal heart rate. Metabolic reactions are accelerated, and enzyme activity is enhanced by moderate increases in temperature; thus, energy production is faster when core temperature is elevated (Åstrand and Rodahl, 1979).

The core temperature rises slowly when compared with the temperature of muscle and blood in the central circulating pool, reflecting the large amount of energy that can be stored within the entire body mass. As the rate of heat dissipation rises to balance the rate of heat production, the core temperature reaches a plateau and remains relatively stable for the duration of exercise. The rise in the core temperature stimulates centrally located thermoreceptors, causing an increase in blood flow to skin and the initiation of sweating. The increase in skin blood flow transfers heat to skin for dissipation. The net transfer of heat from muscles to skin for dissipation depends on the core–to–skin temperature gradient. Skin temperature is initially lower than the core temperature. At the onset of exercise, skin temperature falls slightly due to increased convection resulting from the motion of the subject and then gradually rises. When skin temperature is already high, such as occurs in a warm environment or due to radiant gain from the sun, heat transfer to the skin will be compromised (Rowell, 1986).

Heat is transferred from skin to the environment by convection, radiation, sweat evaporation, and respiratory losses. Environmental conditions, mainly temperature, govern which of these modes is most effective. Loss of heat by convection and radiation depends on a temperature difference between skin and the environment. When environmental temperature is low (~10°C), the mean skin temperature will be approximately 25°C to 28°C, resulting in a temperature difference of 15°C to 18°C. Under these conditions, convection and radiation alone would be sufficient to dissipate the entire heat load imposed by mild-to-moderate exercise. As the ambient temperature increases, the skin–environment temperature gradient falls, becoming negligible at about 36°C and actually reversing at higher environmental temperatures. Thus, heat loss via convection and radiation becomes ineffective, and the body must rely on sweat evaporation for heat loss (Åstrand and Rodahl, 1979).

In moderate ambient temperatures (say, 25°C), approximately 50% of the metabolic heat load is dissipated by radiation and convection and the other 50% by evaporation. However, when skin temperature and ambient temperature are equal, evaporative cooling becomes the only avenue for dissipation of heat. The rate of heat loss by evaporation of sweat depends mainly on the water vapor pressure gradient between skin and the environment and the fraction of the body surface area that is covered with sweat. High environmental humidity will decrease the water vapor pressure gradient and limit the ability of the body to lose heat via sweating. The environmental conditions of high ambient temperature and humidity present a serious threat to the body's mechanisms of heat loss and can result in dangerous elevations of body temperature if exercise continues. This was one of the challenges faced when conducting the equestrian events for the Olympic Games in Atlanta (Adams et al., 1975; Jeffcott and Kohn, 1999; Rowell, 1986).

ESTIMATIONS OF HEAT PRODUCTION DURING EXERCISE

To make estimations of heat production during exercise, calculations can be made using oxygen consumption as an indicator of metabolic rate. The metabolic heat load can be estimated from the following formula:

$$\text{Metabolic heat} = \dot{V}O_2 \text{ (L/min)} \times k \times \text{exercise duration (minutes)}$$

where $\dot{V}O_2$ = oxygen consumption and k = amount of heat liberated per liter of oxygen consumed (k = 4.7 to 5.1 kcal, depending on the substrates used). Assuming a 20% metabolic efficiency, approximately 1 kcal/L of O_2 is available for muscular work (Åstrand and Rodahl, 1979; Mitchell, 1977).

Horses are capable of very high work intensities, and the rate of heat production may exceed basal levels by 40- to 60-fold during racing speeds. The basal metabolic rate of a resting horse has been recorded at 2.2 to 4.2 mL/kg/min. This value is similar to that calculated from the metabolic body size (a function of the body weight to the 3/4 power, 3 kcal/kg$^{3/4}$/hr). The metabolic body size of a 450-kg horse is 97.7 kg, the basal metabolic rate is 285 kcal/hr, equivalent to an oxygen uptake of 58.6 L/hr or 2.2 mL/kg/min. A Thoroughbred racehorse exercising at race speeds runs at approximately 16 to 17 meters per second (m/s), an intensity requiring its maximal oxygen uptake (85 to 90 L O_2/min). This exercise level would be associated with a heat production of 450 kcal/min (90 L/min × 5 kcal/L). If this metabolic heat load was not dissipated, an elevation of body temperature of 1°C for every minute of exercise could occur (i.e., 60°C/hr), assuming the heat capacity of the horse is the same as that of humans (0.83 kcal/kg/°C) (Hodgson et al., 1993).

In contrast, an endurance horse exercising at a mean speed of 4 to 5 m/s consumes approximately 25 L of O_2 per minute (about 40% of maximal oxygen uptake), producing a heat load of about 100 kcal/min. This would result in an increase in body temperature of about 0.25°C/min, or 15°C/hr, if no dissipation occurred.

Despite the lower rate of heat production during endurance exercise, heat dissipation is more important than during racing because of the prolonged duration of the exercise. A Thoroughbred racehorse typically races at maximal speed for 1 to 3 minutes. This would he associated with heat production of 1350 to 2250 kcal (450 kcal/min). The body is able to store a large amount of heat (0.83 kcal/kg/°C), 415 kcal/min for a 500-kg horse. If all the heat produced during a race was stored, the body temperature would rise 3.25°C to 5.42°C. Measurements of exact heat production during this type of exercise have been made, but estimates using treadmill exercise provide useful insights. At the trot and canter, heat production was 78 and 131 kcal/min, respectively. These levels of heat production would result in body temperature elevations of 0.13°C/min at the trot and 0.23°C/min at the canter. Recorded rectal temperature rises of only 0.02°C/min and 0.035°C/min occurred, indicating storage of approximately 15% of the heat produced (Hodgson et al., 1993).

Heat that is stored during exercise is dissipated after cessation of exercise. The core temperature continues to rise within

the first few minutes of recovery from maximal exercise as heat is redistributed from muscles.

AMOUNT OF HEAT DISSIPATED BY SWEATING

Evaporation of 1 L of sweat dissipates approximately 580 kcal of heat. The amount of heat loss by evaporation of 1 L of sweat is equivalent to the heat generated by 1 to 2 minutes of maximal exercise (450 kcal/min) or 5 to 6 minutes of submaximal endurance exercise (100 kcal/min). To dissipate the heat produced by prolonged submaximal exercise, substantial volumes of sweat must be evaporated. An hour of submaximal exercise would produce 6000 kcal (60 min × 100 kcal/min), and to dissipate this heat by evaporative processes, 11 L of sweat would be required (McCutcheon and Geor, 1998). Any process that adversely influences evaporation of sweat can have potentially antagonistic effects on performance because of increased demand on the thermoregulatory and cardiovascular systems. The effectiveness of evaporative cooling is dependent on the environmental temperature, the relative humidity, wind velocity, and body surface area to body weight ratio. Horses have a relatively low ratio of surface area to body weight compared with other species. A 60-kg human has a surface area of approximately 1.7 m^2 compared with a 500-kg horse with a surface area of only 5 m^2. Undoubtedly, this will present a physical limitation to the efficacy of sweating in the horse (Hodgson et al., 1993; Rowell, 1986).

Heat loss from sweating in humans results in a decrease in skin temperature of about 2°C and in horses, a temperature 2.5°C below that of the core temperature has been recorded from a thermocouple measuring the temperature of blood draining primarily from body or flank skin. Thus, sweating results in significant cooling of blood flowing through skin. For this mechanism of heat loss to be utilized, skin blood flow must be high. In exercising humans, up to 15% of cardiac output may be directed to skin. Data from ponies exercising in controlled conditions in the heat demonstrate that up to 20% to 25% of the $\dot{Q}$° is distributed to skin. If one extrapolates these data to the horse, heat loss via sweating can be estimated. Assuming that the $\dot{Q}$° of an exercising endurance horse is approximately 160 L/min (40% $\dot{V}O_{2max}$) and the specific heat capacity of blood is 0.9 kcal/L/°C, cooling of the blood due to sweating represents a loss of 72 kcal/min (0.2 × 160 L/min × 2.5°C × 0.9 kcal/L/°C). The sweat rate necessary to result in this heat loss is 125 mL/min or 7.5 L/hr (580 kcal/L). This heat loss represents only 70% to 75% of the heat load that would result from endurance exercise (100 kcal/min) (Åstrand and Rodahl, 1979; Hodgson et al., 1993; McCutcheon and Geor, 1998).

Heat loss via sweating also can be estimated by measuring the sweat volume lost during exercise. Sweating rates of horses exercising in the heat may reach 10 to 15 L/hr. Thus, by comparing these two methods of estimation, it can be deduced that sweating results in heat dissipation of up to three quarters of total metabolic heat produced. Although mass specific body surface area calculations might suggest otherwise, heat loss via sweating may be more efficient in horses than is the case in humans. This is likely a function of the greater proportion of the $\dot{Q}$° of the horse being distributed to the skin during exercise (McCutcheon and Geor, 1998).

ESTIMATION OF SWEAT LOSSES DURING EXERCISE

Sweating rates have been estimated by weighing horses before and after exercise. Moderate exercise (3.5 m/s) for 6 hours resulted in a 5% to 6% loss of body weight (27 kg), with one horse losing 46.4 kg or 9.1% of body weight. In another study, in cool weather conditions, weight loss of 37 ± 2.6 kg (7.6% ± 0.5%) occurred during exercise at 18 km/hr for 58 to 80 km. During the first 100 km of the Tevis Cup ride, the mean weight loss was 17.5 kg, with a maximal weight loss of 45 kg recorded from one horse, representing 10.5% of the body weight (Carlson, 1983; Schott, 2003).

Thoroughbreds racing over distances of 1 to 2 miles may lose up to 10 L during the warmup, race, and initial recovery periods. Direct measurements of sweat rates on the neck and back of horses exercising on a treadmill at 40% $\dot{V}O_{2max}$ have been shown to be 21 to 34 mL/min/m^2. This corresponds to 6.5 to 9 L/hr for a 450-kg horse, assuming that the total surface area of the horse can be calculated from the equation $SA = 1.09 + 0.008 \times$ body weight (kg) (Hodgson et al., 1993).

AMOUNT OF HEAT DISSIPATED VIA THE RESPIRATORY TRACT

Evaporation of water from the respiratory tract represents an important route of heat loss. Inspired air is both warmed and saturated on its passage through the lungs. Even at the elevated respiratory frequencies associated with high-intensity exercise, expired air is warmed to around 28°C and is at least 85% saturated with water. The heat loss associated with warming inspired air to near body temperature (from 16°C to 33°C) contributes approximately 5% of total heat loss at rest and during exercise. In contrast, the evaporative heat loss associated with humidifying respired gases increases up to fivefold during exercise to remove 10% to 15% of total heat loss. Thus, the combination of estimates for heat loss through sweating (~75%) and up to 20% through respiratory losses accounts for the majority of heat loss during exercise.

During the gallop, biomechanical forces result in synchronization of the respiratory and stride frequencies (Evans and Rose, 1988). Thus, respiratory heat loss can be modified only by altering tidal volume blood flow to the upper respiratory tract, particularly the nasal mucosa, and nasal gland secretion. Despite this limitation, pulmonary ventilation doubles from moderate to maximal speed and can reach >1800 L/min at maximal exercise, which may increase the percentage of respiratory heat loss. In addition, respiration–locomotion coupling reduces the metabolic cost of respiration. Respiratory rate during low-intensity exercise in a cold environment is reduced, which may be associated with a reduced need for heat dissipation.

Respiratory heat loss can be calculated by measuring pulmonary ventilation and assuming that 0.03 g of water is dissipated for every liter of air respired, as reported by Theil et al. (1987). Respiratory heat loss at rest was 4.5 kcal/ min (19 kJ/min), 38% of total heat loss, and at the walk, trot, and gallop, it was 6.2 kcal/min (26 kJ/min), 12.7 kcal/min (53 kJ/min), and 20.5 kcal/min (86 kJ/ min), respectively. At each exercise intensity, the respiratory tract contributed approximately 18% of total heat loss. Similar rates of respiratory heat loss have been reported by other investigators.

Respiratory heat losses also can be estimated from the decrease in blood temperature that occurs as blood passes through the pulmonary circulation. Blood in the carotid artery has been measured in horses exercising on a treadmill and was found to be 0.1°C to 0.3°C lower than blood in the pulmonary

artery. Assuming a $\dot{Q}$° of 160 L/min and a temperature difference of 0.15°C, respiratory heat loss represents 21.6 kcal/min (160 liters/min × 0.15°C × 0.9 kcal/L/min), approximately 22% of the heat load produced during submaximal exercise (100 kcal/min) (Hodgson et al., 1993).

CARDIOVASCULAR FUNCTION RESPONSES TO AUGMENT THERMOREGULATION DURING EXERCISE

The cardiovascular system is vitally important during exercise to meet the increased demand of working muscle while maintaining blood flow to skin to dissipate the heat produced. The cardiovascular system responds to this demand by increasing the total cardiac output, a greater proportion of which is redistributed to exercising muscle (Rowell, 1986).

Skin Blood Flow during Exercise

Skin blood flow plays a major role in the distribution of thermal energy during exercise. Skin blood flow changes in the horse in response to exercise have been measured in ponies but not horses. Measurements in humans report an initial decrease in skin blood flow to increase blood flow to working muscle. However, as core temperature rises above 38°C, the vasodilator drive for heat dissipation is stimulated, and skin blood flow begins to rise, which may be the reason $\dot{V}O_{2max}$ is lower during exercise in the heat (Rowell, 1986).

Cardiac Output during Exercise

The cardiac output increases in proportion to exercise intensity and whole-body oxygen consumption. The increase is due to increased heart rate and stroke volume. Six-fold elevations in $\dot{Q}$° and 41% elevations in stroke volume (SV) have been recorded during mild-to-moderate exercise. However, during maximal exercise, most of the increase in cardiac output is caused by the increase in heart rate. In horses, the exercising muscles contribute a much larger proportion of the body mass than in humans and demand a greater activation of the cardiovascular system (Evans and Rose, 1988).

Similar cardiovascular responses occur in humans in response to exercise, with increases in $\dot{Q}$° and heart rate; however, SV tends to plateau before maximal heart rate is reached and may even decrease during very severe exercise. SV is maintained during prolonged exercise in horses, and horses are, thus, better adapted than humans for the performance of endurance exercise. This probably relates to postural differences between humans and quadrupeds (Evans and Rose, 1988; Rowell, 1986).

Redistribution of Cardiac Output during Exercise

During exercise, there is an increase in the metabolic requirements of a number of tissues. Apart from exercising muscle, blood flow is increased to the myocardium, trachea, bronchi, and respiratory muscles. In response to these increased demands, there is a primary increase in blood flow to exercising muscles and a relative decrease in flow to skin, the resting muscle, the kidneys, and the splanchnic region in proportion to exercise intensity (McConaghy et al., 1996; Rowell, 1986).

EFFECTS OF COMBINED EXERCISE AND HEAT STRESS

When exercise is undertaken in a warm environment, the demands of muscle for increased metabolic requirements and skin blood flow for heat exchange arise concurrently. The system must be carefully regulated to subserve both these functions, and problems can result when there is competition between the need for blood flow to the working muscles and to skin for heat loss, such as occurs during exercise in the heat. To avoid compromising blood flow to skin, which would limit heat loss and result in hyperthermia, or to the working muscle, which would limit aerobic metabolism, resulting in anaerobic work with its limited substrate supply, $\dot{Q}$° would have to increase continuously (Rowell, 1986).

CARDIAC OUTPUT

The effect of exercise in the heat on cardiac output has been studied in ponies. The cardiovascular responses of horses to exercise differ from those of humans in a number of ways: (1) The cardiac output of horses is maintained during prolonged exercise; (2) horses do not have the problem of maintaining blood pressure associated with an upright posture; and (3) the cutaneous vasculature of horses does not show active vasodilation in response to heat stress. In light of these differences and data in ponies, it seems probable that in horses the cardiac output is maintained during exercise in the heat (Evans and Rose, 1988; McConaghy 1996; McConaghy et al., 1996).

In humans, moderate-to-severe exercise in the heat results in a reduction in $\dot{Q}$° below levels occurring in a thermoneutral environment. This reduction in $\dot{Q}$° during exercise occurs as a result of (1) reduced central blood volume caused by increased cutaneous venous volume in response to thermoregulatory drives, (2) decreased plasma volume caused by loss of plasma water to the extravascular compartment in response to tissue hyperosmolality and increased filtration pressure in active muscles, and (3) loss of body fluid in sweat. The reduction in cardiac output seen in humans, caused by decreased plasma volume and loss of body fluid in sweat, also may occur in horses during prolonged exercise in the heat. However, this is not the case in ponies exercising at moderate and high intensities in conditions of high ambient heat load (Rowell, 1986).

REDISTRIBUTION OF CARDIAC OUTPUT

Redistribution of the cardiac output of horses in response to exercise in the heat has been measured in ponies, and these data are extrapolated to horses. The thermoregulatory responses of horses, ponies, and humans are somewhat similar, and as such, blood flow redistribution in response to exercise in the heat is likely to be similar between the species, although ponies do appear to reduce the proportion of cardiac output redistributed to the viscera and the kidneys to augment blood flow to the skeletal muscle, myocardium, skin, and brain (McConaghy, 1996).

The exact redistribution of cardiac output in humans cannot be measured; only estimates can be made. As a result, there is some dispute in the literature as to whether blood flow to the working muscles in humans is reduced to maintain skin blood flow during exercise in the heat.

Thus, the response of the cardiovascular system to exercise is somewhat modified when exercise is carried out in the heat because of the additional environmental heat load. Cutaneous vasodilation occurs at a lower level of exercise because of the earlier attainment of a critical core temperature elevation. To

supply this volume of blood, there is an increased redistribution of blood away from splanchnic and renal vascular beds as well as a heat-induced increase in $\dot{Q}$. This occurs in humans as well as in ponies (Rowell, 1986; McConaghy 1996; McConaghy et al., 1996).

However, the skin of humans must be relatively vasoconstricted during heavier exercise in the heat because, according to Rowell, "there is simply not enough cardiac output or regional blood flow to raise skin blood flow to the levels seen at rest at equivalent levels of core temperature." Thus, either blood flow to skin must be reduced, which would impair heat loss, or there must be a reduction in blood flow to the working muscles, which would reduce metabolic performance. There is evidence that blood flow to the working muscles in humans is not compromised during exercise and heat stress; thus, skin blood flow must be reduced. The same effect has been demonstrated in ponies. It appears that a cutaneous vasoconstrictor response occurs when central blood volume decreases to levels regarded as critical as a mechanism for avoiding circulatory collapse. This can be interpreted to suggest that circulatory regulation appears to take precedence over thermoregulation. Thus a major factor limiting the ability to exercise or to tolerate heat is the threat of circulatory collapse (Rowell, 1986).

Experiments have been performed in sheep to measure exact blood flow redistribution during exercise in the heat. Significant decreases in blood flow to both skin and the exercising muscles occurred, "in contrast to the findings in humans, suggesting that either physiologic responses of humans and sheep differ or that the estimations derived from studies in humans are inaccurate." The reliance of sheep on panting for thermoregulation does imply that significant differences in thermoregulatory responses between sheep and humans may exist (Hales, 1973). Documentation of redistribution of the cardiac output of ponies in response to exercise in the heat has assisted in clarifying this dilemma. Indeed, humans and ponies and, therefore by implication, horses appear to manifest considerable similarities in their thermoregulatory mechanisms. Distribution of the cardiac output of horses at rest and during exercise in a thermoneutral and a warm environment is depicted in Figure 8-2. Responses of ponies in terms of blood flow distribution when undertaking moderate and intense exercise in thermoneutral and hot ambient conditions is shown in Figure 8-3.

CLINICAL PROBLEMS ASSOCIATED WITH HEAT STRESS AND EXERCISE IN HORSES

A number of clinical problems, including exhaustive disease syndrome, synchronous diaphragmatic flutter, heat stress (stroke), rhabdomyolysis, and anhidrosis, can occur as a result of prolonged heat and work stress (Carlson, 1983; Jeffcott and Kohn, 1999).

EXHAUSTIVE DISEASE SYNDROME

Protracted submaximal exercise is associated with the production of a significant heat load. As detailed in an earlier section of this chapter, in certain situations up to 11 L of sweat would have to be evaporated to dissipate the heat produced by each hour of submaximal exercise. The substantial

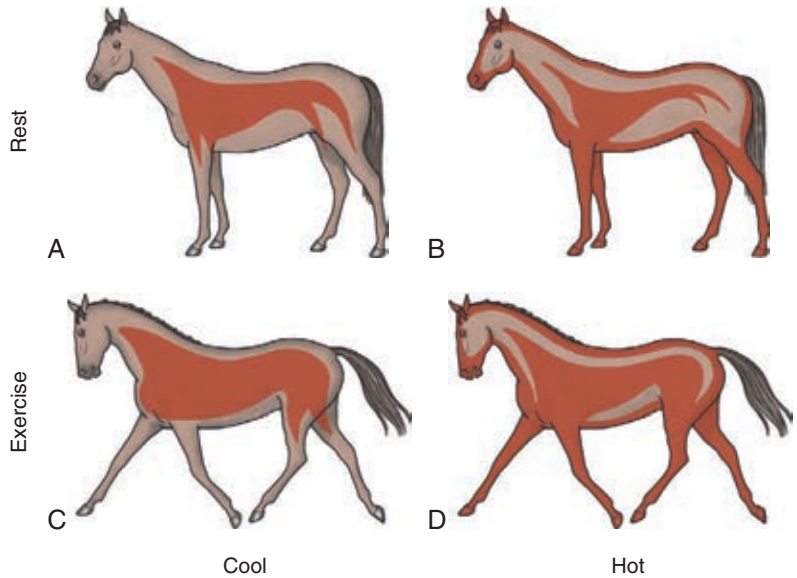

FIGURE 8-2 Schematic illustration of altered distribution of blood volume in response to heat stress at rest and during exercise. **A,** The horse at rest in a cool environment. **B,** The horse at rest exposed to high ambient temperatures. There is a mild increase in cardiac output with a substantial proportion of this increase redistributed to skin to aid in heat loss. **C,** The horse exercising in a cool environment. Most heat is lost without the need for sweat production. As a result, the proportion of cardiac output directed to skin remains relatively low. **D,** Exercise in the heat. Environmental and exercise-induced heat loads impose great demands on thermoregulatory function necessitating redistribution of a much larger proportion of the cardiac output to skin for heat loss.

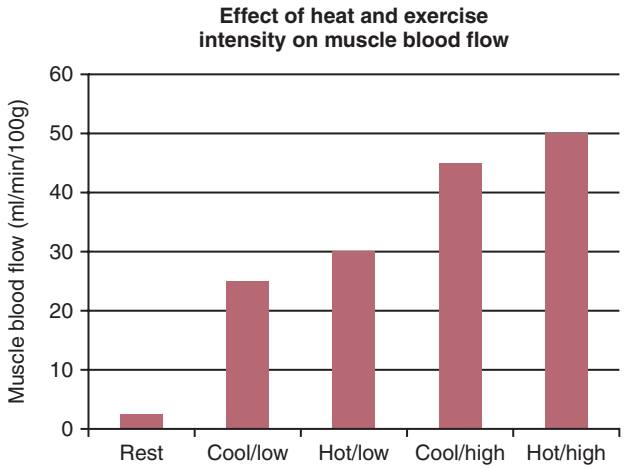

FIGURE 8-3 Duration of exercise required for horses to reach a core temperature (pulmonary arterial blood) in horses exercising in cool/dry, hot/dry and hot humid conditions. This graph demonstrates the effect of heat and humidity on diminishing the capacity of the horse to dissipate exercise induced increases in body temperature.

loss of fluid and electrolytes associated with sweating at this rate results in significant adverse effects on thermoregulatory and cardiovascular mechanisms. Endurance horses forced to exercise for prolonged periods in hot environments can become severely dehydrated, and this may progress to development of hypovolemic shock. This condition, when combined with energy depletion and profound fatigue, has been referred to as *exhausted horse syndrome* (Carlson, 1983).

Effect of Sweat Loss on Body Fluid Composition

The total body water of a normal 450- to 500-kg horse is approximately 300 L, consisting of approximately 200 L of intracellular fluid (ICF) and 100 L of extracellular fluid (ECF). Water moves freely between the ECF and the ICF, its distribution between the compartments depending on the respective content of exchangeable cations, the principal cations being sodium in the ECF and potassium in the ICF. Despite distinct differences in electrolyte composition, these compartments maintain similar osmolalities (McCutcheon and Geor, 1998).

The major components of the ECF are plasma, interstitial fluid, lymph, and transcellular fluid, mainly gastrointestinal fluid. Sweat is derived from interstitial fluid, with subsequent transfer of plasma and cellular fluid to the interstitial space to maintain interstitial fluid volume. Consequently, sweating during exercise depletes both the extracellular and cellular fluid compartments. A water loss total body water of 40 L, such as may occur during an endurance ride, would be derived from both ECF and ICF volumes. This represents a loss of over 15% of the total body water.

In horses, absorption of up to 20 L of fluid from the substantial gastrointestinal tract fluid reserve has been shown to help maintain plasma volume. This may explain the only relatively minor elevations in packed cell volume (PCV) and total plasma protein concentration (TPP) shown by some well-conditioned endurance horses following losses of 30 to 40 kg during a ride (Schott, 2003).

In addition to significant water losses, substantial losses of electrolytes occur. Electrolyte losses occurring as a result of sweat loss during prolonged exercise in horses in a warm environment involve reductions in plasma chloride, potassium, calcium, and magnesium. The chloride loss in sweat is 20% greater than the sodium loss, and as a result, the greatest reduction occurs in plasma chloride, with potassium concentration decreasing moderately and sodium concentration decreasing only slightly. Thus, an ECF deficit of 25 L would be associated with a chloride deficit of about 4000 millimoles (mmol; milliequivalent [mEq]). To maintain electrical neutrality, this hypochloremia is associated with an increase in plasma bicarbonate, inducing metabolic alkalosis. In addition, mild metabolic alkalosis often occurs secondary to the chloride ion depletion accompanying sweat production (Schott, 2003).

When endurance rides are conducted in cool climates, sweat losses may be minimal, and changes in PCV, TPP, and electrolyte concentration insignificant. Many endurance horses develop transient fluid and electrolyte imbalances but are able to recover and replace losses via feed and water consumption. Alterations tend to be more severe in very competitive horses that are pushed at a fast pace, resulting in a greater degree of dehydration, higher heart rates, and a more significant degree of metabolic alkalosis at the end of the ride (Schott, 2003).

Pathogenesis

Major alterations of fluid and electrolyte balance adversely affect athletic performance and may result in a life-threatening metabolic state. Electrolytes are essential for control of membrane potential, muscle contraction, nerve conduction, and enzyme reactions, and they play a central role in the physiologic processes of exercise. Na^+ deficit associated with sweat loss results in a reduction of plasma volume, as ECF Na^+ is the principal determinant of ECF volume. Na^+ depletion in combination with dehydration results in decreased plasma volume, increased blood viscosity, inadequate tissue perfusion, and inefficient oxygen and substrate transport. This may contribute to impaired renal function and partial renal shutdown, effectively resulting in renal failure. Hyponatremia also can lead to intermittent muscular cramping, possibly from inhibition of Na^+-Ca^{2+}-ATPase. Severe hyponatremia results in fatigue, diarrhea, central nervous system signs, and muscular spasms in humans, and similar effects have been reported in horses (Carlson, 1983).

K^+ depletion modifies membrane potentials and may reduce the response of vascular smooth muscle to catecholamines, resulting in peripheral vasodilation and reduced central blood volume. This imposes an additional burden on the heart, already affected by hypokalemia and increased oxygen demand associated with hyperthermia. Hypokalemia also can have a direct pathologic effect on renal nephrons, which may contribute to the development of renal failure.

Metabolic alkalosis associated with depletion of K^+, Cl^-, Ca^{2+}, and Mg^{2+} during exercise may alter membrane potential and neuromuscular transmission and contribute to gastrointestinal stasis, cardiac arrhythmias, muscle cramps, and synchronous diaphragmatic flutter.

Dehydration results in a significant impairment in the efficiency of evaporative cooling which when prolonged results in reduced skin blood flow and sweating with resultant

elevations in the core temperature. Dehydration is the most important single predisposing factor for the development of heat illness. In humans, dehydration affects both thermoregulatory mechanisms, reducing skin blood flow and the sweat response and energy utilization via accelerated depletion of glycogen stores and resulting in an excessive rise in body temperature and heart rate. Dehydration is likely to have similar effects in horses, resulting in hyperthermia and the potential for damage to the central nervous system.

The combined effects of energy depletion, electrolyte loss, acid–base imbalance, and dehydration are likely to alter gastrointestinal and central nervous system functions, reducing the ability to voluntarily replace fluid losses. Medical intervention is necessary if untoward sequelae resulting from these changes are to be avoided (Geor and McCutcheon, 1996; Schott, 2003).

Clinical Signs

Affected horses often show severe signs of depression, usually having no interest in feed and water. Dehydration is expressed clinically as decreased skin turgor, sunken eyes, dry mucous membranes, firm, dry feces (if any are passed), and decreased urine output. Rectal temperature is often markedly elevated (40°C to 42°C). Respiratory rate is elevated (with some horses appearing to *pant*), possibly in an attempt to increase respiratory heat loss, as is heart rate to help maintain cardiac output. Cardiovascular compromise results in increased capillary refill time, decreased pulse pressure and jugular distensibility, and cardiac irregularities. Intestinal stasis commonly occurs with reduced borborygmi and a loss of anal tone. Muscle spasms and cramps are frequently present, and synchronous diaphragmatic flutter ("thumps") may develop in some cases (Geor and McCutcheon, 1996).

A number of serious complications may develop a day to several days following exhaustion. These sequelae are most likely to occur if horses fail to receive immediate and thorough treatment at the time of development of clinical signs. Exertional rhabdomyolysis, renal failure, hepatic dysfunction, gastrointestinal dysfunction, laminitis, central nervous system disorders, and death have been reported. Intensive and immediate treatment at the time exhaustion develops will reduce the risk of occurrence of these problems.

Clinicopathologic Alterations

Exhausted endurance horses have clinicopathologic alterations indicative of severe dehydration, with hematocrit elevations to 0.45 to 0.60 L/L. Plasma protein concentrations are generally in the range 72 to 82 g/L but may reach 100 to 120 g/L. Hematology reveals a stress neutrophilia with a left shift, increased immature neutrophils, lymphopenia, and a marked eosinopenia (Carlson, 1983).

Electrolyte alterations include primarily hypochloremia, with plasma chloride concentrations often in the range 80 to 90 mmol/L, and moderate hypokalemia and hyper- or hyponatremia may be present. Ca^{2+} and Mg^{2+} concentrations tend to decline, which are proposed to contribute to the risk of the development of synchronous diaphragmatic flutter (Carlson, 1983).

The plasma activities of creatine phosphokinase (CK), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and aspartate amino transferase (AST) may be elevated. Marked elevations of CK, AST, and LDH will be present if exertional rhabdomyolysis has developed. Plasma creatinine, urea, and bilirubin may be transiently elevated during endurance rides and will be persistently elevated if renal damage develops (Geor and McCutcheon, 1996; Schott, 2003).

Treatment

Early recognition and treatment will greatly reduce the severity of the exhaustive disease syndrome. During the course of an endurance ride, signs of a problem developing may first be noticed during veterinary examination at mandatory veterinary examination or check points or in the course of the ride. Affected horses may show signs of distress, fatigue, significant dehydration, and sustained elevation of heart rate, respiratory rate, and rectal temperature. In well-conditioned horses that retain good thermoregulatory function, heart rate should fall to less than 55 beats/min, the respiratory rate to less than 25 breaths/min, and the rectal temperature to below 39.5°C within the first 20 to 30 minutes following cessation of exercise. Any horse showing a significant and persistent elevation of heart rate, respiratory rate, or rectal temperature should not be allowed to continue exercise, according to the generally accepted rules of endurance riding competitions (Schott, 2003).

Horses suffering mild signs will usually respond to rest and simple therapy, including cold hosing, standing the horse in shade, and voluntary rehydration. The horse should be offered small amounts of cool water at frequent intervals and be given access to palatable feed. Some recommend offering horses concentrated electrolyte pastes. These animals should be watched closely until recovery is complete. Once homeostasis is restored, the animal can replenish accumulated deficits by voluntary consumption. This process may take several days.

Horses with marked elevations of rectal temperature (greater than 40.5°C) should be cooled as quickly as possible. Hosing down with cold water, allowing the water to warm, scraping it off, and repeating the process on a number of occasions is a very effective way of enhancing conductive heat loss to cool the horse (Figure 8-4). Providing the horse with ventilation via natural breeze in an open space or fanning (misting fans can also be used) will encourage heat

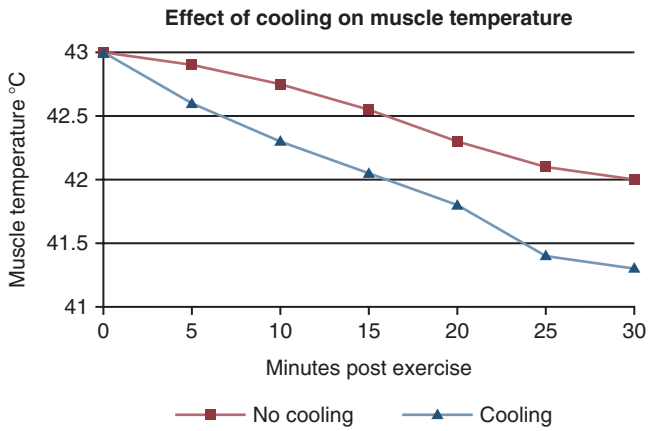

FIGURE 8-4 Effect of aggressive cooling, that is, repeated applications of cold water, on body core temperature in horses following exercise under controlled conditions.

loss via convection and evaporation. Particular attention should be given to cooling the head, neck, and large subcutaneous vessels between the hind legs. Shade, cool airflow, and cool water will dramatically increase radiant, evaporative, and convective heat losses. Some recommend cold water enemas and administration of cool fluids via a nasogastric tube to help lower the core temperature (Jeffcott and Kohn, 1999).

Fluid administration is an essential component of therapy. Some studies have shown that horses suffering significant dehydration following prolonged exercise may only replace two thirds of their water deficit voluntarily by water consumption in the first few hours after exercise. The principal drives for voluntary water consumption are volume depletion and Na^+ concentration. Plasma Na^+ concentrations are often within normal limits, which may reduce the drive for voluntary rehydration. Thus, active (interventionalist) fluid therapy may be required to fully replenish fluid deficits. As a general rule, rehydration fluid should be a "replacement" fluid, therefore having similar composition to plasma, since hyponatremia and hypochloremia can be exacerbated if the entire deficit associated with sweat loss is replaced with water (Geor and McCutcheon, 1996; Schott, 2003).

Fluid therapy should be instituted immediately in any horse showing severe signs or failing to respond to conservative treatment within 30 minutes. Fluid can be administered by nasogastric tube, giving 8 L of isotonic fluid continued at 30- to 60-minute intervals, as required. This assumes no reflux or pain associated with the fluid administration. In severe, acute cases, if colic is present or if there is ileus, intravenous administration is preferable (Geor and McCutcheon, 1996; Schott, 2003).

The exact volume, rate, and route of fluid administration necessary depends on the severity of the presenting signs. Volumes required may be up to 50 L, which can be administered at flow rates of up to 10 L/hr. A severely affected horse may have a Na^+ deficit of around 4000 mmol, which will require 30 L of Plasmalyte solution for replacement. Horses that develop synchronous diaphragmatic flutter (SDF) and gastrointestinal atony will benefit from intravenous calcium therapy (see Treatment for Synchronous Diaphragmatic Flutter) (Schott, 2003)

Response to therapy includes improved mucous membrane color and capillary refill time and increased pulse pressure. With the return of gastrointestinal motility, there should be an improvement in the animal's attitude and desire to eat.

Horses affected by exhaustive horse syndrome should not be transported for 12 to 24 hours because a high degree of muscular activity is associated with prolonged transport, which puts the horse at further risk of postexhaustive problems. Some of these include exertional rhabdomyolysis, laminitis, and renal failure.

Treatment for exertional rhabdomyolysis, a severe complication that may be associated with the exhaustive horse syndrome, will not be dealt with at length here. In general, management consists of strict rest, nonsteroidal antiinflammatory drugs (NSAIDs), fluid therapy followed by careful monitoring, and tailoring of therapy (Geor and McCutcheon, 1996).

PREVENTION OF HEAT STRESS IN HORSES

The risk of this problem developing will be reduced by adequate preparation and careful management at competitions, with consideration of the effect of the ambient weather conditions at the time of the event.

Preparation for the Event

Physical Training

Physical training in humans and horses results in a number of physiologic alterations that result in improved heat tolerance and thus performance in the heat. Training acts as a form of heat acclimation by imposing a heat load on the body, repeatedly stimulating heat-loss mechanisms (Geor and McCutcheon, 1998; Jeffcott and Kohn, 1999).

There is a lowered threshold for the initiation of sweating and peripheral vasodilation, with earlier activation of heat loss mechanisms. The sensitivity of both these responses is also enhanced, so there is greater sweat production and increased skin blood flow for the same elevation of body temperature. These adaptations result in enhanced transfer of heat from the core to the periphery and increased efficiency of evaporative cooling. As a consequence, less heat is stored, a steady thermal state is reached sooner, and a lower internal temperature is maintained. A lower core temperature increases the core–skin temperature gradient so that a smaller skin blood flow is needed for transfer of metabolic heat to skin. Thus, a smaller fraction of cardiac output needs go to skin, cardiac demand is reduced, and the capacity for continued skeletal muscle perfusion is improved.

Training also results in an expansion of plasma volume, which improves thermoregulatory function by enhancing sweat production and assisting conductance of heat, as well as reducing cardiovascular demand associated with working in the heat. Increased blood volume decreases competition between the working muscles and skin; thus, blood flow to skin can be maintained during exercise. In addition, there is an increase in work capacity resulting from a decreased rate of glycogen utilization in response to training mechanisms (Geor and McCutcheon, 1998; Jeffcott and Kohn, 1999).

Adequate training for endurance rides should help reduce the risk of exhaustive horse syndrome, as well as exertional rhabdomyolysis (Geor and McCutcheon, 1998). The reader is referred to Chapter 22, on Training the Endurance Horse.

In humans also, training results in decreased salt concentration in sweat in response to salt depletion from losses in sweat. Aldosterone released in response to fluid and electrolyte losses acts at the level of the sweat duct to reduce the salt concentration of sweat. This response occurs also in the horse but is somewhat less pronounced than is the case in humans.

Training also decreases the levels of subcutaneous fat, which can improve heat dissipation because this adipose tissue layer acts as an insulator, limiting the rate of heat loss from the body and also reducing the ratio of body surface area to mass. Thus, not surprisingly, well-conditioned endurance horses have very low proportions of subcutaneous fat and total body fat.

Heat Acclimatization

In humans, heat acclimatization by training in humid heat further improves heat tolerance during exercise, as well as improving cardiovascular function for work performance. This effect has been shown to occur in the horse also. Thus, horses participating in endurance type activities (endurance

racing, 3-day eventing) have been shown to benefit from undertaking repeated work in the heat if they are going to be competing in hot environments (Geor and McCutcheon, 1998).

It is recommended that human athletes training for competition in hot environments (e.g., athletes preparing for the Atlanta, Barcelona, Beijing Olympics) commence their heat acclimation at least 2 or, preferably, 3 weeks prior to leaving for the event. About 10 and 14 days of work in the heat is sufficient to induce many of the physiologic improvements associated with heat acclimatization in humans, although 3 weeks would be optimal. Similar responses were noted in horses in studies conducted prior to the Olympic Games in Atlanta. As in the case of humans, it is now recommended that horses should be exposed to these conditions for a minimum of 3 weeks to induce an appropriate degree of acclimatization. As such, it is now common practice for horses participating in international competitions in regions where hot and humid conditions are to be expected, to acclimatize well in advance and to arrive at the venue at least a week prior to competition (Noakes, 2003).

Electrolyte Supplementation

Electrolyte supplements are recommended during training if horses are sweating heavily. Free-choice salt should be available, and supplementation can occur via addition to the feed. Supplements should contain equal amounts of Na^+ and Cl^-, with half as much K^+ (Geor and McCutcheon, 1998; Schott, 2003).

Management at the Event

Weather Conditions at the Event

Attention should be paid to weather conditions at the time of the event, and race speed should be adjusted accordingly. High levels of humidity will reduce sweat evaporation, severely limiting this important mechanism for heat dissipation. During competitions in adverse environmental conditions, horses should be carefully monitored for signs of heat stress. Riders should monitor their horses, heart rate and respiratory rate should be measured at frequent intervals, and the general attitude and willingness of the horse should be observed. If marked or sudden elevations of heart rate and respiratory rate occur or the horse appears distressed or unwilling to maintain speed, exercise should be stopped and careful examination carried out. Exercise should be continued at a reduced speed only if the horse recovers immediately. If the horse remains distressed, it should be walked quietly to a rest stop for veterinary attention (Jeffcott and Kohn, 1999).

Watering the Horse during the Ride

In humans, exercise in the heat without water intake results in rising rectal temperature and heart rate with a sudden drop in skin blood flow, falling heart rate, and advancing heat exhaustion. All of this is caused by dehydration resulting in partial breakdown of homeothermy during exercise in the heat. Similar effects appear to occur in horses.

Dehydration can be reduced by careful attention to voluntary intake of water, electrolytes, and energy sources. Electrolyte and energy replacements are recommended for human athletes during prolonged exercise. This is now common practice in endurance horses. Addition of electrolytes to administered fluids replaces electrolyte losses and can aid fluid absorption from the gastrointestinal tract. Addition of carbohydrates, in itself, may benefit certain types of athletic performance in humans and is likely to assist in the uptake of water and electrolytes from the small intestine. In humans, small amounts of glucose or glucose polymers may help in the performance of prolonged exercise by delaying glycogen depletion and exhaustion. This benefit does not appear to translate to the horse, however, as addition of carbohydrate to fluids administered via nasogastric tube are no more readily absorbed than isotonic electrolyte solutions mechanisms (Geor and McCutcheon, 1998; Jeffcott and Kohn, 1999; Schott, 2003).

Consumption of plain water during exercise is not as beneficial as consumption of isotonic fluid because water will decrease plasma Na^+ concentration, resulting in removal of this mechanism as a stimulus for continued water consumption. Hyperhydration prior to commencement of exercise, for example, by consumption of fluids, including glycerol, may aid in decreasing thermoregulatory strain during exercise in humans. However, the benefits of this practice in horses appears to be more equivocal in terms of its efficacy and, as such, is not widely practiced. More emphasis is given to ensuring horses are trained to drink frequently during the competition and at each of the mandatory veterinary check points (Schott, 2003).

Cooling the Horse during the Ride

The hyperthermia of exercise promotes glycogen depletion and blood lactate accumulation, which may contribute to fatigue and early exhaustion. Kozlowski and Salon (1964) found that accomplished exercising dogs significantly reduced the core temperature, decreased the rate of glycogen depletion, and increased exercise duration. Cooling the body during competitions by pouring water over the head and neck at regular intervals is recommended for human athletes. The head and neck are the most appropriate areas, since these areas have a large blood supply and minimal vasoconstriction occurs during exercise. Many investigators have shown that cooling the head and neck during hyperthermia reduces the rise in core temperature. Aggressive cooling of horses can be accomplished during the ride by pouring cool or iced water over the whole body. The water is allowed to warm on the horse, is scraped off, and the process repeated, often on many occasions. This process has been shown to result in much more rapid reductions in the core temperature when compared with other less aggressive forms of cooling (Figure 8-4). As such, this mechanism is now regarded as being essential in horses participating in the speed and endurance phase of a 3-day event, particularly immediately after exercise when the horses are at risk of significant postexertional hyperthermia. This process will be undertaken in shaded areas and in the presence of misting fans, if available (Geor and McCutcheon, 1996; Jeffcott and Kohn, 1999; Noakes, 2003).

SYNCHRONOUS DIAPHRAGMATIC FLUTTER

As the term implies, synchronous diaphragmatic flutter (SDF) is a spasmodic contraction of the diaphragm synchronous with the heartbeat. This condition is seen most commonly in endurance horses performing in hot, humid weather. However, SDF also has been reported in horses after other forms of exercise and also in those with transit tetany, lactation

tetany, and electrolyte imbalances secondary to digestive disturbances (Carlson, 1983).

Clinical Signs

The primary sign is a spasmodic twitch in the flank area unassociated with normal respiratory movements. This movement may be strong enough to produce an audible thump, which has led to the condition being known as "thumps." This twitch is palpable when a hand is placed at the flank, and synchrony with the heart beat will be noted if the heart is auscultated simultaneously. Gastrointestinal atony also may be present, probably caused by hypocalcemia and associated electrolyte losses (Geor and McCutcheon, 1996).

Pathogenesis

It has been proposed that alterations in electrolyte levels and acid–base balance alter the membrane potential of the phrenic nerve, allowing it to discharge in response to the electrical impulse produced by atrial depolarization. The phrenic nerve tracks very close to the atrium, and as such, this hypothesis is plausible. Hypocalcemia, hypokalemia, and alkalosis may all contribute to the development of the condition. Hypocalcemia lowers the depolarization threshold of nerves to electrical stimulation, whereas hypokalemia can cause hyperirritability of long nerves. Alkalosis can result in a decrease in both total and ionized serum calcium. All these conditions can contribute to phrenic nerve hypersensitivity, allowing it to discharge in response to the electrical impulse generated during atrial depolarization (Carlson, 1983).

Treatment

SDF is not life threatening but may indicate metabolic alterations that should be treated promptly. The problem will often resolve spontaneously with rest. Enteral or parenteral administration of balanced electrolyte solutions will assist resolution. If necessary, intravenous calcium can be administered. Calcium borogluconate (20%) is readily available for the treatment of hypocalcemia ("milk fever") in cattle. The solution commonly is supplied in 500-mL bags, which should be diluted 1:4 in isotonic saline and administered slowly to effect. Auscultation of the heart should be carried out during administration, and the infusion should be stopped if any irregularities in heart rate or rhythm develop (Geor and McCutcheon, 1996; Schott, 2003).

Chronic Synchronous Diaphragmatic Flutter

SDF can be a recurrent problem in some horses. Administration of electrolytes during prolonged exercise should help reduce the risk of recurrence, since volume depletion and metabolic alkalosis are predisposing factors. Chronic recurrent SDF may be due to an alteration in the anatomic structures surrounding the phrenic nerve, such as adhesions and fibrosis secondary to pleuropneumonia (Carlson, 1983).

HEAT STRESS (STROKE)

Heat stress occurs when the heat load exceeds the capacity of the thermoregulatory system to effect appropriate heat dissipation.

Predisposing Factors in Humans

A number of factors are associated with an increased risk of heat stress in humans, and it is likely that similar conditions also will adversely affect the thermoregulatory system of the horse. Any factors that reduce heat loss or increase heat gain will predispose to heat stroke in humans. High exercise intensities and ambient temperatures in excess of 37°C (99°F) result in high levels of thermal stress. High relative humidity limits the process of evaporative cooling (Hales, 1987; Robertshaw, 1983).

Individuals who are not exposed to repetitive heat stress because they live in temperate climates or who do not regularly exercise at a rate sufficient to induce sweating will be more at risk. Subcutaneous fat in large amounts in unfit individuals acts as an insulator, decreasing heat loss from the body and decreasing the ratio of body surface area to mass. Infectious disease results in the production of endogenous pyrogens, which cause an elevation of the thermoregulatory setpoint, resulting in heat intolerance. Other stressors include hypoglycemia, fatigue, abrupt changes in daily routine, psychological stress, and transcontinental flights leading to changes in circadian rhythms. Horses are likely to be adversely affected by similar factors (see below) (Hales, 1987; Robertshaw, 1983).

Pathogenesis

The pathogenesis of heat stress in humans has been studied in detail. Failure of the temperature regulatory system at a critical level is attributed to be the cause of heat stroke. During exercise and heat stress, there are competing demands for blood flow between the exercising muscles, the myocardium (to perform work), fat (to provide substrates), and skin (to dissipate heat). These demands, together with sweat losses and fluid redistribution to the interstitial fluid space of active muscles, result in a falling central venous volume and pressure. Subsequently, low-pressure baroreceptors are activated, and vasoconstrictor stimuli for blood pressure regulation overcome the thermoregulatory drives for cutaneous vasodilation. In addition, sweat output declines with severe heat stress because of decreasing blood volume and increasing osmotic pressure. The combination of reduced skin blood flow and sweating results in a progressive elevation in the core temperature. Falling central venous pressure elicits a reduction in blood flow to fat, reducing substrate availability as well as blood flow to the working muscles and the central nervous system. A further decline in central venous pressure enforces further reductions in skin blood flow, uncontrollable elevation of core temperature, and collapse from central nervous system dysfunction or the other effects of severe hyperthermia (detailed below). High body temperature appears to be the cause of collapse, since there is significant evidence that heat has deleterious effects on body tissues (Hales, 1987; Robertshaw, 1983).

The principal damaging effects of heat are liquefaction of membrane lipids, damage to cellular mitochondria and nuclei, increases in cellular energy requirements, and impairment of blood supply. Animal tissues can be destroyed by acute exposure to temperatures of 44°C to 46°C or prolonged exposure to lower temperatures, for example, 42°C to 45°C. Specific tissues affected by heat are the brain, skin, heart, kidney, liver, gastrointestinal tract, adrenal gland, lung, blood vessels, and blood. RBCs are damaged, resulting in erythrocyte sphering, increased blood viscosity, formation of microemboli, and the development of disseminated intravascular coagulation. Vascular lesions, membrane impairments, and circulatory alterations caused by heat stroke result in impairment

of blood supply to most organs, with tissues with a high metabolic rate being most affected (Hales, 1987).

Horses at Risk of Developing Heat Stress

Heat stress occurs most commonly in poorly trained horses forced to exercise for prolonged periods in hot, humid weather, but it can occur after short-duration, high-intensity events such as racing. Horses transported in hot, humid conditions in poorly ventilated transport vehicles also may be affected. Horses kept in cool stalls and exercised in the hottest part of the day may be at risk. Exercising horses in weather conditions that have abruptly become excessively hot and humid will increase the risk of heat stress (Carlson, 1983; Jeffcott and Kohn, 1999).

Excessive warm-up in bright sun before a competition can result in rapid elevations of core temperature and shorten the time to reaching critical levels of hyperthermia. Hyperthermia can develop rapidly in young, inexperienced horses that may become overly excited, with the presence of high levels of catecholamines and an increased metabolic rate.

Racehorses receiving furosemide prophylactically to control exercise-induced pulmonary hemorrhage are mildly dehydrated prior to exercise. Similarly, many racehorses have water withheld several hours prior to competition. In humans, dehydration is a very significant factor predisposing to the development of heat stroke. Excessive elevations in body temperature during exercise occur in dehydrated subjects (Schott, 2003). With reduced efficiency of sweating, there is a decrease in stroke volume and cardiac output, resulting in a reduction in peripheral blood flow and, thus, inefficient conduction of heat from the body core to the periphery. Although during exercise horses have the capacity to selectively cool their brains, when cooled blood from the face flows to the base of the brain to cool the hypothalamus (Figure 8-5), it appears that a point is reached where even these mechanisms are overcome, and critical brain hyperthermia is reached. This likely is a significant contributor to heat stress or stroke (Hales, 1987; Noakes, 2003).

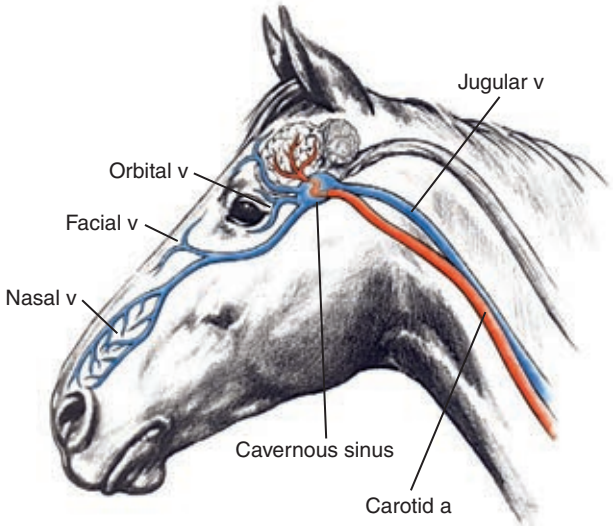

FIGURE 8-5 A schematic representation of the mechanism for selective brain cooling in the horse. Cooled blood from the nares bathes the cavernous sinus and thereby cools the pituitary region keeping this up 1°C cooler than surrounding tissues during hyperthermia induced by intense exercise or ambient temperature.

It seems probable that dehydration would have similar effects on the thermoregulatory capacity of horses. Furosemide has been shown to decrease heat transfer from the body core to the periphery. It would seem prudent to monitor horses receiving furosemide for signs of impending heat stress during exercise in the heat (Geor and McCutcheon, 1996).

Clinical Signs

Horses affected by heat stress frequently show signs of depression and weakness and may refuse to continue to exercise. Heart rate, respiratory rate, and rectal temperature are elevated, with rectal temperatures of up to 43°C. Peripheral vasodilation, in an attempt to increase heat loss, results in congestion of mucous membranes and increased capillary refill time. The sweating response is often inadequate, with hot, dry skin. Muscular disorders such as exertional rhabdomyolysis also may develop. The condition may progress to ataxia, collapse, convulsions, coma, and death, possibly as a result of CNS hyperthermia and changes in membrane permeability (Geor and McCutcheon, 1996).

Treatment

Treatment is directed toward immediate reduction of body temperature. The horse should be moved to a well-ventilated area and should be fanned, if possible, to increase convective heat loss from the skin. As mentioned in the preceding section, large volumes of cool, cold, even icy water should be showered over the horse, with particular attention to the large superficial vessels of the head, neck, and legs, taking care not to omit the rest of the body. The water should be scraped off and the process repeated until the horse appears to show signs of improvement. Ice packs can be placed over large subcutaneous vessels to assist cooling. This also may promote cooling of deeper tissues as the venous blood flows toward the heart (Jeffcott and Kohn, 1999).

Cool water administered via nasogastric intubation, as an enema, or both may be useful in assisting cooling of the body core. However, care needs to be exercised if the horse has any evidence of ileus. Intravenous fluid therapy is considered important also, as plasma volume expansion will assist heat dissipation and replenishment of lost electrolytes (Carlson, 1983).

Antipyretic agents such as NSAIDs should be used carefully and only in conjunction with fluid therapy. High doses should be avoided in dehydrated animals because the risk of NSAID toxicity is greatly increased if perfusion to vital organs is compromised (Geor and McCutcheon, 1996).

Prevention

Assessment should be made of the factors that contributed to the condition developing, and if necessary, adjustments to the training and management of the horse(s) should be instituted. If adverse weather conditions played a significant role in the pathogenesis, exercise in the heat should be avoided or the exercise conditions modified. It is advisable for horses to be exercised only during the cooler times of the day such as mornings and evenings (Jeffcott and Kohn, 1999).

If a competition is to be held during extreme heat or humidity, organizers of the event should be advised of the risk to animals, and thought should be given to postponing the event. Alternatively, strategies to minimize the risk of heat stress

at the competition should be instituted. This may involve reducing the effort required (as has been the case at Olympic Games) with special care taken to monitor horses carefully and provide effective mechanisms for aggressive cooling once the horses have completed the competition. A horse that has suffered from heat stress previously is at a higher risk and possibly should be withdrawn from the competition.

Animals should be transported in well-ventilated trucks and, if possible, only during the cooler periods of the day. Sick, febrile animals should be kept in a quiet, thermoneutral environment (~18°C to 25°C). Horses moved from temperate climates to tropical climates should be allowed to acclimatize before being exercised at high intensities (Jeffcott and Kohn, 1999).

ANHIDROSIS

This condition manifests as an inability to sweat in response to an appropriate stimulus. The disorder occurs mainly in horses in hot, humid climates, particularly in horses bred in temperate areas and transported to the tropics. It has been estimated that 20% of horses in tropical climates (e.g., Florida, Hong Kong) may develop partial or complete anhidrosis if housed in non–air-conditioned environments. The loss of the ability to sweat affects a major avenue for heat dissipation, may result in a severe limitation to exercise performance, and is logically the forerunner of severe exercise hyperthermia in some affected horses (Warner, 1982).

Clinical Signs

Affected horses are unable to sweat normally, which results in heat intolerance and possibly reduced exercise capacity, particularly during exercise lasting more than a few minutes. Following exercise, horses show excessive elevations in rectal temperature and respiratory rate. Fatigue, signs of depression, anorexia, and weight loss also may occur. Skin may be affected, becoming dry and flaky, and alopecia may occur, particularly on the face. Most horses retain some ability to sweat under the mane and the saddle area and between the hind legs. Affected horses develop the problem in summer when ambient temperature and humidity peak and often recommence sweating in the cooler months (Warner, 1982).

Diagnosis

Diagnosis is based primarily on clinical signs. Intradermal skin testing is also available. Intradermal injection of 0.5 mL of 1:1000 epinephrine will produce a marked sweat response in normal horses and is delayed, decreased, or absent in anhidrotic horses. There are several other diagnostic tests in use, one being the terbutalene sweat test. Histologic examination of skin samples may be unrewarding, although some ultrastructural changes in sweat glands, including contraction of the duct lumen and obstruction with cellular debris, may be found. However, this is thought to be an effect rather than a cause of the condition. Histologic examination also may help predict the severity of the condition and the potential for recovery (Warner and Mayhew, 1983).

Pathogenesis

The exact etiology of this condition is unknown. Hypothyroidism, hypochloremia, elevated blood epinephrine concentrations, and exhaustion of the sweat glands have all been suggested. Heat stress is associated with the development of the disease. Prolonged stimulation of the sweat glands by heat exposure is thought to induce a gradual decrease in sweat production. Prolonged high sweat output may alter sweat gland receptor responsiveness to adrenergic stimulation (Warner and Mayhew, 1983)

Treatment

There is no effective treatment other than removal of the horse to a more temperate area or housing the animal in a temperature-controlled (cool) stable. Subsequently, a return to normal sweating may occur, but the condition will often recur if the horses return to hot, humid environments. Some owners have reported an improvement with electrolyte supplementation (Geor and McCutcheon, 1996).

CONCLUSION

The racehorse has an exceptional capacity for exercise. This can involve some of the highest speeds achieved by land mammals. Other breeds within this species are also capable of amazing feats of endurance, whereas many others are used as working horses. The thermoregulatory system of the horse is capable of maintaining homeothermy in the face of the substantial heat loads generated during all types of exercise.

Heat generated within exercising muscles is redistributed to the rest of the body via the circulation and then dissipated from the respiratory tract and the body surface by a number of mechanisms. Heat is transferred from the body surfaces to the environment by conduction, radiation, and evaporation, with evaporation of sweat being the principal method utilized.

To maintain heat loss during prolonged exercise, sweat losses may be significant, up to 40 L, equivalent to 10% of the body weight. Failure to recognize the potential for fluid losses of this magnitude can have serious consequences for the animal. Prolonged exercise in combination with fluid and electrolyte imbalances can result in the development of a number of pathologic conditions. An understanding of thermoregulatory mechanisms will both reduce the risks associated with strenuous exercise and aid in development of strategies to enhance athletic performance.

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CHAPTER

The Respiratory System: Anatomy, Physiology, and Adaptations to Exercise and Training

9

PIERRE LEKEUX, TATIANA ART, AND DAVID R. HODGSON

INTRODUCTION

IMPORTANCE OF THE RESPIRATORY SYSTEM IN THE ATHLETIC HORSE

During the last several decades, research in exercising horses provided growing evidence that the respiratory system may be a limiting factor for maximal performance, even in healthy animals. (Art et al., 1990a; Bayly et al., 1983; Bayly et al., 1987; Erickson et al., 1991; Hodgson et al., 1990; Jones and Lindsdtedt, 1993; Knight et al., 1991; Poole, 2004; Thornton et al., 1983; Wagner et al., 1989). Therefore, any pulmonary dysfunction, even subclinical or moderate, may significantly impair the aerobic metabolism of exercising horses. The observation that respiratory abnormalities frequently are responsible for the "poor performance" syndrome in the horse (Arthur, 1990; Evans and Marlin, 1999; Franklin et al., 2012; McNamara et al., 1990; Morris and Seeherman, 1991) confirms the importance of optimal pulmonary function in the athletic horse. A good understanding of the peculiarities of equine respiratory structure and function is essential for a comprehensive evaluation of the respiratory system and possible correction of its dysfunction.

FUNCTIONS OF THE RESPIRATORY SYSTEM IN THE HORSE

Gas exchange is the major function of the lung, which ensures the transport of oxygen (O_2) from air into blood and of carbon dioxide (CO_2) in the reverse direction. Some parts of the respiratory system also play a role in other nonpulmonary functions such as humidification, warming, and filtering of the inhaled air; swallowing; phonation; olfaction, as a blood reservoir; blood filtering; defense mechanisms against environmental antigens; surfactant production; acid–base regulation; thermoregulation; and the synthesis, release, modification, inactivation, or removal of bioactive substances such as amines, serotonin, histamine, norepinephrine, kallikreins, eicosanoids (prostaglandins, thromboxanes, leukotrienes), neuropeptides (vasoactive intestinal polypeptide, substance P, etc.), enzymes (bradykinase, converting enzymes, etc.), and cytokines (tumor necrosis factor, interleukin 2, etc.).

EXTERNAL FACTORS INFLUENCING PULMONARY FUNCTION

If the respiratory system is able to influence some nonrespiratory functions, the opposite is also true. Pulmonary function may be disturbed by factors that are not directly related to the integrity of the respiratory system such as the quality of the inspired air, the position of the head and neck, the abdominal mass, locomotion-respiration coupling, cardiac function, the equipment used, and a range of other factors. All these factors must be taken into account when evaluating equine pulmonary function.

STRUCTURAL PECULIARITIES OF THE EQUINE RESPIRATORY SYSTEM AND THEIR FUNCTIONAL IMPACT

The function of an organ is highly influenced by its anatomy, and vice versa. This is particularly true with regard to the respiratory system, where external respiration induces important structural changes, mainly during exercise-induced hyperpnea. It is, therefore, useful to remember some structural peculiarities that do influence pulmonary function in the athletic horse. More morphologic details can be obtained from relevant anatomy handbooks (e.g., Hare, 1975).

AIRWAYS

The main function of the airways is to carry air from the nose to the gas-exchanging regions of the lung during inspiration and the opposite during expiration. Therefore, any change in structure that modifies the permeability of the airways to air flow also has a direct impact on pulmonary function.

Nostrils

The nostrils are large and mobile. Their particular structure allows expansion during inspiration, with activation of muscles resulting in flaring of the nostrils and collapse of the nasal diverticulum. This is particularly notable and of some significance during exercise-induced hyperpnea. In addition, function can be disturbed, for example, by injury to the facial nerve which provides the motor efferents to the nostrils.

Nasal Cavities

Because of their large turbinates and important vascularization, the nasal cavities provide a large surface area for heat and water exchange, but they also provide a large source of air flow resistance. Both sympathetic and parasympathetic nerve fibers are distributed to the nasal cavities. Their stimulation may, respectively, vasoconstrict and vasodilate the vascular sinuses, which can induce a decrease or increase in nasal resistance. In contrast, factors inducing an increase of the resistance in the nasal cavities (such as injuries due to nasal intubation, a nasal septum defect

due to a too narrow halter in foals, a tight nose band, etc.) will have deleterious effects during exercise. Hemorrhages resulting from upper airway endoscopy or passage of a nasogastric tube frequently are caused by injuries to the nasal septum and ventral conchal veins.

Pharynx

The soft palate divides the pharynx into the nasopharynx and the oropharynx. Numerous lymphoid follicles are present in the mucous membranes of the dorsal and lateral walls of the nasopharynx and the dorsal surface of the soft palate. The number and size of these follicles are particularly important in young horses and usually regress in mature horses.

The natural tendency of the soft structures of the nasopharynx to collapse during inspiration is limited by tensor muscle contraction.

The guttural pouches are paired diverticulae of the eustachian tubes that communicate with the pharynx via slitlike openings. They are unique to equidae with their function still unknown although debated. These pouches do not seem to influence the passage of air through the upper airways directly. However, they contain strategic structures such as blood vessels (internal and external carotid arteries), cranial nerves (glossopharyngeal, vagus, spinal accessory, and hypoglossal nerves), and cervical sympathetic trunk and retropharyngeal lymph nodes. Any abnormality, even subclinical, of the guttural pouches may potentially damage these highly sensitive structures and consequently induce some dysfunction. For example, lesions of the glossopharyngeal nerves, the vagus nerves, or both may induce a soft palate paralysis and result in its dorsal displacement, which, in turn, will obstruct the airway during exercise.

At a functional level, the most important structural peculiarity of the equine upper airways is the intrapharyngeal ostium, which is an opening in the soft palate formed caudodorsally by the palatopharyngeal wall, laterally by the pillars of the soft palate, and rostrally by the visible border of the soft palate. The laryngeal structures, that is, the corniculate cartilages and the epiglottis, articulate with the ostium like a button in a button hole (Cook, 1989) forming an airtight seal when the horse breathes (Figure 9-1). This peculiar

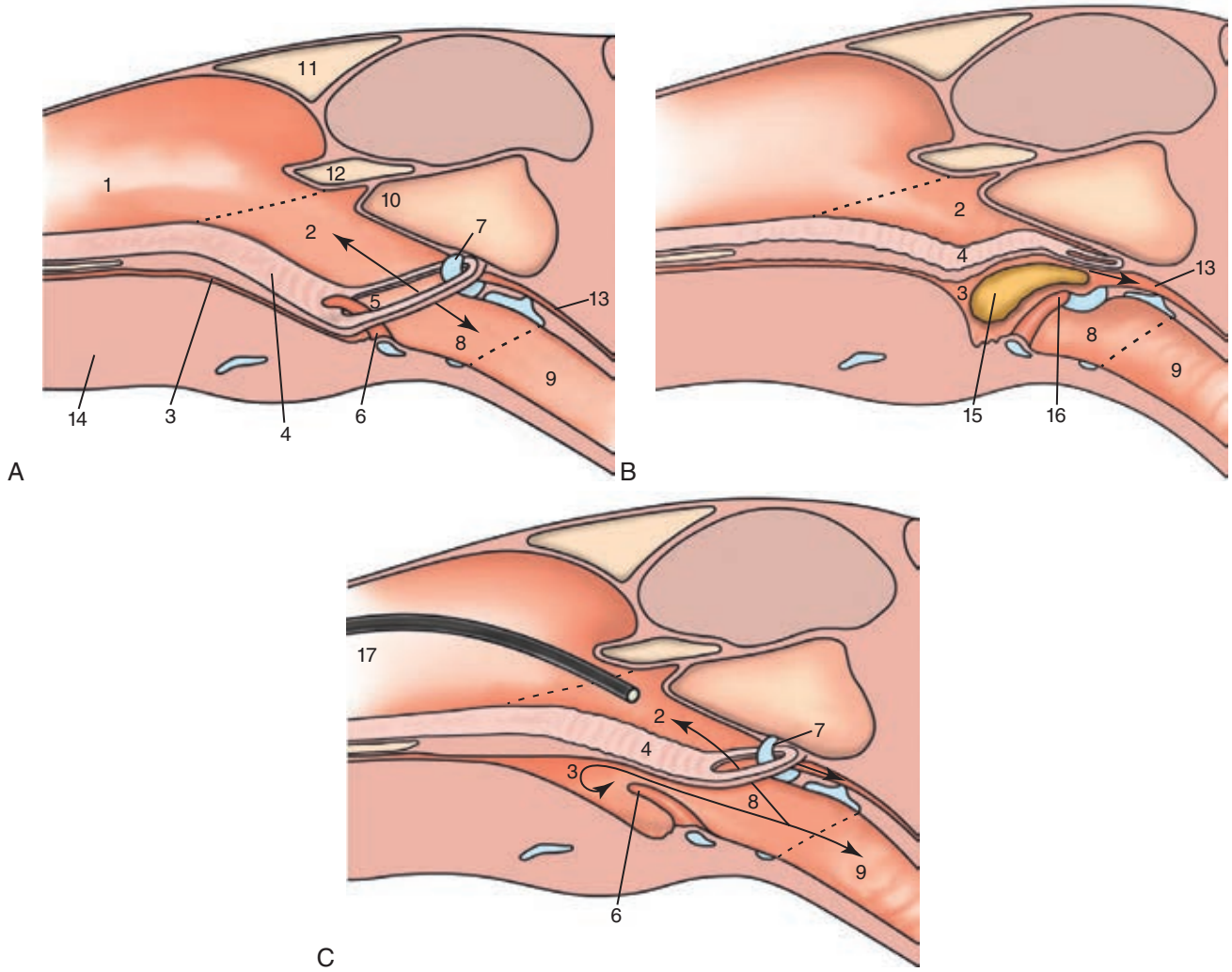

FIGURE 9-1 Lateral view of the pharyngolaryngeal area during (*A*) normal breathing, (*B*) swallowing, and (*C*) dorsal displacement of the soft palate. (l: nasal cavity; 2: nasopharynx; 3: oropharynx; 4: soft palate; 5: intrapharyngeal ostium ("button hole"); 6: epiglottis; 7: corniculate cartilage; 8: larynx; 9: trachea; 10: guttural pouch; 11: frontal sinus; 12: sphenopalatine sinus; 13: esophagus; 14: tongue; 15: food bolus; 16: closed larynx; 17: endoscope.) (*Modified with permission from Cook WR:* Specifications for speed in the racehorse: in the airflow factors, *Menasha, WI, 1989, Russell Meerdink.)*

arrangement explains why the horse is an obligatory nasal breather. Indeed, because of this anatomic characteristic, the horse, unlike humans and dogs, is not able to switch from nasal breathing to oronasal breathing when the nasal resistance to airflow becomes too high, as occurs during exercise-induced hyperpnea.

In horses, the displacement of the caudal border of the soft palate to a position above the epiglottis, called *dorsal displacement of the soft palate,* is not physiologic, except when it occurs during swallowing, coughing, or whinnying (see Figure 9-1). In all other conditions, this dorsal displacement is abnormal and will induce dyspnea, especially during strenuous exercise. It induces a narrowing of the upper airways and causes a soft palate flapping, sometimes resulting in a dramatic asphyxia in racing horses. In some parts of the world, this dysfunction is called "choking down."

Larynx

The structural and functional peculiarities of the equine larynx make it a potential bottleneck in the upper airways, that is, the narrowest portion of the upper airway. The rostral protrusion of the laryngeal cartilages through the intrapharyngeal ostium constitutes the aditus laryngis. This pharyngeal opening is formed dorsally by the corniculate cartilages, laterally by the vocal folds, and ventrally by the epiglottis (Figure 9-2). The aditus laryngis can vary from tight seal, that is, full adduction of the laryngeal structures during swallowing to protect the lower airways from ingesta, to a maximal opening, that is, full abduction of these structures during exercise-induced hyperpnea to decrease the resistance to air flow (see Figure 9-2). This full opening is completed by a dilation of the larynx caused by contraction of the intrinsic muscles, which eliminates the opening of the laryngeal ventricles.

Any impairment of this laryngeal dilatation due to structural factors (such as rostral displacement of the palatopharyngeal arch) or functional factors (such as laryngeal hemiparesis) will be responsible for inadequate ventilation during heavy exercise (Bayly et al., 1984; Franklin et al., 2012) and will generally induce abnormal respiratory noises related to increased air flow resistance (see Figure 9-2).

Trachea

The horse's trachea is a 70- to 80-cm-long flexible tube consisting of 48 to 60 cartilaginous rings that are open dorsally (Figure 9-3). The free ends of these plates overlap in the cervical part, but not in the thoracic part, of the trachea

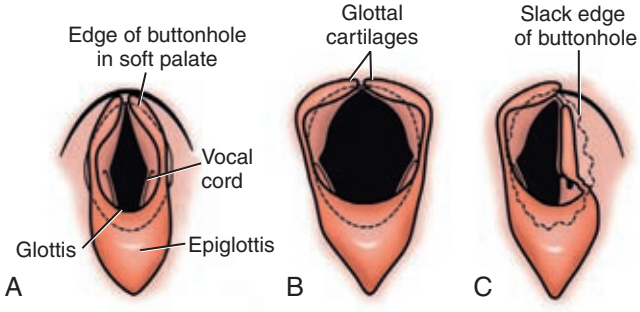

FIGURE 9-2 Front view of the larynx in a healthy resting (*A*) and exercising (*B*) horse. *(Modified, with permission, from Cook WR:* Specifications for speed in the racehorse: in the airflow factors, *Menasha, WI, 1989, Russell Meerdink.)*

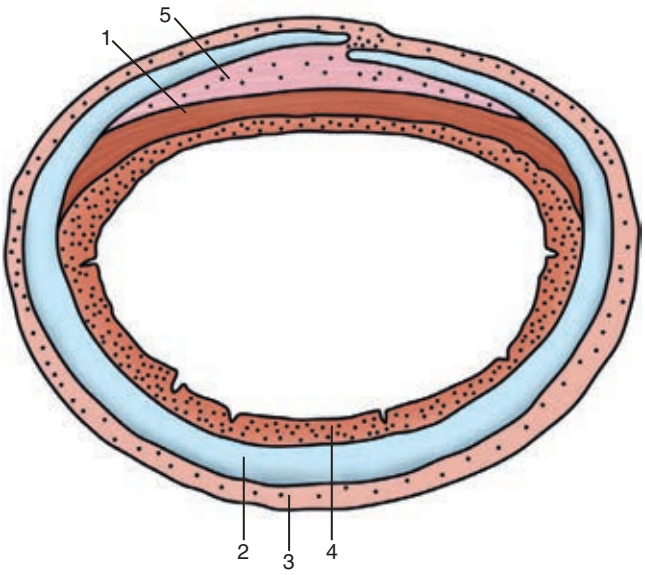

FIGURE 9-3 Cross-sectional view of the cervical trachea (l: tracheal muscle; 2: cartilage; 3: adventice; 4: mucosa; 5: connective tissue). *(Modified with permission from Collin B:* Atlas d'anatomie, *Liege, Belgium, 1976, Universite de Uege.)*

(Hare, 1975). Its cross-section is almost circular proximally and distally (transverse diameter 5.5 cm; sagittal diameter 5 cm) but is more flattened dorsoventrally (transverse diameter 7 cm, sagittal diameter 5 cm) between the two extremes. Routine endoscopic evaluations of the lower airways of horses examined for exercise intolerance show evidence that there is sometimes a sharp lateral narrowing of the intrathoracic trachea just cranial to the tracheal bifurcation.

Tracheal smooth muscles lie in the dorsal tracheal membrane. They are innervated by the autonomic nervous system.

Despite its cartilaginous structure, the extrathoracic trachea is quite compliant and is susceptible to collapse during the highly compressing transmural pressure that occurs during forced inspiration (Art and Lekeux, 1991a; Franklin et al., 2012). Its compliance (and, therefore, its collapsibility) is significantly decreased, however, by smooth muscle contraction (due to exercise-induced adrenal discharge) and by tracheal extension (due to exercise-induced head and neck stretching). Moreover, the resistance to collapse is also dependent on the shape of the tracheal cross-section. In this regard, all horses do not seem to be equal. Indeed, the transverse to sagittal diameter ratio varies from 0.9 (i.e., circular shape) to 2 (i.e., an elliptical shape) with a mean of 1.4 (Art and Lekeux, 1991b) (Figure 9-4). The more ellipsoid the trachea, the more compressible it is. Therefore, horses with a transverse to sagittal diameter ratio higher than 1.5 are disadvantaged and more susceptible to exercise intolerance caused by insufficient ventilation. The size and age of the horse do not influence the compliance of the trachea (Art and Lekeux, 1991b; Franklin et al., 2012).

Bronchi

After the bifurcation of the trachea into the right and left principal bronchi, the bronchial tree branches many times to the periphery of the lung via the primary bronchi, the segmental bronchi, the bronchioles, and the terminal bronchioles (Figure 9-5).

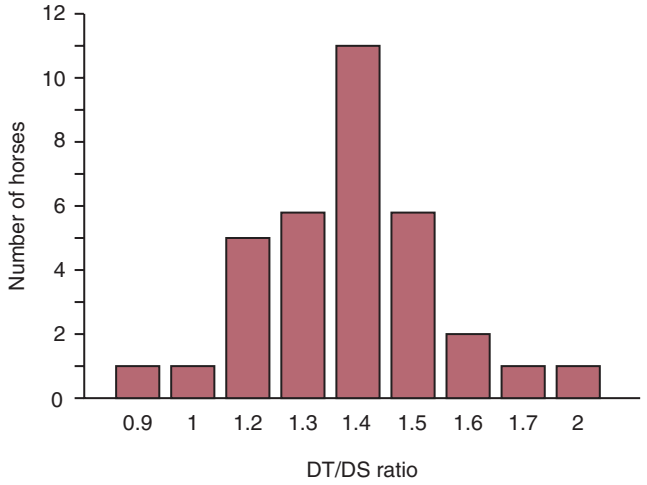

FIGURE 9-4 Distribution of 33 tracheas, according to their transverse sagittal diameter ratio.

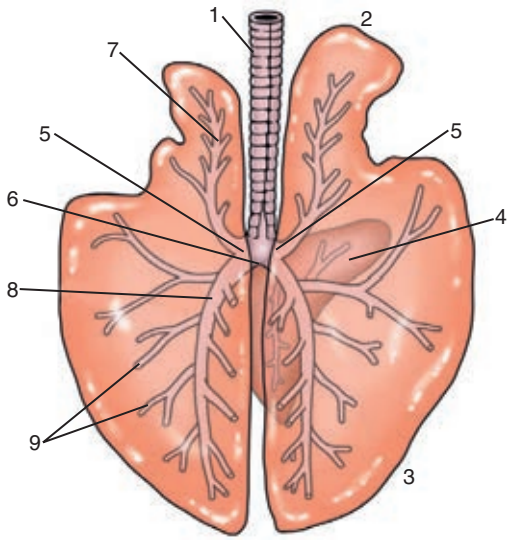

FIGURE 9-5 Dorsal view of the bronchi and lungs (l: trachea; 2: right apical lobe; 3: right diaphragmatic lobe; 4: azygos lobe; 5: principal bronchi; 6: carina; 7: apical lobe bronchus; 8: diaphragmatic lobe bronchus; 9: segmental bronchi). *(Modified with permission from Collin B:* Atlas d'anatomie, *Liege, Belgium, 1976, Universite de Liege.)*

These intrathoracic conducting airways are also susceptible to collapse when the transmural pressure exerted on their walls is compressive. This is particularly true at the level of the small airways, which do not have cartilaginous support. However, in contrast with extrathoracic airways, where partial dynamic collapse occurs only during inspiration, the collapse of small airways occurs only during forced expiration, that is, when the extraluminal pressure is more positive than the intraluminal pressure (Figure 9-6).

LUNGS

With airway generation, the individual diameter and length of the airways decrease, but the total cross-sectional area increases. Therefore, the small airways are not a bottleneck (the larynx is the major impediment) in healthy horses and represent only a small part of the total resistance to air flow.

Respiratory bronchioles are poorly developed in the horse, and most of the gas exchange occurs at the alveolarcapillary unit, which is well designed in this species. The horse's lung is highly developed and represents about 1% of the body weight. It contains at least 10^7 alveoli and probably 1000 times more capillary segments. The alveolar surface density is large and alveolar septa thin compared with other mammals (Gehr and Erni, 1980).

Although the equine lung is not really divided by fissures into lobes, an apical lobe, a diaphragmatic lobe, and an accessory lobe in the right lung and an apical lobe and a diaphragmatic lobe in the left lung are usually described (see Figure 9-5) The lungs are covered by a thick pleura.

The connective tissue septa between lobules are not complete. This allows some collateral ventilation, that is, transfer of air between adjacent lobules via accessory pathways such as the interalveolar pores of Kohn, the canals of Lambert, and communicating respiratory bronchioles and alveolar ducts. The advantage of this collateral ventilation is to partially compensate for reduced ventilation in areas with small airway obstructive diseases (Robinson, 1982). However, in horses, these accessory pathways present a high resistance to air flow and are able to provide only a maximum of 16% of the required volume, a very small proportion compared with the 90% recorded in the human lung (Franklin et al., 2012). Therefore, these pathways are probably of limited value for equine pulmonary function, except possibly for the prevention of atelectasis in horses suffering from airway obstruction.

BLOOD SUPPLY

The lung receives blood from two circulations (Table 9-1). The *pulmonary circulation* receives the total cardiac output from the right side of the heart. The branches of the pulmonary artery carry venous blood to the lung, accompany the bronchi, and form rich capillary plexuses on the walls of the alveoli. Here the blood is arterialized (oxygenation and release of carbon dioxide are two main functions) and returned to the left side of the heart by the pulmonary veins.

The equine pulmonary arteries adjacent to the bronchioles and the alveolar ducts are muscular and have a rather thick medial smooth muscle layer (thinner than the cow and pig but thicker than the dog and sheep). This amount of smooth muscle determines the reactivity of the vessels to hypoxia and thus explains why a horse may present with pulmonary hypertension caused by a hypoxic vasoconstriction (Bisgard et al., 1975).

The *bronchial circulation,* a branch of the systemic circulation, carries arterial blood for the nutrition of the airways and other lung structures. It originates from two arteries: (1) the bronchoesophageal artery, supplying the airways and interlobular septa of most of the lung, and (2) the right apical bronchial artery, supplying the airways of the right apical lobe. Bronchial arteries form a circulatory plexus in the connective tissues along the airways. Branches from this plexus penetrate the bronchial walls to form a subepithelial vascular plexus, the role of which is probably to ensure some heat dissipation. The bronchial circulation is drained by either the azygos or the pulmonary veins.

At the level of the terminal bronchioles, pulmonary and bronchial circulations anastomose. Most of these anastomoses occur at the level of the capillaries and veins rather than the arteries (Magno and Fishman, 1982; McLaughlin, 1983).

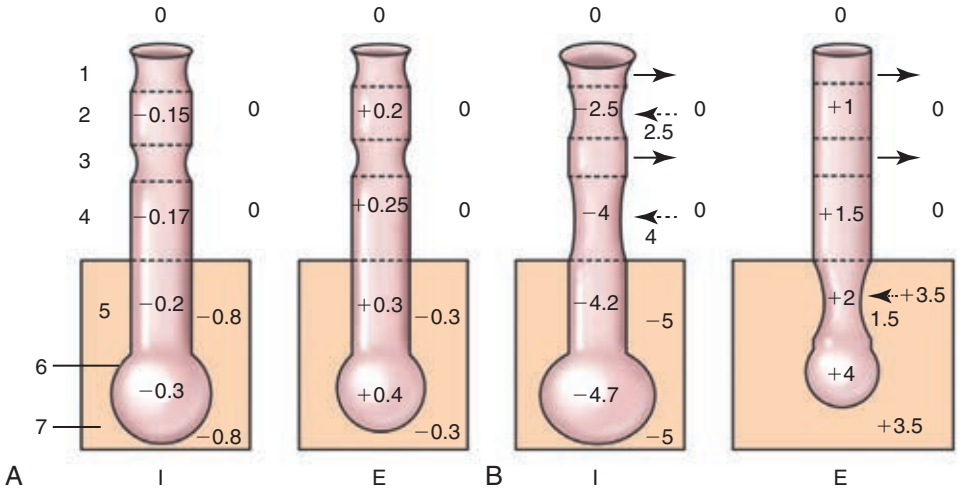

FIGURE 9-6 Schematic illustration of the active dilating (due to tensor muscles contraction) (*solid arrows*) and passive compressing (due to transmural pressure) (*dashed arrows*) mechanisms on the airways during resting (A) and exercising (B), inspiration (I) and expiration (E). Pressures are given in kPa. (0 = atmospheric pressure). (1: nasal cavity; 2: pharynx; 3: larynx; 4: extrathoracic trachea; 5: intrathoracic airways; 6: alveoli; 7: pleural cavity).

TABLE 9–1

Comparison Between Pulmonary and Bronchial Circulations

	Pulmonary	Bronchial
Aims	• Gas exchange • Venous blood filtration • Blood reservoir	• Nutrition of airways, vessels, and visceral pleura • Thermoregulation
Structure	Right ventricle ↓ Pulmonary artery ↓ Pulmonary arterioles ↓ Pulmonary capillaries ↓ Pulmonary veins	Left ventricle ↓ Bronchial and bronchoesophogeal arteries ↓ Peribronchial plexus ↓ Subepithelial plexus ↓ Pleural, vascular, and ganglia plexi ↓ Azygos veins
Blood flow (liters/min)	±99 percent of the right ventricle: 30 (280)	±2 percent of left ventricle: 0.6 (6)
Pressure (mmHg)	Arterial: 30 (100) Capillary: 20 (80) Venous: 10 (60)	Arterial: 100 (200) Capillary: 20 (>60) Venous: 15 (60)
Capillary flow	Pulsatile	Constant
Vascular resistance (mmHg/liter/min)	0.7 (0.25)	140 (20)
Effect of hypoxia	Vasoconstriction	Vasodilation
Effect of hyperthermia	-	Vasodilation
Effect of pleural pressure changes	+++	+

Note: Data given in this table are only indicative. Exercise values are in parentheses. a: anastomoses

The *lymph vessels* are numerous and arranged in two sets: (1) a superficial one, forming a network in and under the pleura, and (2) a deep one, accompanying the bronchi and pulmonary vessels.

THORACIC CAVITY

The thoracic cavity is roughly triangular, with its caudal base formed by the diaphragm. The roof is formed by the thoracic vertebrae and the ligaments and hypaxial muscles connected with them; the lateral walls are formed by the 18 ribs and the intercostal muscles; and the floor is formed by the sternum, the cartilages of the sternal ribs, and their associated muscles. A longitudinal septum, termed the *mediastinum,* extends from the roof to the floor, and the diaphragm divides the cavity into two lateral chambers, each containing a lung. The pleura provide a continuous cover over the surface of the lung (visceral pleura) and extend to provide a lining for the internal surface of the thoracic walls (parietal pleura) and to form the mediastinal cavity. Practically, all the organs in the thorax are in the mediastinal cavity, with the exception of the lungs, the caudal vena cava, and the right phrenic nerve.

Compared with the dorsoventrally flattened thorax of the human, the horse has a rather rounded thorax. Moreover, cranially, its thorax is laterally compressed to facilitate locomotor function. The differences in size, shape, and position of the abdomen in relation to the lungs, as well as the high rigidity of the equine thorax (Leith, Gillespie, 1971), are probably advantageous for locomotion and for stabilization of the relaxation volume of the respiratory system during postural changes.

Respiratory Muscles and Their Innervation

Inspiratory Muscles

The diaphragm is the main inspiratory muscle. It is a domed musculotendinous sheet separating the abdomen and the thorax. It consists of a costal portion, arising from the xyphoid process and the costochondral junctions of the eighth rib to the fourteenth rib, and a crural portion, arising from the ventral surface of the first several lumbar vertebrae and extending toward the tendinous center of the diaphragm. The apex of the dome extends to the eighth intercostal space at the level of the base of the heart. The external intercostal muscles, which join the ribs, are also active during inspiration. However, during exercise, the serratus ventralis participates in inspiration much more than do the external intercostal muscles (Manohar, 1991).

Other inspiratory muscles include those connecting the sternum or the ribs and the head (sternomandibularis, scalenus). When they contract, they pull the sternum or the ribs forward and participate consequently in enlargement of the thorax (De Troyer and Loring, 1986).

Expiratory Muscles

The abdominal muscles (external and oblique abdominis, transverse and rectus abdominis, transverse thoracis) and the internal intercostal muscles are expiratory muscles. When they contract, they increase the abdominal pressure, forcing the relaxed diaphragm forward and reducing the thoracic volume (De Troyer and Loring, 1986).

Other Respiratory Muscles

Some muscles such as the abductor muscles, which dilate the nares, the pharynx, and the larynx, are able to modify the size of the airways.

Innervation

The *phrenic nerves,* which innervate the diaphragm, come from the cervical spinal cord and traverse down both sides of the heart. In horses suffering from electrolyte imbalances (e.g., after prolonged exercise or diarrhea), the phrenic nerve may become hyperexcitable, and sometimes a diaphragmatic flutter ("thumps") may be observed (i.e., diaphragmatic contraction in phase with cardiac depolarization) (Refer to Chapter 8). This is usually transient but often responds to intravenous administration of polyionic replacement fluids augmented with calcium borogluconate.

The laryngeal muscles are innervated by the *recurrent laryngeal nerves*. The recurrent laryngeal nerve is one of the longest nerves of the body. The left laryngeal nerve originates from the brain and travels down the neck into the chest as a component of the vagus nerve. At the level of the heart, the recurrent laryngeal nerve branches off the vagus and becomes an individual nerve, which travels back up the neck before finally reaching the larynx. The high incidence of left laryngeal hemiplegia in the horse has been related to neural injuries because of the specific course of the left laryngeal nerve. However, this theory may be challenged by the occasional occurrence of right hemiplegia (Franklin et al., 2012).

FUNCTIONAL PECULIARITIES OF THE EQUINE RESPIRATORY SYSTEM AT REST AND ADAPTATIONS DURING EXERCISE

The main respiratory processes involved in gas exchange are ventilation (i.e., how air gets to the alveoli), perfusion (i.e., how gas is removed from the lungs by the blood), ventilation to perfusion ratio (i.e., how matching of air and blood in the lung influences the gas exchange), diffusion (i.e., how gas gets across the air–blood barrier), gas transport (i.e., how gases are moved from lungs to the tissues), mechanics of breathing (i.e., how the lungs are moved), and control of breathing (i.e., how the supply of gas exchange is adjusted to the demand).

VENTILATION

Lung Volumes

Expired Minute Volume

Equine lung volumes are illustrated in Figure 9-7. The volume of air inhaled or exhaled during a normal breath is termed the *tidal volume* (Curtis et al., 2006; Gallivan et al., 1989a; Ramseyer et al., 2010). Its value in the healthy resting athletic horse is about 12 milliliters per kilogram (mL/kg of body weight. Multiplying the tidal volume by the respiratory frequency gives the *expired minute ventilation.*

Exercise imposes a potent stress on the ventilatory pump: As speed increases, minute ventilation increases almost linearly, and the expired minute ventilation, which averages 80 liters per minute (L/min) at rest (Table 9-2), may reach values in the vicinity of 1800 L/min during heavy exercise (Art and Lekeux, 1993; Art and Lekeux, 1995; Katz et al., 2005; Knight et al., 1991). Indeed, this may be an underestimation. The change in minute ventilation necessary to meet the gas exchange requirements can be reached by changing tidal volume, respiratory frequency, or both.

In horses, when trotting, the increase in minute ventilation is achieved by a simultaneous increase in tidal volume and respiratory frequency at low exercise intensities and mainly by an increase in respiratory frequency at high exercise intensities (Art and Lekeux, 1988a; Curtis et al., 2006; Evans and

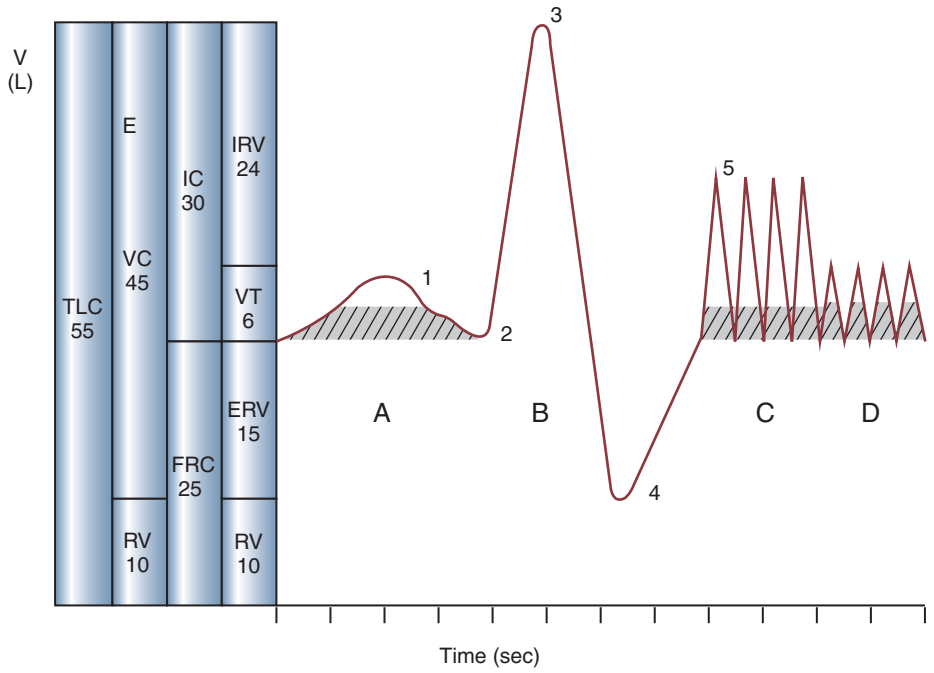

FIGURE 9-7 Mean lung volumes (L) in healthy adult horses under several conditions (*TLC*: total lung capacity; *VC*: vital capacity; *RV*: residual volume, i.e., the volume of gas which remains in the lung after a forced expiration; *IC*: inspiratory capacity; *FRC*: functional residual capacity, i.e., the volume of air which remains in the lung after a normal expiration; *IRV*: inspiratory reserve volume; *VT*: tidal volume; *ERV*: expiratory reserve volume). **A,** Rest breathing. **B,** Forced breathing. **C,** Exercise breathing. **D,** Recovery breathing. (1: resting inspiratory level; 2: resting expiratory level; 3: maximal inspiratory level; 4: maximal expiratory level; 5: exercising inspiratory level.) The hatched zones represent the dead-space portion of tidal volume. This figure supposes that exercise does not change FRC, which remains to be investigated in the horse.

TABLE 9–2

Mean Respiratory Values in Healthy Thoroughbred Horses Considered Average for Their Quality, Fitness, Age (5 Years), and Size (Body Weight 470 kg) and Running on a Treadmill under Temperate Climatic Conditions (Lung and Gas Volumes are Expressed as BTPS and STPD Respectively).

Value	Unit	Rest	Walk	Slow trot	Canter	Fast gallop	Recovery -5
A. VENTILATION*							
$\dot{V}o2max$	%	3.3	14	18	60	100	20
V_T	liters	5.6	5.8	6.2	9.2	13.2	6.5
V_D	liters	3.4	3.4	3.5	2.6	2.6	3.5
V_A	liters	2.2	2.4	2.8	6.6	10.6	3.0
V_D/V_T	%	60	58	57	28	20	54
f	breaths/min	14	65	91	113	121	110
t_i	seconds	1.9	0.45	0.34	0.27	0.25	0.29
t_e	seconds	2.4	0.47	0.32	0.27	0.25	0.26
t_i/t_{tot}	%	44	49	52	50	50	53
$\dot{V}_E$	liters/min	78	377	564	1040	1598	715
$\dot{V}_D$	liters/min	47	219	321	291	320	386
$\dot{V}_A$	liters/min	31	158	243	749	1278	329
$\dot{V}_E/\dot{V}_{O2}$	liters/liter	35	40	47	26	24	55
$\dot{V}_E/\dot{V}_{CO2}$	liters/liter	43	48	51	27	23	51
mean $\dot{V}_i$	liters/s	2.9	13	19	34	53	26

Continued

TABLE 9–2

Mean Respiratory Values in Healthy Thoroughbred Horses Considered Average for Their Quality, Fitness, Age (5 Years), and Size (Body Weight 470 kg) and Running on a Treadmill under Temperate Climatic Conditions (Lung and Gas Volumes are Expressed as BTPS and STPD Respectively).—cont'd

Value	Unit	Rest	Walk	Slow trot	Canter	Fast gallop	Recovery -5
mean V_e	liters/s	2.3	12	20	34	53	30
$\dot{V}_i$ max	liters/s	4.1	14	27	45	64	32
$\dot{V}_e$ max	liters/s	4.2	18	30	52	79	39
$\ddot{V}_i$	liters/s^2	8	240	632	1124	1685	650
$\ddot{V}_e$	liters/s^2	7	225	566	1086	1595	744
B. MECHANICS OF BREATHING[†]							
$P_{pl\ min}$	kPa	−0.78	−1.19	−1.78	−3.19	−4.85	−1.68
$P_{pl\ max}$	kPa	−0.34	0.31	0.55	2.42	3.62	0.53
maxΔP_{pl}	kPa	0.44	1.5	2.33	5.61	8.47	2.21
P_{in}	kPa	0.02	0.54	1.16	2.61	4.26	1.71
$\Delta P_{\dot{V}}=0$	kPa	0.24	−0.29	−0.90	−2.42	-3.88	−1.90
C_{dyn}	Liter/kPa	23	−20	−6.8	−3.8	-3.4	−3.4
R_L	Pa/liter/s	25	26	30	48	57	27
R_{UA}	Pa/liter/s	20	21	23	38	46	21
R_{LA}	Pa/liter/s	5	5	7	10	11	6
W_{vis}	J	1.4	6.2	14	36	82	14
$\dot{W}_{vis}$	J/min	17	403	1274	4068	9922	1540
$\dot{W}_{vis}/\dot{V}_E$	J/liter	0.22	1.07	2.2	3.9	6.2	2.2
$\dot{W}_{vis}/\dot{V}_{O2}$	J/liter	7.7	42	106	102	148	118
C. GAS EXCHANGE [∞]							
$\dot{V}_{O2}$	ml/kg/min	4.7	20.2	25.5	85.1	142.5	27.7
$\dot{V}_{CO2}$	ml/kg/min	3.8	17.0	23.4	80.9	146.8	29.8
R	-	0.82	0.85	0.92	0.95	1.03	1.07
Venous P_{O2}	mmHg	39	32	30	25	16	62
Venous P_{CO2}	mmHg	47	49	50	64	96	43
HCO_3^-	mmol/liter	28.8	28.9	27.5	26.6	23.0	19.2
Arterial P_{O2}	mmHg	95	101	99	83	69	115
Arterial P_{CO2}	mmHg	45	44	43	46	50	32
$(A\text{-}a)\Delta_{O2}$	mmHg	4	2	4	16	29	6
pH_a	-	7.39	7.40	7.40	7.39	7.26	7.36
PCV	%	38	42	44	48	58	56
Hb	g/liter	140	150	160	175	220	220
Arterial S_{O2}	%	97	97.5	97	95	90	98.5
Arterial C_{O2}	liter%	20	22	22	25	28	33
Venous C_{O2}	liter%	14	11	11	7	5	21
$C(a\text{-}v)_{O2}$	liter%	6	11	11	18	23	12
Alveolar P_{O2}	mmHg	99	103	103	99	98	121
Alveolar P_{CO2}	mmHg	44	43	42	45	49	31
Fme_{O2}	%	16.2	17.0	17.7	14.9	14.3	18.4
Fme_{CO2}	%	2.6	2.6	2.5	4.6	5.4	2.1
Fet_{O2}	%	14.0	14.5	14.5	13.9	13.9	17.2
Fet_{CO2}	%	6.3	6.1	6.0	6.4	7.0	4.5

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Value	Unit	Rest	Walk	Slow trot	Canter	Fast gallop	Recovery -5
D. PULMONARY HEMODYNAMICS[§]							
HR	beats/min	35	75	103	155	210	83
SV	liters	1.05	1.07	1.12	1.39	1.36	1.32
$\dot{Q}$	liters/min	37	80	115	215	285	110
PaPmax	mmHg	37	69	78	99	152	53
PaPmin	mmHg	22	30	39	47	50	26
PaPm	mmHg	28	43	52	65	82	35
Pw	mmHg	16	25	30	37	49	20
P (aP-w)	mmHg	12	18	22	28	33	15
PVR	mmHg/liter/min	0.32	0.22	0.19	0.13	0.11	0.14
$t^{O}a$	°C	37.5	37.9	38.1	38.7	41.3	39.7
$\dot{V}_{O2}$/HR	ml/kg/beat	0.06	0.25	0.25	0.55	0.68	0.34
$\dot{V}_A/\dot{Q}$	liters/liter	0.84	1.98	2.11	3.48	4.48	3.00

*$\dot{V}_{o2}$max %: percent of maximal oxygen uptake; V_T: tidal volume; V_D: physiologic dead space; V_A: alveolar volume; V_D/V_T: ratio of dead space tidal volume; f: breathing frequency; t_i: inspiratory time of the breathing cycle; t_e: expiratory time; t_i/t_{tot}: ratio of inspiratory to total time for the breathing cycle; $\dot{V}_E$: minute volume; $\dot{V}_D$: dead space ventilation; $\dot{V}_A$: alveolar ventilation; $\dot{V}_E/\dot{V}_{O2}$: ventilator equivalent for oxygen uptake; $\dot{V}_E/\dot{V}_{CO2}$: ventilator equivalent for carbon dioxide output; mean V_i: mean inspiratory flow or inspiratory drive; mean $\dot{V}_e$: mean expiratory flow; $\dot{V}_{i\,max}$: peak inspiratory flow; $\dot{V}_{e\,max}$: peak expiratory flow; $\ddot{V}_i$: volume acceleration at the onset of inspiration; $\ddot{V}_e$: volume acceleration at the onset of expiration.

†P_{p1min}: peak intrapleural pressure recorded during inspiration; $P_{pl\,max}$: peak intrapleural pressure recorded during expiration; maxΔP_{pl}: maximum change in intrapleural pressure; P_{in}: inertial pressure; $\Delta P_{\dot{V}}=0$: intrapleural pressure gradient between the two points of zero flow; C_{dyn}: dynamic lung compliance; R_L: total pulmonary resistance; R_{UA}: upper airway resistance; R_{LA}: lower airway resistance; W_{vis}: viscous work of breathing; $\dot{W}_{vis}$: minute work of breathing; $\dot{W}_{vis}/\dot{V}_E$: work of breathing per ventilated liter; $\dot{W}_{vis}/\dot{V}_{O2}$: work of breathing per liter of oxygen uptake.

‰ $\dot{V}_{O2}$: oxygen uptake; $\dot{V}_{CO2}$: carbon dioxide output; R: respiratory exchange ratio; venous P_{O2}: venous oxygen partial pressure; venous P_{CO2}: venous carbon dioxide partial pressure; arterial P_{O2}: arterial oxygen partial pressure; arterial P_{CO2}: arterial carbon dioxide partial pressure; HCO_3^-: bicarbonate content: (A-a)Δ_{O2}: alveolar-arterial oxygen gradient; pH_a: arterial pH; PCV: packed cell volume; Hb: blood hemoglobin concentration; arterial S_{O2}: percent saturation of hemoglobin with oxygen; arterial C_{O2}: arterial oxygen content; venous C_{O2}: venous oxygen content; C(a-v)$_{O2}$: arteriovenous oxygen content gradient; alveolar P_{O2}: alveolar oxygen partial pressure; alveolar P_{CO2}: alveolar carbon dioxide partial pressure; Fme_{O2}: mixed expired oxygen fraction; Fme_{CO2}: mixed expired carbon dioxide fraction; Fet_{O2}: end-tidal oxygen fraction; Fet_{CO2}: end-tidal carbon dioxide fraction.

[§]HR: heart rate; SV: stroke volume; $\dot{Q}$: cardiac output; PaPmax: maximal pulmonary artery pressure; PaPmin: minimal pulmonary artery pressure; PaPm: mean pulmonary artery pressure; Pw: pulmonary artery wedge pressure; P (aP-w): pulmonary driving pressure; PVR: pulmonary vascular resistance; t^0a: arterial blood temperature; $\dot{V}_{O2}$/HR: oxygen pulse; $\dot{V}_A/\dot{Q}$: global ventilation/perfusion ratio.

Marlin, 1999). Values as high as 133 breaths/min have been reported in Standardbred horses running on a treadmill (Dahl et al., 1987; Franklin et al., 2012).

In galloping horses, the respiration and the locomotion are compulsorily coupled (Bramble and Carrier, 1983; Franklin et al., 2012). Step and respiratory frequencies average 110 to 130 per minute with maximum values of 148 per minute reported (Franklin et al., 2012; Hörnicke et al., 1983; Hörnicke et al., 1987). Therefore, when the horse gallops, the increase in minute ventilation with increasing speed is due mainly to the increase in tidal volume rather than in respiratory frequency. Tidal volumes between 12 L and 15 L are reported in fast galloping horses (Art et al., 1990a; Bayly et al., 1987; Franklin et al., 2012; Hörnicke et al., 1987; Landgren et al., 1991).

Alveolar and Dead Space Ventilation

Only a portion of the inspired volume reaches the area of the lung where gas exchange takes place; this is the *alveolar ventilation.* The remaining part of the minute ventilation is wasted in the regions of lung where no gas exchange occurs; this is the *physiologic dead space ventilation,* which includes the conducting airways (anatomic dead space) and the alveoli that are ventilated but not perfused (alveolar dead space). The dead space to tidal volume ratio averages 50% to 60% in the resting horse (Curtis et al., 2006; Franklin et al., 2012; Gallivan et al., 1989b; Lekeux et al., 1992), a percentage twice as large as reported in other athletic species such as humans and dogs.

For any given minute ventilation, the lower the physiologic dead space ventilation, the higher is the alveolar ventilation and the better is the gas exchange. However, the evidence that adequate gas exchange is maintained with very low tidal volume and very high respiratory frequency (600 breaths/min, i.e., high-frequency or jet ventilation) suggests that such factors as mass convection, convective dispersion, and molecular diffusion may provide an adequate gas transport between the atmospheric air and the alveoli despite a very high dead space to tidal volume ratio (Chang, 1984; Froese and Bryan, 1981).

Exercise-induced changes in alveolar ventilation and dead space to tidal volume ratio in horses depend on the type of exercise performed (Bayly et al., 1987; Curtis et al., 2006; Franklin et al., 2012; Pelletier et al., 1987; Pollman and Hörnicke, 1984; Rose and Evans, 1987). During mild-to-moderate exercise, the dead space volume does not change significantly (Curtis et al., 2006; Franklin et al., 2012; Pelletier et al., 1987). Therefore, the increase in tidal volume will increase the alveolar ventilation and decrease the dead space to tidal volume ratio.

If the exercise is prolonged at a constant rate, the dead space ventilation will increase by a simultaneous increase in

respiratory frequency and in the dead space to tidal volume ratio (Curtis et al., 2006; Franklin et al., 2012; Pelletier et al., 1987; Rose and Evans, 1987). This adaptation probably reflects the thermoregulatory role of the respiratory system (see Chapter 8). Lastly, during intense effort, there is a decrease in this ratio from about 60% to 20% (Curtis et al., 2006; Franklin et al., 2012; Lekeux et al., 1992; Rose and Evans, 1987). In absolute terms, the physiologic dead space is reduced from 3.5 L at rest to 2.5 L during heavy exercise. Because the anatomic dead space averages 2.5 L at rest and is expected to remain unchanged during exercise, the exercise-induced difference in the dead space (l L) is probably attributable to the disappearance of the alveolar dead space (i.e., alveoli that are ventilated but not perfused) induced by the recruitment of previously nonfunctional pulmonary capillaries (Curtis et al., 2006; Franklin et al., 2012; Lekeux et al., 1992).

Distribution of Ventilation

The distribution of ventilation is not uniform in the lung, even in healthy horses. This occurs for two different reasons. The main reason is that the intrapleural pressure changes are not uniform all over the thoracic cage. Because of gravitational effects, pressure is more negative in the dorsal than in the ventral part of the lung (Derksen and Robinson, 1980; Franklin et al., 2012). Consequently, the dorsal alveoli are more distended, less compliant, and receive less air during inspiration at any ventilatory rate.

A second reason may be the occurrence of some inequalities in the regional small airways resistance, alveoli compliance, or both; inhaled air preferentially enters the areas of the lungs with low resistive airways and highly compliant alveoli. Figure 9-8 displays how the alveoli that follow highly resistive airways (B), or which have a lower compliance, will fill up much more slowly than the others (A) (Otis et al., 1956). This ventilatory asynchrony is moderate in healthy horses and does not have significant effects on gas exchange at low respiratory frequencies. However, in horses with significant asynchrony (i.e., subclinical small airways disease) and with a high respiratory frequency (i.e., during exercise), this phenomenon will significantly impair gas exchange and may result in poor performance (Curtis et al., 2006; Derksen et al., 1992; Franklin et al., 2012) (see Figure 9-8).

Factors That Tend to Reduce Ventilatory Asynchrony

The interdependence between adjacent lung regions tends to limit the nonuniform changes in regional ventilation (Franklin et al., 2012; Robinson and Sorenson, 1978). These mechanical interactions are the result of the intricate mesh of interconnecting elastic and collagenous tissue fibers in the lung. Collateral ventilation between adjacent lung areas is also potentially able to reduce the nonuniformity of the ventilation distribution. However, in the horse, these collateral pathways, because of their high resistance to air flow, are of limited usefulness at low respiratory frequencies and probably of no functional importance at all at high respiratory frequencies (Robinson and Sorenson, 1978).

Factors That Tend to Increase Ventilatory Asynchrony

All factors that either interfere with the properties of the lungs, such as decrease in the compliance of the alveoli (interstitial edema), increase in the resistance of small airways (collapse, bronchospasm, hypersecretion, blood), or increase in

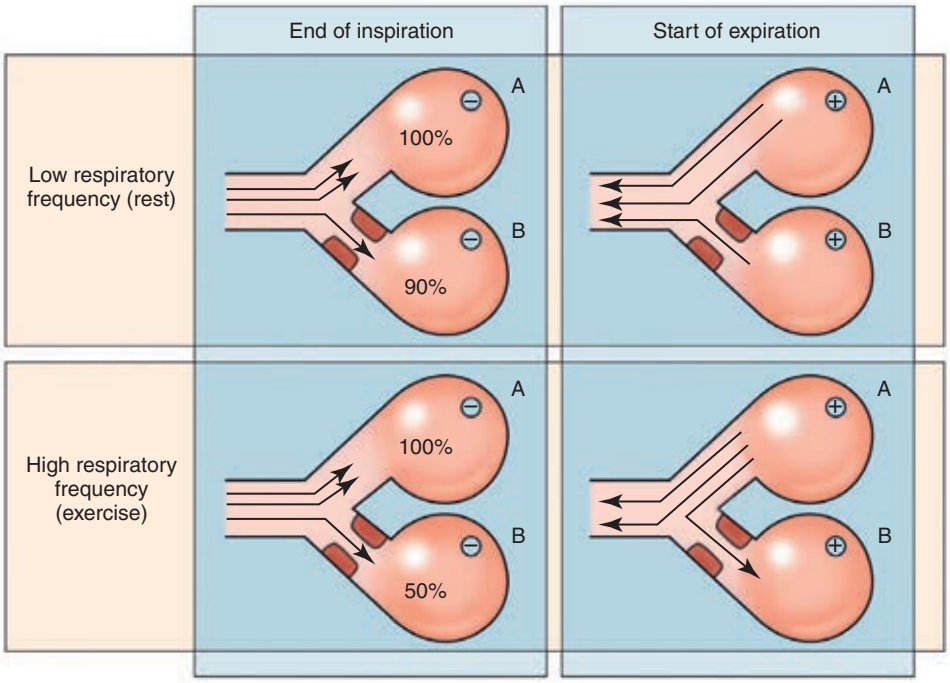


FIGURE 9-8 Schematic illustration of the negative effect of ventilatory asynchrony on alveolar ventilation. The higher the respiratory frequency, the lower is the ventilation in obstructed alveoli (*B*), which receive less fresh air because of the high resistance of their conducting airways. At the start of expiration and because of the pressure gradient due to the shift in respiratory phase, some expired air from the normal alveoli (*A*) may momentarily become (poor quality) inspired air for the obstructed alveoli (*B*).

respiratory frequency (during exercise), are likely to increase the ventilatory asynchrony and impair the gas exchange.

Exercise, by increasing the respiratory frequency, probably magnifies the regional differences in ventilation (see Figure 9-8). The lobules, which have a long time constant for filling, do not fill adequately before expiration begins, and consequently, ventilation–perfusion mismatching and hypoxemia result. Moreover, because of the interdependence between adjacent lung areas, the lobules with increased airflow resistance, decreased compliance, or both are stretched and compressed by the surrounding lung parts (Mead et al., 1970; Franklin et al., 2012). This may induce abnormal stresses on the tissues of these lobules. It has been suggested that exercise-induced pulmonary hemorrhage could sometimes be a consequence of this pulmonary overstretching (Robinson, 1979).

PULMONARY PERFUSION

The lungs are perfused by the pulmonary artery, which supplies the gas-exchange regions. The distribution of blood flow throughout the lung depends on the pressure difference between the pulmonary artery and the pulmonary vein and the vascular resistance. This, as in other species, is influenced by the gravitational forces. Blood flow to the conducting airways, interlobular septa, and pleura is supplied by the bronchial artery, and the filtrated fluid is drained by the lymphatic circulation.

The differences between pulmonary and bronchial circulations are outlined in Table 9-1. Pulmonary vascular resistance is very low; it is estimated at only one seventh the resistance of the systemic circulation. Consequently, the mean pressure throughout these vessels is much less than in the systemic circulation (~25 versus ~125 mm Hg). Although the vascular resistance in the systemic circulation is totally due to the precapillary vessels, the pulmonary vascular resistance is equally distributed between the pre- and postcapillary vessels. Because of the peculiar distribution and the low value of the pulmonary vascular resistance, pressure in the pulmonary capillaries remains pulsatile.

During strenuous exercise, pulmonary blood flow increases five- to eightfold (Franklin et al., 2012; Parks and Manohar, 1983; Thomas and Fregin, 1981; Thomas et al., 1983; Thornton et al., 1983). A marked simultaneous pulmonary hypertension is a feature of exertion in horses and ponies; the mean pulmonary arterial pressure rises about threefold, from 28 mm Hg at rest to about 84 mm Hg at a fast gallop, with maximal reported values of 100 mm Hg (Erickson et al., 1990; Erickson et al., 1992; Evans and Rose, 1988a, b, c; Franklin et al., 2012; Goetz and Manohar, 1986; Wagner et al., 1989). The pulmonary right-to-left shunt of the cardiac output is approximately 1% at rest. It may decrease up to 0.4% during heavy exercise (Franklin et al., 2012; Wagner et al, 1989).

Factors Influencing Pulmonary Vascular Resistance

Factors capable of modifying the pulmonary vascular resistance are either extravascular or intravascular. Changes in lung volume represent the main *extravascular factor.* The pulmonary vascular resistance increases at extreme lung volumes because of the compression of the lung vessels during forced expiration (small lung volume) or during forced inspiration (lung distended) (Fishman, 1985). The increase in blood viscosity related to the exercise-induced rise in packed cell volume is another extravascular factor influencing pulmonary vascular resistance; each increase of 1% in the packed cell volume induces an increase of 4% in the pulmonary vascular resistance (Taylor et al., 1989).

Vasoactive compounds or changes in the local composition of blood are *intravascular factors* that regulate the pulmonary vascular resistance by modifying vascular smooth muscle tone. Pulmonary vascular resistance decreases with an increase in blood flow, pulmonary arterial pressure, or both (Franklin et al., 2012; Taylor et al., 1989). This results from a combination of dilation of the perfused vessels (increase in their cross-sectional area and consequently decrease in their flow resistance) and recruitment of previously unperfused vessels. Therefore, despite the substantial increase in packed cell volume, there is an approximately threefold decrease (from 0.32 to 0.11 mm Hg/L/min) in pulmonary vascular resistance with strenuous exercise (see Table 9-2).

Factors Influencing Pulmonary Perfusion Distribution

Gravitational Factors

It has been shown that there is a vertical gradient of pulmonary blood flow, with the ventral regions receiving more perfusion per unit lung volume than the dorsal regions (Amis et al., 1984; Franklin et al., 2012). According to the relative magnitudes of pulmonary arterial, venous, and alveolar pressures, blood flow in the lung can be divided into four zones (Fishman, 1985).

In *zone 1*, at the top of the lung, there is no blood flow, because the mean pulmonary arterial pressure is too low to overcome the hydrostatic pressure imposed by the column of blood connecting the pulmonary artery to the apical blood vessels. Therefore, alveolar pressure exceeds both pulmonary arterial and venous pressures, and the collapsible capillaries remain closed. However, because the mean pulmonary arterial pressure is about 15 to 18 mm Hg (i.e., 20 to 25 cmH_2O), it may be sufficient to perfuse the vertical height of the lung above the heart. This zone is probably small in most horses. Because this lung region is unperfused, it does not participate in gas exchange and represents the "alveolar dead space." During exercise, the increased pulmonary arterial pressure probably improves the recruitment of the vessels in zone 1 and therefore makes the distribution of perfusion more homogeneous. The alveolar dead space is estimated to be 0.8 L to 1 L in the resting horse or about 3.5% of the functional residual capacity and is very likely to disappear in the exercising horse (Franklin et al., 2012; Lekeux et al., 1992).

In *zone 2*, pulmonary arterial pressure is greater than the alveolar pressure, the latter being, in turn, greater than venous pressure. Therefore, the capillary is open for a part of its length, until the point where alveolar pressure exceeds intravascular pressure. Consequently, blood flow in zone 2 is determined by the respective values of alveolar and arterial pulmonary pressures (and is independent of venous pressure); it, therefore, increases down this zone of lung, according to the progressive increase of pulmonary arterial pressure as a result of the hydrostatic gradient.

In *zone 3*, both pulmonary arterial and venous pressures exceed alveolar pressure; capillaries are perfused throughout their length and are increasingly distended down this zone.

A *zone 4* is sometimes described in which the pulmonary blood flow decreases as a result of a compressing interstitial pressure on the vessel. Although it is well established that pulmonary blood flow is distributed in a vertical direction with respect to gravity, Hakim et al. (1987) suggested that in humans there also could be a gradient from the center to the periphery. Because local or peripheral vascular resistance rises in proportion to distance from the lung hilum, the center of each lobe will be better perfused than its periphery.

Humoral and Neural Factors

Although predominantly passive mechanical forces determine regional blood flow distribution, the smooth muscles in arteries and veins respond to vasoactive compounds. The magnitude and mechanism of these responses depend on numerous factors such as the pre-existing level of pulmonary vascular tone and the integrity of the pulmonary vascular endothelium (Bray and Anderson, 1991).

A modest autonomic innervation, with both adrenergic and cholinergic components, is found in the muscular vessels of the pulmonary circulation. Stimulation of the sympathetic nervous system constricts the blood vessels of the lung, whereas parasympathetic stimulation causes vasodilation.

Hypoxic Vasoconstriction

The modification of ventilation in some regions of the lung or in the whole lung also influences pulmonary perfusion. In unventilated regions of the lung, alveolar hypoxia occurs, inducing a local hypoxic vasoconstriction. This constriction provides a mechanism to redistribute pulmonary blood flow from less-ventilated regions to well-ventilated regions and therefore improves the ventilation to perfusion ratio and the gas exchange. The magnitude of the response to hypoxia depends on the thickness of the pulmonary arterial smooth muscle layer; the response of the horse is intermediate between such species as cattle and pigs, in which the response is quite vigorous, and sheep and dogs, in which the response is minimal (Bisgard et al., 1975; Robinson, 1982). The mechanism for the occurrence of this constriction is still unclear. A combination of the action of a vasoactive agent with cellular mechanisms has been suggested as a possible cause. Hypoxic vasoconstriction is generally advantageous when occurring locally but may become unfavorable when occurring through the whole lung, as occurs in acute hypoxia. Exercise-induced pulmonary hypertension occurring in the horse does not appear to be caused by hypoxic pulmonary vasoconstriction (Franklin et al., 2012; Pelletier and Leith, 1991).

Bronchial Circulation

The bronchial circulation receives approximately 1% to 2% of the cardiac output from the left side of the heart (i.e., oxygenated blood). It supplies the airways, large pulmonary blood vessels, septa, pleura, and other lung structures. However, the lung does not suffer from a partial obstruction of the bronchial circulation. The numerous anastomoses between the pulmonary and bronchial circulations may provide blood flow to the bronchial circulation (Franklin et al., 2012; Taylor et al., 1989). Conversely, when the pulmonary perfusion is locally impaired, the bronchial circulation may proliferate and maintain some blood flow throughout the lung, thus contributing partly to gas exchange (Lilker and Nagy, 1975). Such vascular proliferations also may occur in response to pulmonary inflammation. Because these may be extensive in the dorsocaudal regions of the lungs, which are the regions that sometimes bleed during intense exercise, they have been related to the occurrence of exercise-induced pulmonary hemorrhage. In addition, the role played by pulmonary hypertension, bronchial hypertension, or both in exercise-induced pulmonary hemorrhage is strongly suspected but has not been demonstrated so far (Franklin et al., 2012).

Postpulmonary shunts may result from anastomoses between pulmonary and bronchial circulations and, theoretically, may be partly responsible for the fall in the arterial oxygen partial pressure (pO_2) observed during exercise. However, a shunt of 1%, which is a reasonable approximation, would reduce arterial pO_2 by 5 mm Hg, whereas the actual decrease during exercise is much larger (Franklin et al., 2012; Wagner et al., 1989).

Lymphatic Circulation

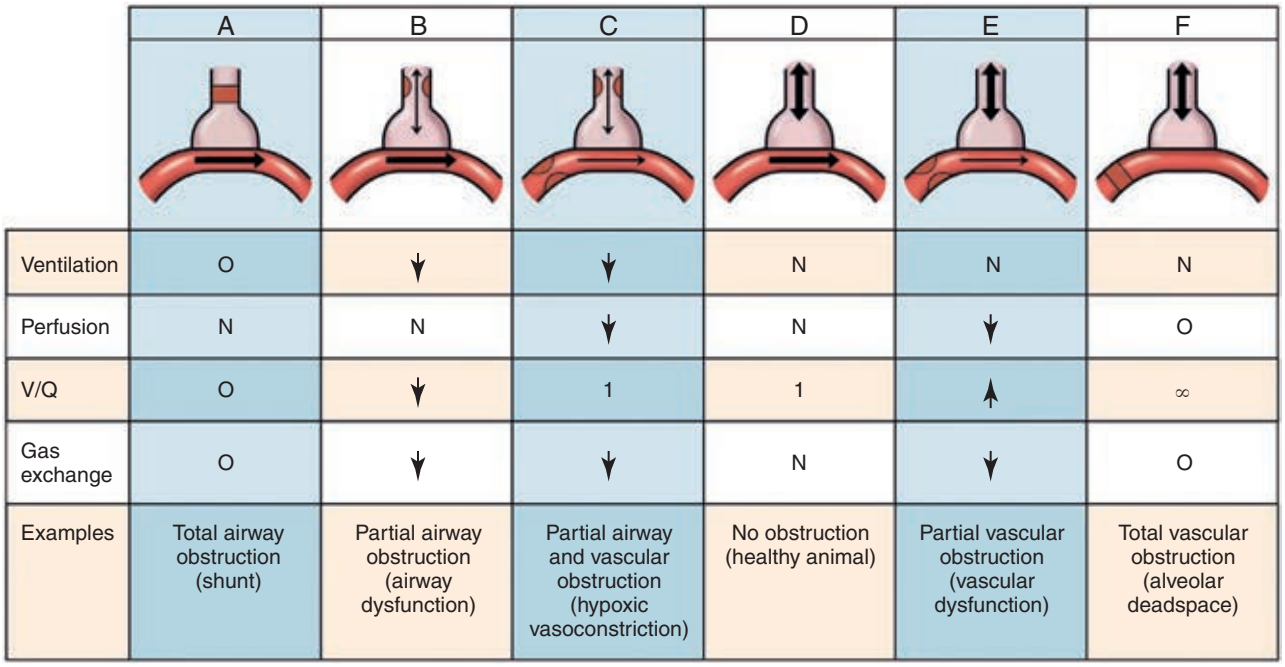
Fluid filtration occurs between the capillaries and the interstitial tissue. The alveolar endothelium is less permeable than the capillary endothelium. Therefore, the fluid does not leak into the alveoli unless the epithelium is damaged or unless there is a considerable accumulation of fluid in the interstitial tissue. Fluid filtered from the capillaries moves through the interstitium toward perivascular and peribronchial tissues, where lymphatic vessels are located. During exercise, the marked increase in the pulmonary arterial pressure probably induces an increase in the rate of fluid filtration across the capillary walls of the lung. The fact that this condition is not associated with pulmonary edema suggests that in horses, as in other species (Coates et al., 1984), the lymphatic system has a substantial capacity to drain the pulmonary interstitial space (Taylor et al., 1989). Moreover, the "pumping" action associated with the large and frequent pressure changes in the equine thoracic cage during exercise could contribute to the adequacy of the lymphatic drainage.

VENTILATION TO PERFUSION RATIO

Gas exchange ultimately depends on optimization of the ventilation to perfusion ratio; efficient gas exchange occurs in the lung regions where the ratio is 0.8:1.0.

Most lung regions have a ventilation to perfusion ratio of 0.8, but regions exist that are excessively perfused (ventilation/perfusion ratio <0.8) or ventilated (ventilation to perfusion ratio >0.8). When ventilation is impaired (i.e., partial airway obstruction), the ventilation to perfusion ratio decreases, and in extreme cases of total airway obstruction, the ventilation to perfusion ratio is 0. This corresponds to a right-to-left shunt. If perfusion is inadequate (because of pulmonary vasoconstriction or hypotension) the ventilation to perfusion ratio will increase, and in extreme cases of underperfusion caused emboli or in zone 1, the ventilation to perfusion ratio becomes infinite (Figure 9-9).

In quiet, resting horses, the ventilation to perfusion ratio is not influenced by the gravity; that is, it is uniform from the top to the bottom of the lung, suggesting that the gradient in lung ventilation is matched by the gradient in lung perfusion (Amis et al., 1984). In horses exercising heavily, there is only a very slight mismatch of ventilation and perfusion. This mismatch accounts for 25% of the increase in the alveolar–arterial pressure difference in O_2 (Franklin et al., 2012; Wagner et al., 1989). The physiologic characteristics of the ventilation to perfusion ratio of horses contrast with those in humans. In



	A	B	C	D	E	F
Ventilation	O	↓	↓	N	N	N
Perfusion	N	N	↓	N	↓	O
V/Q	O	↓	1	1	↑	∞
Gas exchange	O	↓	↓	N	↓	O
Examples	Total airway obstruction (shunt)	Partial airway obstruction (airway dysfunction)	Partial airway and vascular obstruction (hypoxic vasoconstriction)	No obstruction (healthy animal)	Partial vascular obstruction (vascular dysfunction)	Total vascular obstruction (alveolar deadspace)

FIGURE 9-9 Illustration of several cases of ventilation-perfusion mismatching at the bronchoalveolar level (0: zero; N: normal).

the latter, the ventilation to perfusion ratio is nonuniform at rest, and a true mismatching occurs during exercise (Franklin et al., 2012; Gale et al., 1985).

In subjects suffering from airway disease, exercise will enhance ventilation–perfusion mismatching, mainly the result of impairment of ventilation. This is particularly true in horses, in which the collateral ventilation has little ability to compensate for the nonpermeability of small airways (Robinson and Sorenson, 1978). Therefore, the lobules that take a longer time to fill will have an inadequate ventilation to perfusion ratio with resultant hypoxemia and, sometimes, hypercapnia.

PULMONARY DIFFUSION

Composition of the Respiratory Gases

The ambient pO_2, that is, the relative contribution of the O_2 pressure to the total pressure of the ambient gas mixture (air), is about 20.93% of the total pressure exerted by air (air has a pressure of 760 mm Hg at sea level). The partial pressure of CO_2 in ambient air is negligible (0.03%) (Figure 9-10).

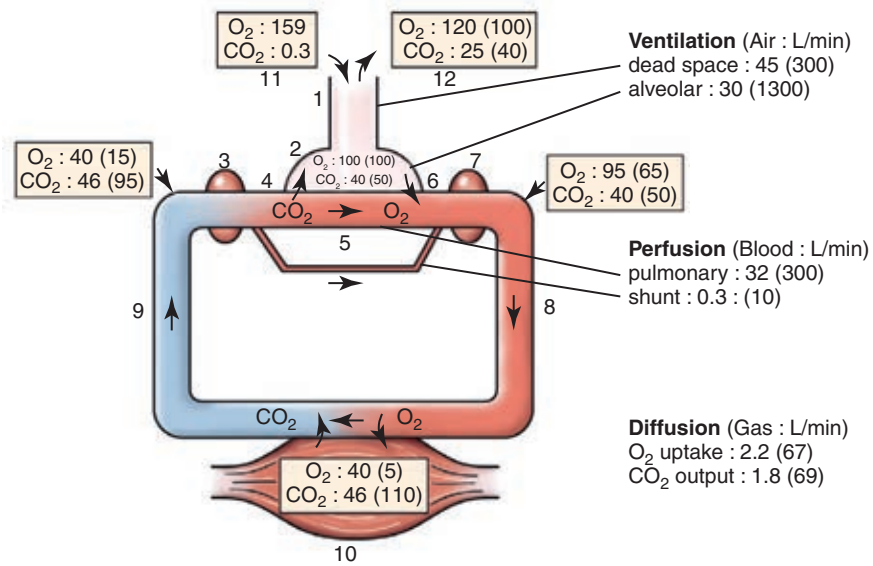


FIGURE 9-10 Partial pressures of gases (mm Hg) in relation to ventilation, perfusion, and diffusion processes liters per minute (L/min) in resting and exercising (data between parentheses) conditions (1: airways; 2: alveoli; 3: right heart; 4: pulmonary artery; 5: pulmonary capillaries; 6: pulmonary veins; 7: left heart; 8: arteries; 9: veins; 10: locomotor muscles; 11: inspired air; 12: expired air).

After passing through the nasal cavities and the upper airways, air is saturated with water (H_2O). At near normal body temperature (37°C), the partial pressure of the water vapor represents about 6.1% of the total pressure, or 47 mm Hg. Therefore, the other gases exert a total pressure of only 713 mm Hg (or 760 mm Hg minus 47 mm Hg). Therefore, the actual O_2 pressure of air in the trachea decreases from 159 mm Hg (room air) to 149 mm Hg.

The composition of alveolar air is quite different from inspired air because CO_2 is continuously expelled across the alveolar membrane into the alveoli with O_2 taken up from the alveolar lumen. Composition of alveolar air is changing constantly owing to cyclic variations depending on the respiratory phase (alveolar pO_2 increases during inspiration and decreases during expiration). Consequently, the following values for alveolar air composition are mean values: 13.6% O_2, 5.3% CO_2, 74.9% N_2, and 6.2% H_2O. However, after expiration, the remaining air in the lungs will be mixed with the air of the next inspiration, damping the variation in alveolar gas composition (see Figure 9-10).

Alveolar Diffusion

Diffusion is the passive process whereby O_2 passes from alveoli to capillary blood and CO_2 passes in the reverse direction. As indicated by the following formula, the rate of diffusion *v* is influenced by the pressure gradient of the gases between alveoli and capillary (*PA - P*cap), by the physical properties of the gases (*D* = diffusion coefficient), by the surface area available *A*, and by the thickness *X* of the alveolar–capillary barrier:

$$v = \frac{D \times A(P_A - P_{cap})}{X}$$

The alveolar–arterial pressure gradient is not constant; on the one hand, it decreases progressively as the blood passes through the alveolar capillaries, and on the other, it varies according to the stage of the respiratory cycle. The maximal pressure gradients that can be encountered are about 60 and 6 mm Hg of O_2 and CO_2, respectively. The coefficient of diffusion of O_2 in the horse averages 0.45 L/min/mm Hg (Hare, 1975); CO_2 is 25 times more diffusible than O_2.

The horse, along with other athletic animals, appears to have an appropriately enlarged alveolar–capillary surface area (Weibel, 1979). Moreover, during exercise, this area is enlarged by the recruitment of unperfused vessels induced by the increase in pulmonary arterial pressure. At rest and moderate exercise, no measurable diffusion limitation is observed in the healthy horse with the alveolar–arterial O_2 tension difference averaging 4 mm Hg. In contrast, during heavy, but not necessarily maximal, exercise (from 60% of $\dot{V}_{O2max}$), arterial hypoxemia and hemoglobin desaturation occur in horses (Bayly et al., 1983; Franklin et al., 2012; Thornton et al., 1983). Simultaneously, the alveolar–arterial O_2 tension difference widens and may reach values as high as 30 mm Hg (Franklin et al., 2012; Lekeux et al., 1992).

Hypoxemia is primarily related to one of the following factors: (1) decrease in the pO_2 in the inspired air, (2) right-to-left vascular shunts, (3) ventilation–perfusion mismatching, (4) diffusion impairment, or (5) alveolar hypoventilation. Right-to-left shunts and mismatching of ventilation and perfusion have been shown to be accessory factors in producing hypoxemia during exercise (Franklin et al., 2012; Wagner et al., 1989). A mild ventilation to perfusion ratio inequality accounts for 25% of the widening of the alveolar–arterial O_2 tension difference. Alveolar–capillary diffusion disequilibrium is responsible for the remaining 75% of this widening.

During heavy exercise, different and opposite factors may influence the diffusion capacity. Capillary transit time is greatly shortened, as is the time for O_2 equilibration which will impair diffusion (Figure 9-11). However, other changes actually improve diffusion. First, a mixed venous O_2 pressure as low as 16 mm Hg has been reported during a fast gallop (Bayly et al., 1989). Because the alveolar O_2 pressure remains steady (Figures 9-11 and 9-12), the pressure gradient between the capillary blood and the alveolar air is increased, improving O_2 extraction and diffusion. Second, the increased blood flow results in functional new alveoli previously unperfused at rest and, therefore, increases the exchanging alveolar surface. It also distends the pulmonary capillaries and, thus, increases the capillary blood volume. In humans, the capillary blood volume is able to expand about three times its resting value with only a four- to fivefold increase in pulmonary blood flow (Dempsey, 1986). In horses, the blood flow increases eightfold, and it can be presumed that the capillary blood volume expands considerably more than that in humans. Lastly, the increase in the packed cell volume promotes O_2 diffusion by increasing the number of binding sites available. The fact that the horse shows evidence of diffusion limitation during heavy exercise (Wagner et al., 1989) suggests that the physiologic adjustments improving the pulmonary diffusion are overcome by the short capillary blood transit time, which averages 0.4 to 0.5 seconds in this species. However, other athletic species have a short transit time during exercise (0.29 seconds in the dog, 0.35 seconds in the pony, and 0.5 seconds in humans), but neither the dog nor the pony demonstrates diffusion limitation during exercise (Parks and Manohar, 1984). Therefore, it may be hypothesized that these species are able to compensate for their short capillary transit time by other physiologic adaptations, which may be absent in the horse.

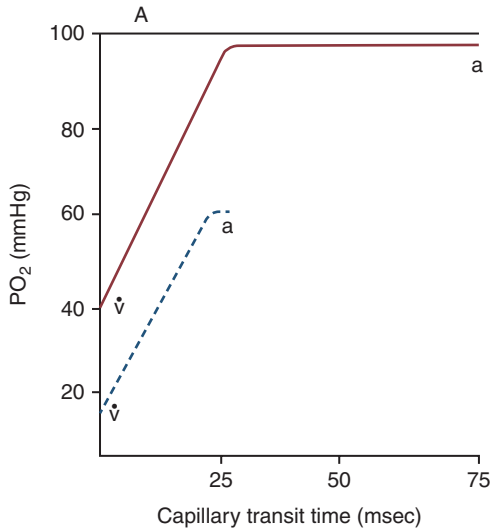


FIGURE 9-11 Schematic illustration of oxygen (O_2) diffusion across the alveolar–capillary membrane in resting (*solid line*) and exercising (*dashed line*) conditions. The capillary transit time in milliseconds (ms) is decreased about threefold during strenuous exercise (*A*: alveolar air; *v*: mixed venous blood; *a*: arterialized blood).

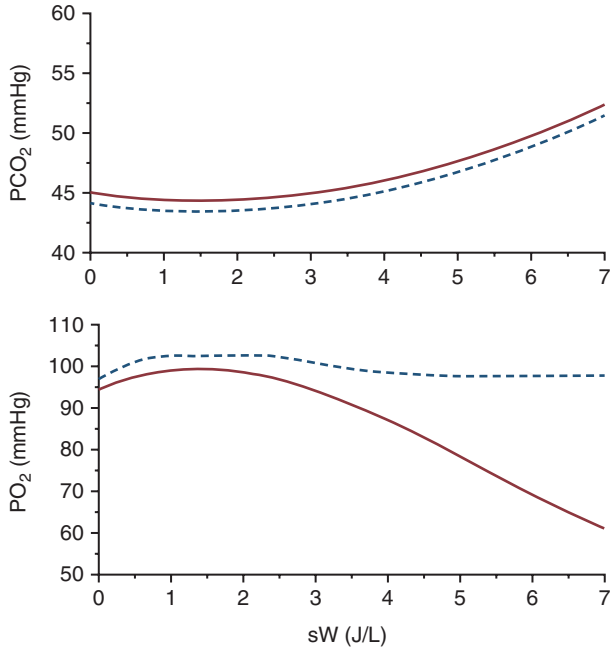


FIGURE 9-12 Relation between the specific work of breathing (W_{rm}/V_E) and the partial pressure of arterial (*solid line*) and alveolar (*dashed line*) oxygen partial pressure (pO_2) and carbon dioxide partial pressure (pCO_2). Note the increasing alveolar–arterial gradient for O_2 but not for CO_2.

Tissue Diffusion

Arterial blood enters the tissue capillary with an arterial pO_2 of 85 to 100 mm Hg and an arterial pCO_2 of 40 to 44 mm Hg. The tissue O_2 and CO_2 tensions are determined by the level of metabolic activity but generally average 40 and 46 to 48 mm Hg, respectively. Therefore, O_2 and CO_2 will diffuse down their respective pressure gradients (see Figure 9-10). Once released into the tissues, O_2 will be bound to myoglobin, an iron-containing pigment, the main function of which is the transfer of O_2 within the muscle cells.

Because the arterial pO_2 in blood returning from all tissues averages 16 mm Hg during heavy exercise, it may be assumed that O_2 tension at the level of the exercising muscle is much lower. The dramatic decrease in O_2 tension in the tissues increases the driving pressure for oxygen diffusion at this level. Overall, the release of O_2 from hemoglobin at the level of the working muscles is promoted by the right shift of the oxyhemoglobin dissociation curve resulting from acidosis, hypercapnia (venous pCO_2 = 96 mm Hg), and hyperthermia ($t°a > 41.5°C$). Tissues with high aerobic metabolic needs are more vascularized than are others, and accordingly, the surface area for gas exchange is greater.

Gas Exchange

Oxygen Uptake

Under steady state conditions, oxidative metabolism supplies the body with essentially all the metabolic energy needed to maintain the supply of high-energy phosphate compounds required for homeostasis. Oxygen molecules enter the body and pass through each of the serial transport steps of the respiratory system (ventilatory convection, pulmonary diffusion, circulatory convection, and peripheral tissue diffusion) at a rate that is exactly matched to the rate at which they are consumed by oxidative phosphorylation in the mitochondria. The measurement of the rate of the whole-body aerobic metabolism at steady state, or O_2 uptake, is, therefore, a direct measure of the rate at which the pulmonary gas exchanger is functioning to meet the body's demand. The O_2 transport system functions as a cascade delivery system in which the difference in concentration of O_2 at the top of the cascade (ambient air) and the bottom (mitochondrial inner membrane) drives the flux of O_2 through the system and the reverse for CO_2.

When the demand for O_2 is set in the peripheral tissues, for example, in the muscle during exercise, gas exchange rates at each of the respiratory system's transport steps must adjust proportionally to maintain the supply of O_2 to the muscles. With increasing exercise intensity, there is a nearly linear increase in the rate of O_2 uptake to a certain point, beyond which O_2 uptake remains constant at higher speed (Art and Lekeux, 1993; Evans and Rose, 1988a, b, c; Franklin et al., 2012; Rose and Evans, 1987) (Figure 9-13). At intensities above that at which the rate of O_2 uptake plateaus, termed $\dot{V}_{O2max}$, the turnover rate of high-energy phosphate compounds continues to increase, but the source of metabolic energy that enables this increased turnover to occur is anaerobic glycolysis, with subsequent increases in the concentration of lactate in muscle and blood. Such exercise is termed *supramaximal*. It is generally assumed that $\dot{V}_{O2max}$ represents the athlete's capacity for the aerobic resynthesis of high-energy phosphate compounds and provides, therefore, a quantitative statement of an individual's capacity for aerobic energy transfer. Compared with mammals of similar size, horses achieve a higher $\dot{V}_{O2max}$ per kilogram by building and maintaining more of the following structures in the O_2 transport chain: heart size, hemoglobin, and peripheral capillary bed. Additionally, they have a larger skeletal muscle mass that contains a higher density of mitochondria than do domestic animals of the same size (Parks and Manohar, 1984). In horses $\dot{V}_{O2max}$ is generally not reached until heart rate exceeds 200 beats/min (Franklin et al., 2012; Rose and Evans, 1987). The measurement of $\dot{V}_{O2max}$ in horses has the disadvantage of requiring relatively sophisticated equipment, but it is undoubtedly the more accurate index for the assessment of fitness and training adequacy in the athletic horse (Art and Lekeux, 1993; Curtis et al., 2006; Evans and Rose, 1988a; Franklin et al., 2012).

Carbon Dioxide Output

The principal end products of aerobic catabolism are CO_2 and H_2O. At rest, these products are voided approximately at the rate they are produced. Because of their chemical composition, each energy substrate requires different amounts of O_2 in relation to CO_2 produced during oxidation. A respiratory value that is potentially diagnostic of energy substrate is obtained by simultaneously measuring both CO_2 output and O_2 uptake and taking their ratio:

$$\text{Moles of } CO_2 \text{ produced per unit time/moles of } O_2 \text{ consumed per unit time}$$

When measured at the lungs, this ratio is called the *respiratory exchange ratio R*. An *R* near 1.0 indicates that the cells are catabolizing mostly carbohydrates, whereas an *R* near 0.7 indicates predominantly lipid catabolism. Under steady-rate exercise conditions (up to 80% of $\dot{V}_{O2max}$ in trained subjects) the exchange of O_2 and CO_2 measured at the lungs reflects the actual gas exchange from nutrient metabolism in the peripheral tissue.

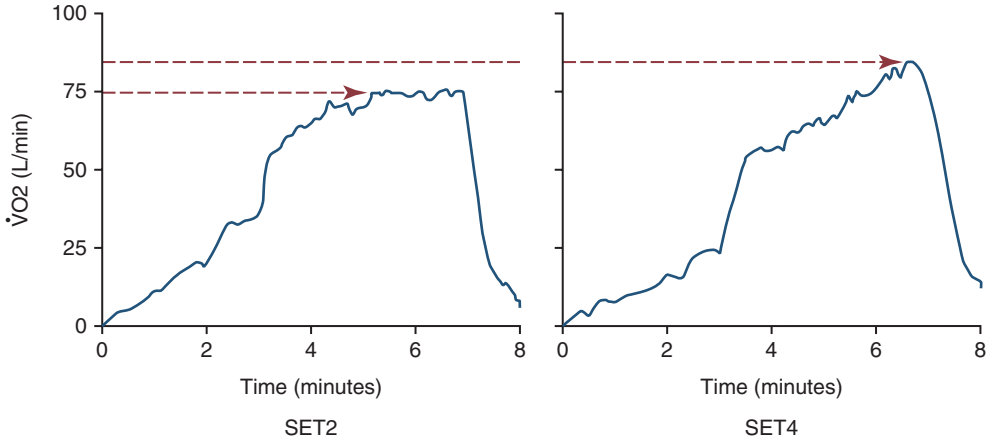


FIGURE 9-13 Oxygen uptake ($\dot{V}O_2$) curves obtained from the same horse (weight 512 kg, age 5 years) on a breath-by-breath basis (each point is the mean of 10 respiratory cycles) during a standaradized treadmill exercise before (SET 2) and after (SET 4) a 6-week period of training. The test consisted of exercise of increasing speed (1.7, 4, 8, 9, 10, 11 meters per second [m/s], 1 minute for each speed) on a treadmill inclined at 6 degrees. The horse galloped at the beginning of the third minute of the test. $\dot{V}O_{2max}$ was reached at 9 m/s during SET 2 and was not reached during SET 4.

Strenuous exercise, above 80% of $\dot{V}_{O2max}$ in a trained subject, presents a situation in which *R* can rise significantly above 1.00. The lactic acid (LA) generated during exhaustive exercise is buffered by sodium bicarbonate and other buffers in the blood to maintain the acid–base balance in the reaction:

$$HLA + NaHCO_3 \rightarrow NaLA + H_2CO_3 \rightarrow H_2O + CO_2$$

During this process, carbonic acid, a weaker acid, is formed. In the pulmonary capillaries, carbonic acid breaks down to its components, CO_2 and H_2O, and CO_2 exits through the lungs. This buffering process adds extra CO_2 to that quantity normally released during energy metabolism, and the *R*, therefore, rises above a value of 1.00.

BLOOD GAS TRANSPORT

Oxygen

The pO_2 represents the relative pressure exerted by the gas in a mixture of gases (in air or in liquids); the saturation of O_2 represents the percentage of the total hemoglobin binding sites occupied by O_2; the O_2 content represents the total amount of O_2 in the blood, which is primarily O_2 bound to hemoglobin with lesser amounts dissolved in the plasma.

Oxygen Dissolved

Once O_2 passes through the alveolar–capillary barrier, it either dissolves in the plasma or combines with hemoglobin, the latter being its main form of transport. At rest, the complete equilibration between alveolar and capillary O_2 tensions occurs before the blood leaves the capillary. O_2has low solubility in plasma; only 0.3 mL of O_2 will be dissolved in 100 mL of plasma when alveolar pO_2 is 100 mm Hg. However, the O_2 dissolved in the plasma determines the arterial pO_2 and therefore plays an important role in both O_2 diffusion and O_2 blood transport.

Oxygen Bound to Hemoglobin

Each molecule of hemoglobin can reversibly bind up to four molecules of O_2, forming the oxyhemoglobin complex. Beyond its primary role of O_2 transport, hemoglobin provides a sink for O_2 and, therefore, contributes to the maintenance of an adequate pressure gradient during alveolar–capillary diffusion. In resting horses, hemoglobin concentration is about 140 to 150 g/L. Each gram of hemoglobin is able to hold 1.36 to 1.39 mL of O_2.

Oxygen Content

The oxygen content of blood is mainly determined by the hemoglobin concentration and its saturation with O_2. When hemoglobin is saturated with O_2, 100 mL of blood carries about 20 mL of O_2, compared with the 0.3 mL of O_2 dissolved per 100 mL of plasma. The saturation of hemoglobin depends on the arterial pO_2, which depends, in turn, on the amount of O_2 dissolved in the plasma. Above a pO_2 of approximately 70 mm Hg, the oxyhemoglobin curve is flat, and any increase in partial pressure will add little O_2 to hemoglobin. At this partial pressure, the hemoglobin is nearly saturated; only a few (3% to 5%) of the binding sites are still available. Below a pO_2 of 60 mm Hg, the oxyhemoglobin curve shows a sharply decreasing slope. This partial pressure is encountered in the tissues (mean pO_2 of about 40 mm Hg). In this state, blood loses about 25% of its O_2 to the advantage of the tissues. When the metabolic rate is high, that is, during exercise, the tissue pO_2 is further lowered, and more O_2 will be released. Lastly, increases in blood temperature, $[H^+]$, pCO_2, and intracellular concentration of certain organic phosphates (2,3-DPG) induce a right shift of the curve, promoting a higher O_2 release at the level of the metabolizing tissues.

Figure 9-14 shows the oxyhemoglobin dissociation curve of horses, which is slightly different from the classic curve reported for humans. The affinity of equine hemoglobin for O_2 is higher than in humans but less influenced by the temperature modifications (Jones et al., 1989).

When the number of erythrocytes (and, therefore, the amount of hemoglobin) is reduced, as in anemia, the O_2 content is reduced despite normal arterial pO_2 and hemoglobin saturation. In contrast, when the packed cell volume increases, as during exercise in horses, the O_2 content increases, even if the arterial pO_2 is reduced (see Table 9-2). The

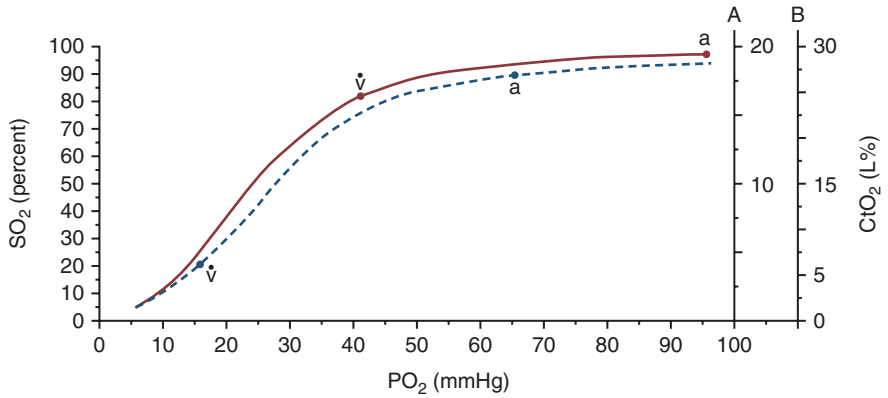


FIGURE 9-14 Oxyhemoglobin dissociation curve in resting (*solid line*) and heavily exercising (*dashed line*) horses. The shift of the curve to the right is caused by exercise-induced hyperthermia and acidosis [pO_2: partial pressure for O_2; SO_2: percent saturation of hemoglobin for O_2; Ct_{O2}: O_2 content at rest (*A*) (hemoglobin = 140 g/liter) and during exercise (*B*) (hemoglobin = 220 grams per liter [g/L]). Values for arterialized (a) and mixed venous (v) blood are also illustrated on the curves.

increase in the packed cell volume and the consequent increase in hemoglobin caused by splenic contraction are an adaptation to exercise specific to the horse, providing almost 50% to 60% more binding sites for O_2 during exercise (Clerbaux et al., 1986). This represents a compensatory adjustment for the fall in arterial pO_2 and hemoglobin desaturation. However, too great an increase in packed cell volume may be disadvantageous from a hemodynamic point of view because it increases the blood viscosity (Jones et al., 1989).

Carbon Dioxide

Carbon dioxide results from metabolic processes occurring in tissues, and once produced, it diffuses from cells into capillary blood. In resting conditions, when blood leaves tissues, the pCO_2 increases from 40 mm Hg in arterial blood to about 46 mm Hg. Approximately 5% of CO_2 is dissolved in plasma; this fraction determines the pCO_2. The remaining CO_2 is transported in two chemical combinations. Most of the CO_2 combines reversibly with H_2O, forming carbonic acid, which then dissociates into bicarbonate and hydrogen ion:

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

Between 60% and 80% of CO_2 is transported as HCO_3^-. The reaction may occur in the plasma but occurs mainly in red blood cells (RBCs), where the presence of carbonic anhydrase accelerates the process several 100-fold. The reverse reaction occurs when blood reaches the lungs.

The formation of carbamino compounds (15% to 20% of the total CO_2 blood content) by coupling of the CO_2 to the –NH groups of proteins (mainly hemoglobin) is the last form of transport for CO_2. At high intensities of exercise, that is, 100% of $\dot{V}_{O2max}$, the large muscular mass produces an extraordinary amount of CO_2, and the lung is unable to completely eliminate it. The development of a relative CO_2 retention that is observed in horses even when they are running without a mask is unique among mammals (Bayly et al., 1989; Franklin et al., 2012). This may be caused by a mechanical limitation to ventilation (Bayly et al., 1999). Human athletes develop a compensatory hyperventilation during heavy exercise to ensure high alveolar pO_2, which, in turn, hastens the rate of equilibrium of alveolar gases with mixed venous blood but also provokes a decrease of the arterial pCO_2 (about 30 mm Hg) (Boucher et al., 1981). Obviously, in exercising horses, there is a lack of truly compensatory hyperventilation, contributing to the development of exercise-induced hypercapnia (Bayly et al., 1989; Bayly et al., 1999; Franklin et al., 2012; Parks and Manohar, 1984).

MECHANICS OF BREATHING

Volume changes in the respiratory apparatus imply that work is being performed on the respiratory system, mainly expanding or compressing the gas in the lungs and displacing it in and out of the airways. The driving forces exerted by the respiratory muscles are opposed mainly by static forces (elastic, gravitational, and surface) and flow-resistive forces (viscous and turbulent resistance of the gases and viscous resistance of the tissues); the inertial forces, negligible at rest, are also of importance in running horses.

Therefore, at each stage of the respiratory cycle, the change in pleural pressure (ΔP_{pl}) is the sum of the elastic (P_{el}), frictional (P_{fr}), and inertial (P_{in}) pressure changes. It is determined by the change in lung volume (ΔV_T), lung compliance (C), airflow ($\dot{V}$), respiratory resistance (R), volume acceleration ($\ddot{V}$), and inertance (I) of the respiratory system:

$$\Delta P_{pl} = P_{el} + Pfr + P_{in} = \Delta V_T/C + R\dot{V} + I\ddot{V}$$

The study of the relationship between the pressures exerted on the respiratory system (which are the causes) and the changes in volume and airflow that result (which are the effects) is the basis of the mechanics of breathing (Figure 9-15).

Breathing Strategy in the Horse

The equine species differs from the other species with regard to breathing strategy (Franklin et al., 2012; Gallivan et al., 1989a; Koterba et al., 1988; Wasserman and Whipp, 1975). Unlike humans, at rest, horses breathe around, rather than from, the relaxed volume of the respiratory system (i.e., the equilibrium position where the tendency of the lung to recoil inward is equal to the tendency of the chest wall to recoil passively outward) (Wasserman and Whipp, 1975). In contrast to other species, the second part of exhalation is active in the horse, and consequently, the very first part of inhalation is

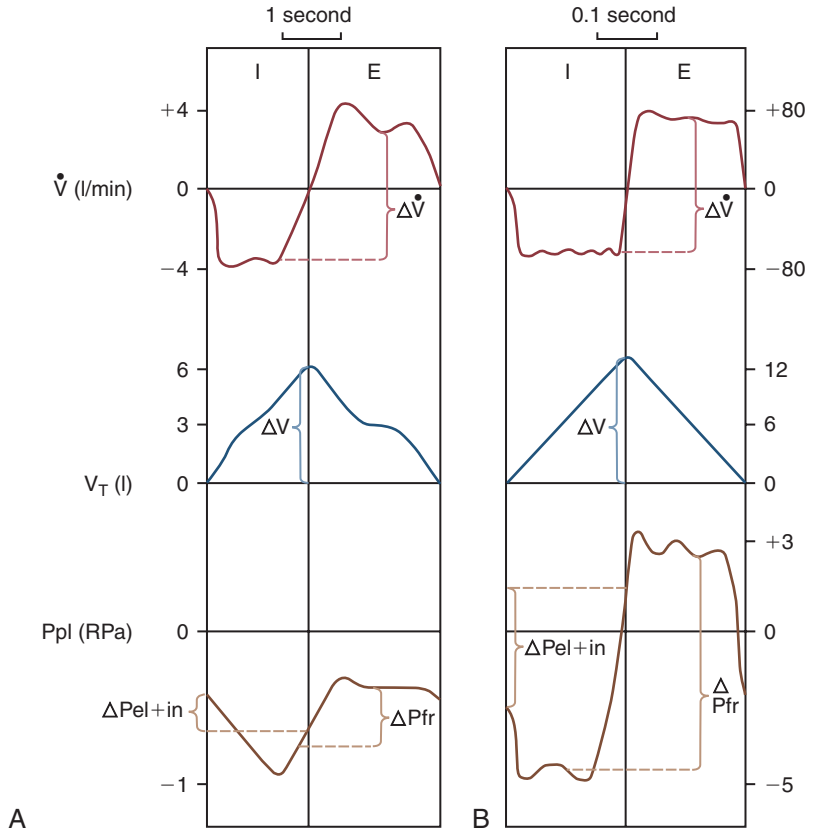


FIGURE 9-15 Simultaneous recording of flow (V), volume (V_T), and pleural pressure (P_{pl},) curves at rest (*A*) and during exercise (*B*). Scales are different on the two graphs (I: inspiration; E: expiration; P_{fr}: frictional forces; P_{el} + in: elastic and inertial forces; total pulmonary resistance [$R_L = \Delta P_{fr}/\Delta V$; dynamic lung compliance (C_{dyn}) = $\Delta V/\Delta P_{el}$ + in]).

passive. The physiologic reason for this strategy is still unknown, but it has been hypothesized that it could minimize the work of breathing. Indeed, the equine chest wall is very stiff compared with other species (Leith and Gillespie, 1971). This implies that the work done to overcome this stiffness during inspiration (called the *elastic work*) will be proportionally higher. Breathing around, rather than from, the relaxed respiratory volume implies that the energy stored during the latter active part of expiration will be restored during the first passive part of inhalation. Thus, abdominal muscles, by performing positive work during expiration, share the total work of breathing with inspiratory muscles.

A direct consequence of this specific breathing strategy is that the pattern of the respiratory airflow is biphasic or polyphasic (Franklin et al., 2012; Gallivan et al., 1989a; Koterba et al., 1988; Wasserman and Whipp, 1975). Exercise induces a sharp increase in the peak respiratory air flow and enlarges the flow-volume loops (Figure 9-16), the shape of each tending toward an increasingly rectangular pattern. The fact that at a fast trot, a plateau occurs during inspiration and expiration suggests a flow limitation when ventilation increases (Franklin et al., 2012; Koterba et al., 1988).

Ventilatory muscle activity has not yet been studied directly during exercise. However, numerous indirect experiments relating measurements of pleural and transdiaphragmatic pressure changes (Art and Lekeux, 1988b; Art et al., 1990) respiratory air flow shape and amplitude, (Manohar, 1991; Slocombe et al., 1991) or blood perfusion of the respiratory muscles (Manohar, 1991; Slocombe et al., 1991) have helped to quantify the magnitude of the increase in ventilatory muscle activity.

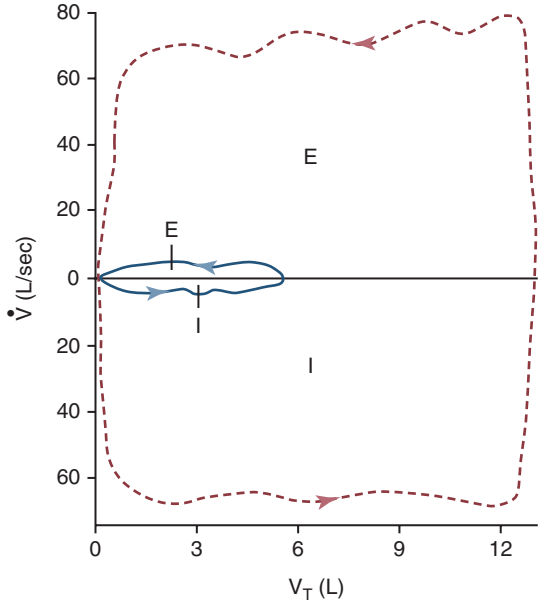


FIGURE 9-16 Flow (V)–volume (V_T) loop in a resting (*solid line*) and exercising (*dashed line*) horse (I: inspiration; E: expiration).

Pleural Pressure

Because the visceral and parietal pleurae are maintained in close apposition, the lung and the thorax interact mechanically. The work performed by the ventilatory muscles induces changes in the pressure of the intrapleural space. During inspiration, the pleural pressure (which, at rest and in quiet conditions, is subatmospheric) decreases, and at the end of inspiration, when the air flow returns to 0, the pleural pressure increases slightly. During expiration, the pleural pressure increases toward its value at functional residual capacity, that is, lung volume before inspiration (see Figure 9-15).

There is a vertical pressure gradient throughout the thorax so that pleural pressure is more subatmospheric in the dorsal than in the ventral regions. Also, the changes in the pleural pressure during respiration are greatest in the middle and in the bottom and less in the top of the thorax. This results in preferential ventilation of the ventral regions of the lungs (Derksen and Robinson, 1980; Franklin et al., 2012).

The force exerted by the ventilatory muscles, especially the diaphragm, also may be estimated by measurement of the transdiaphragmatic pressure, that is, the pressure gradient between the thorax and the gastric pressures, the latter being assimilated to the abdominal pressure (Art and Lekeux, 1988b; Art et al., 1990b; Wasserman and Whipp, 1975).

When exercise starts, the maximal pleural pressure changes increase, and in Thoroughbred horses during maximal exercise, maximal pleural pressure changes as high as 8.5 kilopascals (kPa) have been recorded (Art et al., 1990a). Under these conditions, respiratory frequency is about 120 breaths/min, and therefore, such a pressure swing takes less than 250 milliseconds (ms). Because the transformation of chemical energy into mechanical energy has a limiting rate, less tension can be maintained at high velocities of contraction than at lower speed. The force-speed characteristics of the respiratory muscles could, therefore, be a limiting factor to further increases in ventilation during strenuous exercise (Franklin et al., 2012).

Pulmonary Resistance

Definition and Distribution throughout the Respiratory System

The total pulmonary resistance quantifies the permeability of the airways to air flow. The respiratory flow must be generated mainly against the frictional resistance between the air molecules and the walls of the airways but also against the viscous drag of the tissues.

The resistance, *R*, to air flow passing through a tube is determined by the radius, *r*, and the length, *l*, of the tube, as well as by the physical properties of the gas breathed (μ), according to the following formula:

$$R = \frac{8\mu l}{r^4}$$

This points out the critical importance of the radius; for example, if it is divided by 2, *R* is multiplied by 16.

During quiet breathing, 50% of the total pulmonary resistance results from the nasal passages, 30% from the remaining upper airways, and 20% from the intrathoracic airways (Curtis et al., 2006; Art et al., 1988) (Figure 9-17). The importance of the relative contribution of the nasal cavities is not specific to horses, but the horse, in contrast to other animals, cannot switch from nasal breathing to oronasal breathing.

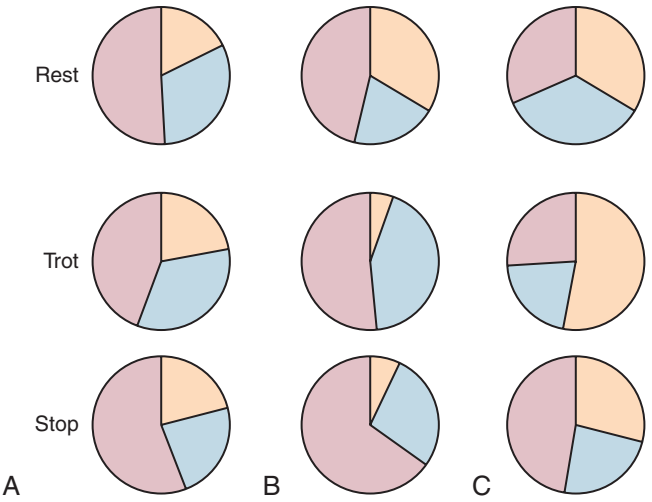


FIGURE 9-17 Relative contribution of nasal (*hatched area*), laryngeal plus extra thoracic tracheal (*light area*), and intrathoracic airway (*dark area*) resistance to total pulmonary resistance under resting and exercising conditions (*A*: total resistance; *B*: inspiratory resistance; *C*: expiratory resistance; *stop*: 20 first seconds of recovery).

Effect of Exercise on Total Pulmonary Resistance

Although it does not significantly modify the relative contribution of each part of the respiratory tract to the total pulmonary resistance, exercise does induce a substantial increase in this resistance (Art et al. 1988; Art et al., 1990a; Curits et al. 2005; Franklin et al., 2012). During exercise, physiologic adjustments such as dilation of the external nares, full abduction of the larynx, and bronchodilation tend to facilitate the increase in flow and decrease the resistance by enlarging the cross-sectional area of the airways and, therefore, their radius. However, despite these exercise-induced adaptations, heavy exercise induces a more than twofold increase in the total pulmonary resistance. The total pulmonary resistance is the result of two kinds of opposing factors: (1) physiologic ones tending to decrease the resistance and (2) physical ones (mainly frictions, turbulences, inhomogeneous distribution of the resistance along airways and alveoli, and airway cross-sectional area changes induced by compressing transmural pressures) tending to increase it (Curtis et al., 2006). When the horse walks or trots slowly, both factors cancel each other out, and the resistance remains unchanged. However, during heavy exercise, the physical factors, depending on flow amplitude and increasing total pulmonary resistance, largely override the physiologic ones, and the resistance increases. Lastly, during recovery, while the ventilatory variables return progressively to their baseline, the physiologic factors overcome the physical factors, and the resistance may be less than during quiet breathing at rest (Art et al., 1988; Curtis et al., 2006; Franklin et al., 2012).

Factors Increasing Resistance to Air Flow during Exercise

Friction and Turbulence

The importance of friction and turbulence to the increase in the total pulmonary resistance has been demonstrated by experiments where exercising horses breathed a helium–oxygen mixture. This mixture has a lesser density than air and therefore minimizes turbulence and friction. During exercise, it

induced a significant increase in minute ventilation because of an increase in respiratory frequency, as well as a 50% decrease in the total pulmonary resistance and mechanical work of breathing (Art et al., 1988; Franklin et al., 2012).

Dynamic Partial Collapse of the Airways

Studies of the inspiratory and expiratory components of the resistance values in trotting horses have shown that during inspiration, the extrathoracic airways account for more than 90% of the total pulmonary resistance, whereas during expiration, the intrathoracic airways are responsible for more than 50% of the pulmonary resistance (Art et al., 1988; Curtis et al., 2006; Franklin et al., 2012) (see Figure 9-17 on page 143). This observation could be explained by the fact that during exercise, a dynamic partial collapse may occur when the pressure surrounding the airways exceeds the pressure within the lumen (Art et al., 1991). When a horse inhales, pressures in the extrathoracic airways may be as low as minus 5 kPa (subatmospheric), whereas the pressure in the surrounding tissues remains atmospheric (see Figure 9-6). During expiration, the intrathoracic pressure becomes greater than the pressure prevailing inside some of the intrathoracic airways (see Figure 9-6). When exposed to compressive pressures, these structures tend to collapse, consequently increasing their resistance to air flow. Because of their bony support, the nasal cavities are less subject to compression than the structures that are less well supported, such as the nares, pharynx, trachea, and bronchi. If this collapse occurs normally in healthy horses, it can be expected to be dramatically worse in horses suffering from airway obstruction, a condition accompanied by substantial transmural pressures during exercise (Curtis et al. 2006; Gillespie, 1974).

It has been shown that both the extra- and intrathoracic parts of the trachea are sufficiently compliant to decrease their cross-sectional areas when submitted to high, but nevertheless physiologic, compressive transmural pressures (Art and Lekeux, 1991a; Art, Lekeux, 1991b; Franklin et al., 2012). Moreover, the shape of the cross-sectional area of the individual's trachea significantly influences collapsibility; tracheae with a circular cross-sectional shape are less compressible than tracheae with a more ellipsoidal shape (Art and Lekeux, 1991b). This is particularly important in view of the variability observed in this shape among individual horses (see Figure 9-4), some horses being probably more susceptible than others to dynamic tracheal collapse. In other words, during intense exercise, when high levels of ventilation are reached, horses having an ellipsoidal trachea may be at a disadvantage compared with those having a more circular trachea.

Lastly, the extension of the trachea decreases its collapsibility (Art and Lekeux, 1991b). This means that hyperextension stiffens the trachea under dynamic conditions, therefore decreasing its resistance to air flow and consequently increasing the maximal air flow. This could explain the benefit of neck extension during maximal exercise; as well as providing maximal patency of the nasopharyngeal airways, the cervical extension and the consequent longitudinal extension of the trachea decrease the tracheal compliance and minimize the phenomenon of dynamic collapse

Nonpathologic Factors Influencing Pulmonary Resistance

A major factor affecting the diameter of the tracheobronchial tree and consequently its resistance to air flow is smooth muscle contraction or relaxation. When irritant materials such as dust are inhaled, the tracheobronchial irritant receptors are stimulated, resulting in bronchoconstriction, elicited by afferents of the parasympathetic nervous system. A practical consequence is that any factor that will impair the mucociliary clearance also will decrease airway permeability. For example, keeping the heads of healthy horses raised for long periods during transportation has been shown to impair the physiologic upper airway clearance and interfere with the airway permeability (Funkquist et al., 1988; see also Chapter 10).

A noncholinergic excitatory system, with substance P as the transmitter and activated by axon reflexes from airway receptors, also may cause bronchoconstriction. Relaxation of smooth muscle occurs following β_2-adrenergic receptor stimulation by circulating catecholamines. The nonadrenergic, noncholinergic inhibitory nervous system serves also as another bronchodilator system. Its efferent fibers are in the vagus nerve, and the neurotransmitter is thought to be nitric oxide (Franklin et al., 2012; Racklyeft and Love, 1990).

Dynamic Compliance

The *dynamic compliance* gives an estimate of the elastic properties of the lung. The lung has an inherent elasticity because of the elasticity of the tissue (the normal lung is an elastic structure that contains a network of elastin and collagen fibers) and the surface tension forces. The latter is lowered by the pulmonary surfactant, a complex material composed of 80% lipids and 20% proteins. The surfactant maintains alveolar stability and prevents pulmonary atelectasis.

The elastic properties of the lung are well documented by the generation of a pressure–volume relation curve constructed by plotting the different driving pressures necessary to inflate the lung up to a given level versus the lung inflation. This relationship, established during spontaneous and tidal breathing, defines the dynamic compliance (see Figure 9-15 on page 142). Although it depends on the intrinsic properties of the lung, it is also influenced by dynamic factors such as lung inflation and respiratory frequency. Dynamic compliance increases with lung inflation and may decrease with increasing respiratory frequency. The latter is especially true in lungs presenting a certain degree of obstruction of the lower airways. Therefore, dynamic compliance measurement is sometimes used as an index of ventilatory asynchrony (Franklin et al., 2012).

Pathologic conditions that induce lung rigidity (i.e., pulmonary edema, pulmonary hypertension, and fibrosis) also induce a decrease in dynamic compliance. The dynamic lung compliance at rest is approximately equal to 23 L/kPa. Calculations of dynamic compliance during intense exercise in horses indicate that the lungs become much less compliant, dropping to about 10% of the resting value (Katz et al., 2005). This change will indubitably increase the work of breathing exponentially.

Pulmonary Inertance

Inertial forces are those necessary to accelerate or decelerate the air in the respiratory airways. In humans, the inertance of the respiratory system is negligible, and so are the pressures necessary to induce accelerations and decelerations of the air in the airways (inertial pressures), even during intense exercise. In contrast, the inertial pressures are not negligible in exercising

horses, which is due to, in particular, the length of the trachea (Kaltman and Johnson, 1992; Franklin et al., 2012).

Tracheal length in mammals is designated by the physiologic and anatomic peculiarities of each species (Art et al., 1989). Horses are grass-eating animals with proportionally longer legs than other domestic grass-eating species and consequently have a longer neck. They also have a deep thorax. The anatomic consequence of this is that they also have a proportionally longer trachea than other species. Therefore, the tracheal diameter must be "chosen" in a way that its design is optimized to satisfy dominant constraints such as tracheal resistance, inertance (the longer the trachea and the smaller its section, the greater is its inertance), flow limitation, dead space, and minimum work of breathing (Art et al., 1989; Franklin et al., 2012). A larger diameter would be advantageous with regard to pulmonary resistance and inertance but disadvantageous for the anatomic dead space.

Physiologic Implications

During quiet breathing, respiratory frequency is about 14 breaths/min, tidal volume is about 5.5 L, the total pressure change to which the lungs are subjected is about 0.44 kPa, and the pressure associated with volume acceleration is about 0.22 kPa (Kaltman and Johnson, 1992). During a fast gallop, respiratory frequency increases up to 120 to 148 breaths/min, tidal volume increases up to 15 L, the maximal pressure change reaches values of about 8.5 kPa, and total volume acceleration reaches more than 3000 L/s (Art and Lekeux, 1995; Bayly et al., 1987; Katz et al., 2005). The pressure required to produce these accelerations is then approximately 4.3 kPa, or 50% of the total pressure change. Therefore, it appears that inertial pressures become of great importance in the exercising horse. They may even be a limiting or at least a constraining factor to any further increase in ventilation (Bayly et al., 1999; Franklin et al., 2012; Kaltman and Johnson, 1992).

Consequences for the Measurement of the Dynamic Compliance

The great importance of the respiratory inertial factors in the running horse also explains why the dynamic compliance becomes negative when measured during exercise (Art and Lekeux, 1988a; Art et al., 1988; Katz et al., 2005) (Figures 9-15 and 9-18). Classically, the dynamic compliance is measured at points of zero flow and is defined by the equation:

$$C_{dyn} = V_T/(\Delta P_{el} - 4\Pi^2 - f^2 \cdot V_T \cdot I)$$

where ΔP_{el} is the elastic pressure change. The term $4\Pi^2 - f^2 \cdot V_T \cdot I$ (i.e., the inertial pressure change) is generally considered as negligible, and the equation simplified is:

$$C_{dyn} = V_T/\Delta P_{el}$$

In exercising horses, $4\Pi^2 - f^2 \cdot V_T \cdot I$ increases, and its absolute value may become $\geq \Delta P_{el}$. This explains why, in these specific conditions, C_{dyn} is overestimated, infinite, or negative.

Mechanical Work of Breathing

A classic approach to the assessment of the mechanical work of breathing is based on measurement of the area of the pressure–volume loops (see Figure 9-18). Although it is well known that the total work of breathing is underestimated by this method, it gives a good estimation of the dynamic components of the work of breathing. It has been shown that the work per respiratory cycle, the work per liter of air ventilated, and the work per minute are increased dramatically during exercise (Bayly et al., 1999; Leith, 1982). For example, the power output (i.e., the work of breathing per minute) increases many hundred times between rest and strenuous galloping (see Table 9-2).

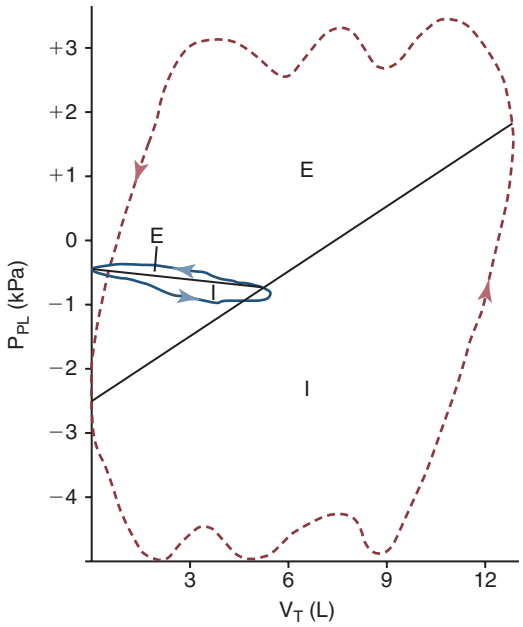


FIGURE 9-18 Pressure (P_{pl})-volume (V_T) loop in a resting (*solid line*) and exercising (*dashed line*) horse. The area of this loop gives an estimation of the work of breathing (I: inspiration; E: expiration; 1 kilopascal [kPa] = 7.5 mm Hg).

The relationship between the mechanical work of breathing per minute and the minute volume in running horses is curvilinear with an upward concavity (Figure 9-19). The curve is of ever-increasing slope, implying that the mechanical cost of breathing for any additional units of air ventilated becomes greater with any increase in ventilation (Art et al., 1990a; Leith, 1982; Franklin et al., 2012).

The sharp increase in the work of breathing with exercise-induced hyperventilation is explained by the increase in resistive, elastic, and inertial work. The increase in the resistive work is related to the increase in the total pulmonary resistance (see Figure 9-19), itself caused by compulsory nasal breathing, to friction and turbulence, and to the dynamic partial collapse of the airways (Art and Lekeux, 1991a; Franklin et al., 2012). The threefold increase in volume probably increases the elastic work significantly owing to chest wall stiffness (Leith, 1982; Franklin et al., 2012). Furthermore, the length of the airways and the magnitude of flow accelerations during exercise make the inertial work nonnegligible (Kaltman and Johnson, 1992; Franklin et al., 2012). These observations strongly suggest that the work of breathing is a limiting or at least a constraining factor to further increases in ventilation during strenuous exercise (Franklin et al., 2012).

Inspiratory Muscles to Total Oxygen Uptake Ratio

The ratio of the mechanical work of breathing to O_2 uptake has been calculated in galloping horses to evaluate the relative respiratory muscle O_2, uptake compared with total O_2 uptake.

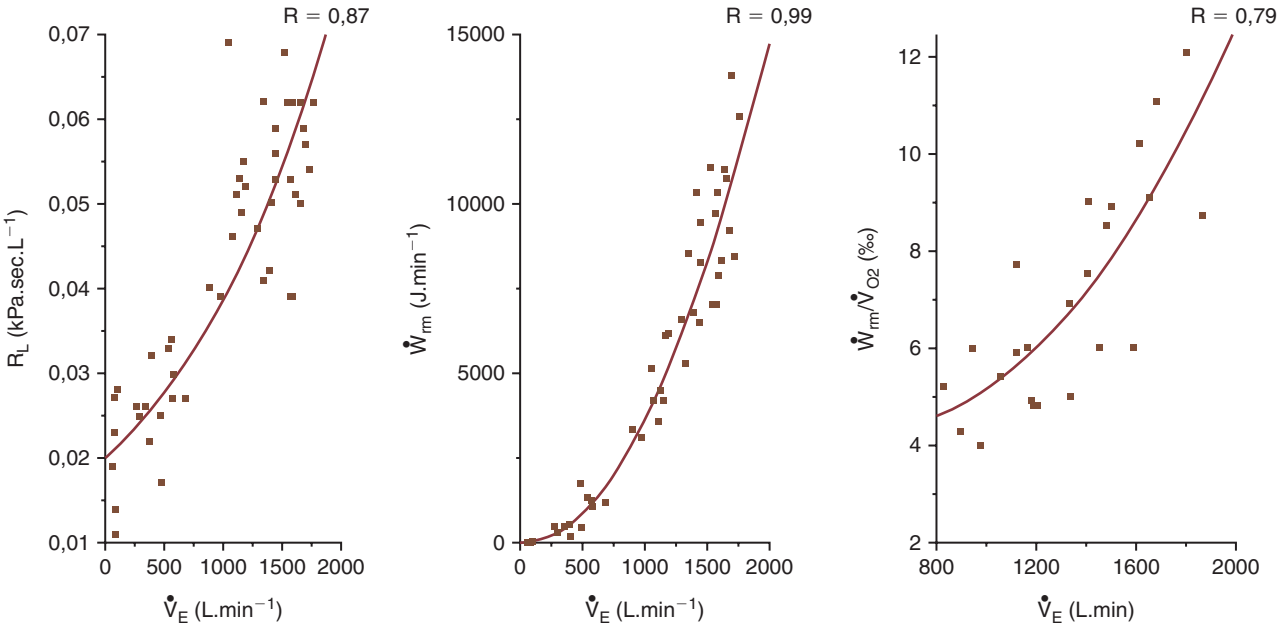


FIGURE 9-19 Effect of the increase of minute ventilation (V_E) on total pulmonary resistance (R_L), minute work of breathing (W_{rm}), and the ratio W_{rm} to O_2 uptake ($W_{rm}/\dot{V}O_2$). This could explain why the energy cost of breathing could become excessive at high ventilatory levels.

This ratio increases exponentially with the minute volume, indicating that during exercise, respiratory muscle O_2 uptake reaches a substantial percentage of the total O_2 uptake, estimated to be up to 20% of total O_2 uptake (Art et al., 1990a) (see Figure 9-19). This may be interpreted to suggest that in horses as in humans there is a "critical level of ventilation" above which any further increase in O_2 uptake would be entirely consumed by the respiratory muscles (Franklin et al., 2012).

Respiration Locomotion Coupling

At the walk and trot, respiratory and step frequencies have been reported to be sometimes coupled, but this coupling is neither constant nor compulsory (Art et al., 1990b; Bye et al., 1983; Franklin et al., 2012). However, when the coupling exists, it seems that the "abdominal piston" acts in synergy with the respiratory pump. Therefore, at these intensities this strategy probably reduces the cost of breathing.

Once the horse gallops, there is a compulsory linkage between step and respiratory rates (Attenburrow, 1983; Young et al., 1992). The mechanisms underlying this coupling are not yet well understood. It is probably caused by a mechanical-energetic linkage resulting in metabolic advantage (Boggs, 2002). It may be that the visceral contents act as a piston and flexion of the back and loading of the thorax by the forelimbs contribute to the mechanical advantages of this synchronization (Bramble and Carrier, 1983). During protraction of the forelimbs, the rib cage is pulled forward and outward, allowing inhalation. During weight bearing, the rib cage absorbs forces and is compressed, resulting in exhalation. Experimental evidence shows that in galloping horses, back flexion rather than the visceral piston mechanism assists breathing (Young et al., 1992).

It must be emphasized that this coupling is not absolute in healthy horses. Indeed, some galloping horses, both on the track and on the treadmill, sporadically show a "big breath" which continues for two or three strides. The reason for this remains to be elucidated.

Respiratory Muscle Recruitment

In mammals, the diaphragm (separating the thorax from the abdomen) and the external intercostal muscles (joining the ribs) are the main inspiratory muscles. However, in horses, the serratus ventralis, on the one hand, has the most important role in assisting the inspiratory effort of the diaphragm both at rest (Hall et al., 1991) and during exercise (Manohar, 1991). On the other hand, the transversalis is the principal muscle actively involved in expiration. Lastly, the intercostal muscles are also activated in the second part of inspiration and expiration (Hall et al., 1991).

A study of respiratory muscle recruitment by electromyography has been performed in healthy resting horses (Hall et al., 1991; Wasserman and Whipp, 1975) and in ponies during normoxia and hypoxia (Brice et al., 1990; Manohar, 1986). During exercise, both inspiratory and expiratory muscle activities increase, and the metabolic needs of the muscles become substantial. The increase in blood flow is likely related to the increase in the metabolic needs. Therefore, the activity of the ventilatory muscles in ponies has been estimated indirectly by the increase in the muscles' perfusion (Manohar, 1990a, b; Manohar, 1991). The ventilatory muscles comprise approximately 5.5% of the total body weight and receive 10% and 15% of the cardiac output at rest and at maximal exercise, respectively. The increase in blood flow in these muscles is accompanied by a precipitous decrease in their vascular resistance (Manohar, 1990b; Manohar, 1991).

During exercise, the costal diaphragm blood flow exceeds the blood flow to all other inspiratory or expiratory muscles; indeed, it increases over 20-fold in maximally exercised ponies. Moreover, during maximal exercise, diaphragmatic blood

flow reaches its upper limit (Manohar, 1987) and adenosine infusion, which causes a marked vasodilation in the pony's diaphragm at rest, fails to elicit any further vasodilation, indicating that vasodilator capacity is completely utilized in these conditions (Manohar, 1986). This may be interpreted to suggest a potential limiting factor of the ventilatory machinery during heavy exercise (Franklin et al., 2012).

CONTROL OF BREATHING

With the exception of the changes induced by strenuous exercise, it can be assumed that the arterial blood gas tension and the chemical composition of blood remain remarkably steady in healthy horses. This means that the metabolic needs are constantly matched by the alveolar ventilation. This matching is under the control of a central controller, which receives afferent information from peripheral and central receptors and which regulates, via motor neurons, the ventilatory muscles according to that information. The respiratory control center is located in the medulla, and its activity is modulated by a variety of neural inputs. Another part of the brain is also involved in controlling the respiratory rhythm, and higher conscious centers can intervene to modify the pattern of breathing.

Respiratory Control at Rest

Central Control

Breathing is controlled by neurons located in the pons and the medulla. The respiratory center in the medulla is divided into two groups of neurons. There is a dorsal group (located in the ventral portion of the nucleus tractus solitarius), which contains mainly inspiratory neurons, and a ventral group (located near the nucleus ambiguous and nucleus retroambiguous), which has both inspiratory and expiratory neurons. If the medulla is experimentally isolated from all neural inputs, the animal continues to breathe rhythmically. The activity of both medullary centers is modulated by two respiratory centers in the pons: (1) the apneustic center, located in the lower pons, which stimulates the inspiratory neurons of the medulla; and (2) the pneumotaxic center, located in the upper pons, which switches off the inspiration.

Chemoreceptors and Humoral Control

Because the primary function of the lungs is to supply O_2 and remove CO_2 it is clear that ventilation ultimately must be controlled, according to information on gas exchange. Chemoreceptors are sensors that detect changes in arterial blood gases or chemical composition. They give feedback about the results of adjustments in ventilation to the respiratory control centers. Changes in arterial pCO_2 and pH are detected by both peripheral and central chemoreceptors, while changes in arterial pO_2 are detected by peripheral chemoreceptors only. Therefore, at rest, sensation of pCO_2, $[H^+]$, or both is paramount in regulating ventilation under usual resting conditions. Small changes in arterial pCO_2, pH, or both are more potent regulators of ventilation than changes in arterial pO_2.

The *peripheral chemoreceptors* are located in the carotid bodies at the bifurcation of the common carotid arteries and in the aortic bodies, near the aortic arch. These chemoreceptors send afferent impulses to the control center via the vagus and the glossopharyngeal nerves. Their activity is enhanced not only by hypercapnia, hypoxemia, and acidosis but also by hyperthermia and decreased blood pressure. They are sensitive to the changes in arterial pO_2 but not to changes in oxyhemoglobin content. Although their response to CO_2 and pH is linear, their response to changes in arterial pO_2 is nonlinear; they only show enhanced activity once arterial pO_2 is below 60 mm Hg.

The *central chemoreceptor* tissue is located near the ventral aspect of the medulla. It lies in the intracerebral interstitial fluid and is separated from blood by the blood–brain barrier. It apparently responds to changes in the interstitial tissue fluid pH. The latter is induced either by changes in arterial pCO_2 giving a fast response (because CO_2 diffuses freely through the blood–brain barrier and induces a fast decrease in the interstitial fluid pH) or by a change in blood pH, giving a delayed response (because the barrier is relatively impermeable to H^+). Therefore, an acute increase in H^+ concentration is at first detected by the peripheral receptors.

Pulmonary and Airway Receptors

Three kinds of receptors with vagal afferents are located in the lungs and play a role in ventilatory control. The *pulmonary stretch receptors* are nerves ending in the tracheal and bronchial smooth muscles. Their activity is enhanced by enlargement of airway cross-section, for example, when the lung volume increases, and results in an inhibition of further inspiratory activity. The stretch receptors could be responsible for the adjustment in the pattern of breathing to minimize the energy cost of breathing. They also could prevent lung overstretching when the ventilatory demand is high, that is, during heavy exercise.

Irritant receptors have a minor role in the control of breathing. They are located between and below the epithelial cells of the airways (larynx, trachea, bronchi, and intrapulmonary airways). They protect the lung against various aggressors or irritants by reacting to a variety of stimulations (such as inhalation of irritant gases, dust, release of histamine) and inducing tachypnea, bronchospasm, cough, and mucus secretion.

Pulmonary C fibers ramify in the pulmonary interstitium close to the pulmonary capillaries, where they may monitor blood composition or degree of distension of the interstitium. They are responsible for the respiratory adjustments occurring in disease states in many species.

Muscle Spindle Stretch Receptors

As with other skeletal muscles, the ventilatory muscles have spindle stretch receptors. The density of these receptors is variable from one muscle to another; they are few in the diaphragm but numerous in the intercostal muscles. These receptors control the strength of ventilatory muscle contraction.

Respiratory Control during Exercise

Gas-exchange requirements, that is, O_2 uptake and CO_2 output, vary with the metabolic rate. Without doubt, exercise is the most potent stress to the body's oxidative machinery, that is, to the muscle and the functions concerned with gas exchange, gas transport, and tissue respiration. When animals exercise at increasing intensity, the O_2 uptake rises linearly to a maximum termed $\dot{V}_{O2max}$. At this exercise intensity, all the available muscles' aerobic capacities are recruited, and with further increase in intensity, there will be no further increase in O_2 uptake. At maximal exercise, the O_2 uptake may increase more than 50 times from resting rates, this increase being satisfied in part by a >30-fold increase in the minute

volume (Art and Lekeux, 1993; Evans and Rose, 1988a; Franklin et al., 2012; Rose et al., 1988).

The question of how the ventilatory rate is controlled during exercise is one of the major unresolved issues in respiratory physiology. The controversy centers on the origin of the stimulus that provides for a rapid and precise adjustment of alveolar ventilation to meet the metabolic demand. In humans, there are several theories proposing either neural stimuli or humoral stimuli or a combination of both (Forster and Pan, 1991). In horses, the specific control mechanisms invoked appear to differ according to the exercise intensity (Franklin et al., 2012).

Respiratory Control during Low-Intensity Exercise

During short-term moderate exercise, arterial blood gases and chemical composition do not change. This stability is the result of the ventilation rate increasing in tandem with the metabolic rate. However, gaseous tensions are far too stable to account for the increase in ventilation on the basis of the simple negative-feedback system existing at rest. Although there is a considerable conviction that gas tensions (especially pCO_2 and associated H^+ concentration) are involved in the control of ventilation during exercise as well, how they are involved remains unresolved. It is likely that other drives are involved in the control of ventilation, for example, mechanoreflexes originating from motion of the working limbs and changes in cardiac output, thermoregulatory drivers, and cortical and psychological factors (Wasserman et al., 1986).

The exercise-induced hyperpnea at mild and moderate exercise intensities has been thoroughly studied in ponies, showing that in this species, the hyperpnea is related to an increase in lactic acidosis (Erickson et al., 1991) and to spinal afferent information (Pan et al., 1990). In contrast, exercise-induced hyperpnea is not related to increases in arterial or venous CO_2 (Forster et al., 1986; Klein et al., 1982; Pan and Forster, 1984; Pan et al., 1983; Powers et al., 1987), a decrease in blood pH (Pan et al., 1986), an increase in H^+ stimulation at the medullary receptors (Bisgard et al., 1978), a decrease in arterial pO_2 (Forster et al., 1983), cardiovascular causes (Pan and Forster, 1984), nor an influence of limb motion (Forster et al., 1984). However, transposition of these observations to horses is questionable because horses and ponies differ in their respiratory adjustments to exercise. Although the horse becomes hypoxemic and often hypercapnic during heavy exercise, the pony does not become hypoxemic and, on the contrary, becomes hypocapnic, with arterial pCO_2 as low as 27 mm Hg (Franklin et al., 2012; Parks and Manohar, 1984).

Respiratory Control during High-Intensity Short-Term Exercise

During strenuous exercise, the horse, independent of its ability and state of fitness, demonstrates a decrease in arterial pO_2 and pH and, if the exercise is intense enough, an increase in arterial pCO_2 (see Figure 9-12). These chemical regulators are supposed to strongly stimulate ventilation, but the stimuli appear to be insufficient to maintain arterial blood gas homeostasis. This is not the case in other species such as humans, dogs, and ponies, which as a rule do not show hypoxemia or hypercapnia during strenuous exercise. However, it must be pointed out that in the elite, endurance human athlete at peak fitness, cardiovascular and muscular adaptations to training reach such an exceptional level that the pulmonary system may be taxed maximally or even lag behind the functional capacity of the remaining aerobic system. Such a condition may result in hypoxemia and hypercapnia during high-intensity exercise in these elite athletes (Dempsey et al., 1984).

Thus, in contrast to other species, horses do not adopt a compensatory hyperventilatory response, that is, hyperventilation that could compensate for the gas-exchange impairment due mainly to diffusion limitation and partly to ventilation–perfusion inequalities (Bayly et al., 1989; Franklin et al., 2012; Wagner et al., 1989). The reason for this hypercapnic hypoventilation in horses remains unclear, with numerous hypotheses having been suggested, none of which have been proven at this time (Franklin et al., 2012).

Hypotheses to Explain the Lack of Compensatory Hyperventilation in Horses Exercising Intensely

Influence of the Locomotion–Respiration Coupling on Exertional Ventilation

This coupling has been suggested as a major constraint to the increase in ventilation. Although there is no doubt that respiratory frequency is totally related to step frequency in galloping horses, several experimental observations rule out the coupling as the unique reason for hypoventilation: (1) Standardbred horses racing at a trot (a gait where a coupling may exist but is not compulsory) also demonstrate hypoxemia and hypercapnia (Franklin et al., 2012; Thornton et al., 1983); (2) the magnitude of hypercapnia is poorly related to the respiratory frequency (Bayly et al., 1989); and (3) the respiratory frequency of the pony is also tightly coupled with its stride frequency and, nevertheless, it adopts a compensatory hyperventilation and becomes hypocapnic during heavy exercise (Parks and Manohar, 1984).

Lesser Sensitivity of Receptors

A slight increase in ventilation in horses running at 10 meters per second (m/s) is observed when the CO_2 concentration in the inhaled air increases from 0% to 3%. However, a further increase in inhaled CO_2 up to 6% does not induce any further changes in ventilation when these horses are not at their maximal ventilation capacity, from which it can be suggested that there is a lessened sensitivity of the chemoreceptors (Landgren et al., 1991). However, the underlying mechanisms of this observation remain to be elucidated.

Influence of Force–Velocity Characteristics of Ventilatory Muscles

Theoretically, the compensatory hyperventilation that would be required for the homeostasis of arterial pO_2 should be ensured by either (1) a tidal volume of about 23 L (up to 15 L are reported), which together with a respiratory frequency of about 120 breaths/min implies mean respiratory airflow of more than 100 L/s, or (2) a 2:1 coupling between respiratory frequency and stride frequency, which implies that the respiratory frequency should reach values of about 240 breaths/min (up to 148 breaths/min reported). In terms of respiratory muscle energetics and force–velocity characteristics, such a level of ventilation seems impossible to reach.

Negative-Feedback Mechanisms Generated by Ventilatory Muscle Fatigue

The increase in the ventilatory activity is accompanied by an exponential increase in both the work of breathing and the O_2

uptake (energy cost) of the ventilatory muscles, mainly because of the increase in lung volume, in respiratory frequency, and in the resistive, elastic, and inertial forces (Art et al., 1990a; Franklin et al., 2012) (Figure 9-20). Consequently, the energy demand of the ventilatory muscles becomes substantial, and the energy supply may be insufficient to satisfy this demand. This could result in a negative metabolic balance at the level of the ventilatory muscles, which could lead to their fatiguing (Franklin et al., 2012; Leblanc et al., 1988). This phenomenon could control the pattern of breathing by a negative-feedback mechanism acting either on the respiratory centers or directly on the ventilatory muscles (Mador and Acevedo, 1991). This negative-feedback would override all the other positive feedback, tending to further increase ventilation (Dempsey, 1988).

During maximal exercise, highly trained human athletes often reach the mechanical limits of their lungs and respiratory muscles for producing alveolar ventilation (Johnson et al., 1992). The occurrence of respiratory muscle exhaustion has been demonstrated to occur in humans after high-intensity short-term exercise (Bye et al., 1984), as well as during prolonged exercise (Loke et al., 1982). This results in a progressive increase in diaphragmatic excitation–contraction decoupling (Bye et al., 1984; Roussos and Macklem, 1986). Although at the onset of exhaustion in the horse during high-intensity short-term exercise there is a sudden decrease in the minute volume and the O_2 uptake (Rose and Evans, 1987; Art and Lekeux, 1993), the occurrence of muscle fatigue remains to be demonstrated in this species.

A Possible Explanation for the Lack of Compensatory Hyperventilation in Horses Performing Intense Exercise

The fact that the increase in ventilation becomes limited during heavy exercise in horses may be explained by several factors. First, it has already been shown that during strenuous exercise, the horse reaches a "critical level of ventilation" above which any further increase in O_2 uptake would likely be entirely consumed by the respiratory muscles (Franklin et al., 2012; Kaltman and Johnson, 1992). The advantage of not increasing ventilation further during strenuous exercise may be that extra flow resistive and elastic work is avoided and that there is a reduction in the O_2 uptake of ventilatory muscles, consequently reducing their energy demand. In terms of performance, hypoxemia and hypercapnia could be less disadvantageous than reaching the critical level of ventilation. Second, this negative-feedback mechanism could protect the ventilatory muscles against exhaustion and irreversible damage to the contractile oxidative machinery. Third, pulmonary overstretching, that is, high lung volume, has been shown to increase the fragility of alveolar and vessel walls. By limiting the increase in lung volume, the horse could minimize the risk of tissue rupture, which if it occurred could possibly lead to severe exercise-induced pulmonary hemorrhage. What is clear from these observations is that the respiratory and not the cardiovascular system is likely to be the limiting factor in exercise capacity in horses performing heavy exercise (Franklin et al., 2012).

Respiratory Control during Submaximal Prolonged Exercise

During prolonged heavy, but not maximal, exercise, the impairment of gas exchange (especially hypercapnia) seems to be progressively compensated for after several minutes of exercise (Bayly et al., 1989; Franklin et al., 2012; Hodgson et al., 1990). This may be interpreted to suggest that horses, like humans, experience a "second wind" phenomenon, that is, the relief of hypoventilation, which could be induced by diaphragmatic fatigue (Scharf et al., 1984). Several

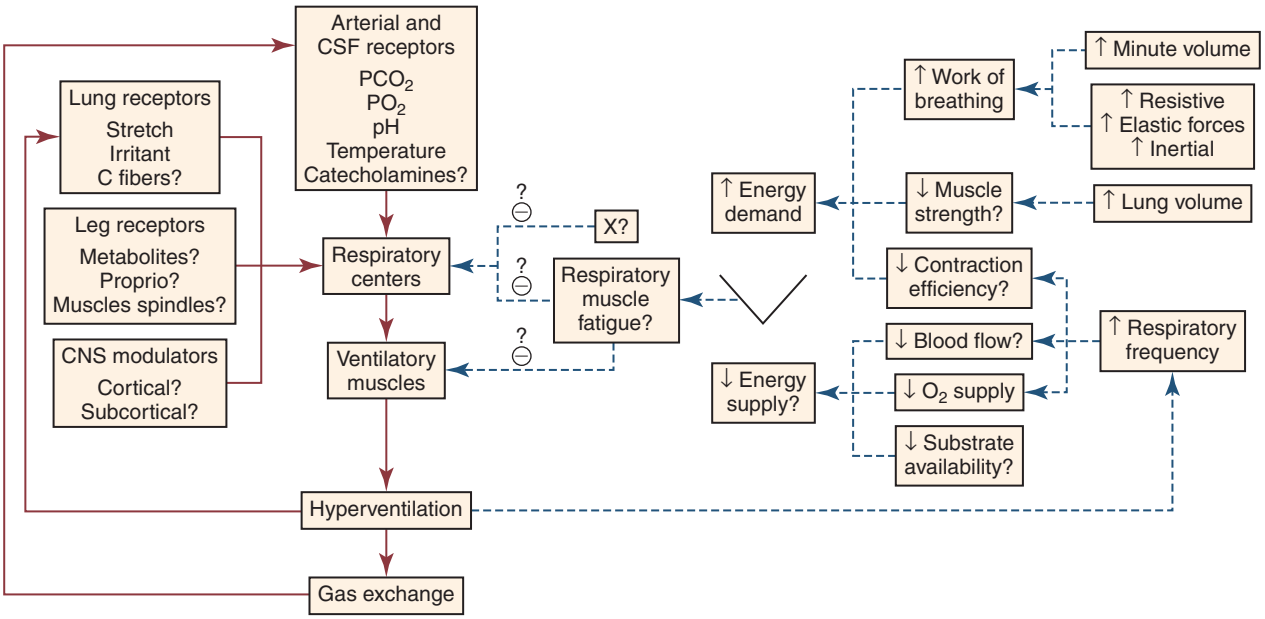


FIGURE 9-20 Hypotheses for the control of the ventilatory response in heavily exercising horses. The physiologic justification of such a negative-feedback mechanism (*dashed line*) could be to protect ventilatory muscles against exhaustion and to avoid a compensatory hyperventilation with negative metabolic balance (i.e., decreased O_2 supply to locomotor muscles due to increased O_2 supply to ventilatory muscles) (?: suspected mechanism which remains to be demonstrated in the horse).

factors have been postulated to explain the improvement in ventilatory muscle function after a few minutes of exercise: (1) The length of the diaphragm may be modified by recruitment of other ventilatory muscles or by changes in the functional residual capacity, resulting in an increase in its force of contraction according to its length or tension characteristics; (2) the contractile function of the diaphragm may be improved; (3) the blood flow to and in the working diaphragm may be more effectively redistributed; and (4) the catecholamine release may lead to increased contractility (Franklin et al., 2012). These factors, however, need to be confirmed.

RESPIRATORY FUNCTION DURING RECOVERY FROM EXERCISE

After exertion, all physiologic measurements return progressively to their resting values. The speed of this return is dependent on intensity and duration of the exercise performed, the state of fitness of the horse, and the bioclimatologic conditions.

The metabolic reasons for the excess postexercise O_2 uptake are discussed elsewhere in this book (see Chapter 3). These include resynthesis of phosphocreatine in the exercised muscles, catabolism or anabolism of blood lactate, persistence of high body temperature, and restoration of hormonal homeostasis (Rose et al., 1988). This excess O_2 uptake is associated with an elevated minute volume that is mainly the result of an increase in respiratory frequency (Art and Lekeux, 1988c; Franklin et al., 2012). In the minutes after cessation of exercise horses hyperventilate, as assessed by the high ventilatory equivalent for O_2. Despite a high dead space to tidal volume ratio, this hyperventilation results in alveolar hyperventilation and a consequent hyperoxia and hypocapnia (Art et al., 1990a). In addition, the end-tidal expired gases have high O_2 (~17% versus 14% at rest) and low CO_2 concentrations (~4.5% versus 6% at rest).

Several explanations may be put forward to explain this post-effort hyperventilatory effect. When the horse stops exercise, the possible influence of the limb motion does not intervene in respiratory control, and other stimulants such as acidosis could become major drivers of this process. The resulting alveolar hyperventilation and respiratory alkalosis could be advantageous to compensate for the profound metabolic acidosis resulting from exercise.

In the postexercise period, pulmonary resistance becomes significantly lower as ventilatory variables return toward their resting values; this means that physiologic adjustments such as bronchodilation or dilatation of the larynx are no longer overcome by those physical phenomena that tend to increase pulmonary resistance.

The thermoregulatory role of the respiratory system also accounts for the posteffort hyperventilation that is caused by an increase in the respiratory frequency rather than by an increase in tidal volume. It has been shown that ponies and horses recovering from the same treadmill exercise test in hot and humid conditions have a significantly higher respiratory frequency than ponies recovering in dry and cold conditions (Art and Lekeux, 1988c; see also Chapter 8). This must be taken into account when horses running endurance courses are examined at the "vetgate"; when bioclimatologic conditions are hot and humid, the respiratory frequency is a poor indication of the actual ventilatory demand of the horse (Rose, 1983).

RESPIRATORY ADAPTATIONS TO TRAINING

The effects of training have received increasing attention during the last decade. The use of standardized treadmill tests has largely contributed to the improvement in knowledge in this field. However, differences in the experimental observations exist and show that physiologic adjustments to training may differ according to various factors such as the age of the athlete together with the previous stage, history, and intensity and duration of training. Indeed, during training, the multiple stages of the oxygen transport chain are stimulated and undergo adaptation, but their respective responses to training occur on differing timetables. The oxidative machinery of the muscles develops faster than the capillarity vascularization of these tissues; circulating hemoglobin concentration increases only in the early stage of training; and the cardiac output increases only after several weeks of training. Adaptations to training also depend on the breed and discipline, as well as on the training programs (treadmill or field; duration and intensity). Furthermore, the kind of test used to illustrate the effects of training also influences the observations; short or prolonged exercise, standardized mild, moderate, submaximal heavy effort, or maximal exercise up to fatigue are used for this purpose. However, despite the problems associated with the study of training effects, some information is now available.

EFFECT OF TRAINING ON GAS EXCHANGE

Without doubt, training rapidly and significantly improves $\dot{V}_{O2max}$ (Art and Lekeux, 1993; Evans and Rose, 1987; Evans and Rose, 1988b; Knight, 1991; Tyler et al., 1996) (see Figure 9-13 on page 140). The cardiovascular mechanisms underlying the improvement in O_2 uptake with training have been elucidated, but the ventilatory mechanisms have received less attention. The improvement in O_2 uptake with training is related to an increase in cardiac output, O_2 extraction, or both. Although, results regarding the effects of training on arteriovenous difference in O_2 content are somewhat conflicting; it is reported to be unchanged during submaximal exercise (Bayly et al., 1983; Thornton et al., 1983) increased during maximal exercise (Knight, 1991) or decreased during maximal exercise (Evans and Rose,1988b). The discrepancy between these studies is largely explained by the difference in the training programs utilized, that is, intensity and duration and the standardized exercise tests used. For example, the decrease in the arteriovenous difference in O_2 content with training during maximal exercise was simultaneous with a sharp increase in cardiac output, which suggests that the transit time in the tissues is reduced by a significant increase in cardiac output so that adequate diffusion is less possible (Evans and Rose, 1988b). In contrast, in the study reporting an increase in arteriovenous difference in O_2 content, there was no change in the cardiac output (Knight, 1991).

Training does not seem to modify the exercise-induced alterations in blood gas tensions during or after heavy exercise (Butler et al., 1991; Evans and Rose, 1988b; Franklin et al., 2012; Thornton et al., 1983) or the pulmonary arterial pressure and pulmonary blood flow velocity during mild standardized exercise (Erickson et al., 1987).

EFFECT OF TRAINING ON VENTILATION

Although all the systems implicated in exercise physiology (i.e., muscles, cardiovascular system, bones, and tendons) can undergo improvements in metabolic efficiency in response to training, the ventilatory capacity, by itself, appears to be only capable of limited adaptations to training. The significant increase in the O_2 uptake induced by training is not accompanied by an equivalent increase in the minute volume. The ventilatory rate is unchanged during a maximal exercise test up to fatigue (Evans and Rose, 1988b) or slightly lower during a standardized exercise test (Art and Lekeux, 1993) after a period of training. This results in a significant decrease in the ventilatory equivalent for O_2 (minute volume to O_2 uptake ratio), obvious even in the early stages of training (Art and Lekeux, 1993; Franklin et al., 2012). The mechanisms underlying this training adjustment remain obscure.

In human athletes, the improvement in the ventilatory equivalent for O_2 is explained by a training-induced reduction in respiratory frequency and increase in tidal volume. Therefore, the time for gas exchange at the level of the alveoli is increased by training, and this induces improved alveolar O_2 extraction; the mean expired O_2 is about 18% in untrained men and 14% in well-trained athletes (Fringer and Stull, 1974; Jirka and Adamus, 1965).

In equine athletes, the decrease in the ventilatory equivalent after training is also associated with an improvement in the alveolar O_2 extraction (Art and Lekeux, 1993; Franklin et al., 2012). However, in contrast with humans, the reason for this improvement seems not to be a change in the pattern of breathing but more probably an increase in the affinity of hemoglobin for O_2. This could be explained by a training-induced shift to the left of the oxyhemoglobin curve resulting from a decrease in the extent of the exercise-induced acidosis and hyperthermia (Art and Lekeux, 1993; Franklin et al., 2012).

Whatever the reason, the decrease in the ventilatory equivalent means that horses breathe less air to ensure a given O_2 uptake. Theoretically, this could imply that (1) the relative energy cost of ventilation is reduced; (2) the fatigue of the respiratory muscles is delayed; (3) the reduction in O_2 uptake by the muscles is profitable to the working locomotor muscles, or all of these (Franklin et al., 2012). However, these assumptions remain to be proven.

EFFECT OF DETRAINING

It was originally presumed that ventilatory adaptations occurring as a result of training were transient and rapidly reversible. After a 3-week period of detraining, most of the training-modified ventilatory parameters, that is, O_2 uptake, ventilatory equivalent for O_2, and minute ventilation during a standardized exercise, return to their pretraining values (Art and Lekeux, 1993; Knight, 1991). However, in more prolonged training over 9 months, there was no significant reduction in $\dot{V}_{O2max}$ during the first 4 weeks of detraining, and after 12 weeks of detraining mean $\dot{V}_{O2max}$ was 8% lower than peak training values, but still 15% above those before training (Tyler et al., 1996). The slow decrease in $\dot{V}_{O2max}$ after prolonged training has implications for the loss of aerobic fitness and performance in horses in training, which may need a period of rest due to injury or disease. If horses have been in training for a long period, a 4 to 6 week rest may not have adverse effects on aerobic capacity. Such horses may be able to resume training at a higher training level more rapidly and return more quickly to racing. However, there have been no studies on the effects of detraining on bone density and soft tissues such as tendons.

THE RESPIRATORY SYSTEM AND ITS THERMOREGULATORY ROLE

Many mammals utilize the respiratory system to lose heat by evaporative cooling. To increase heat loss, they breathe (mainly expire) through the mouth instead of through the nose, and they hyperventilate by increasing the respiratory frequency and reducing tidal volume. In contrast, the horse cannot breathe through the mouth. Nevertheless, there are anatomic and physiologic characteristics that provide evidence that the respiratory system plays a role in body thermoregulation at rest, during exercise, and during recovery.

In resting ponies, changes in ambient temperature, without concomitant changes in body temperature, induce modification in respiratory frequency and tidal volume. Changes in skin and airway temperatures, therefore, appear capable of eliciting changes in breathing (Kaminski et al., 1985).

Prolonged steady exercise induces a progressive increase in respiratory frequency with an increase in the physiologic dead space to tidal volume ratio (Pelletier et al., 1987; Rose and Evans, 1987; Thiel et al., 1987). This may be interpreted to suggest that in horses, as in humans, the respiratory system becomes increasingly involved in thermoregulation during long-term effort. Additionally, the respiratory system has been shown to have an integral role in selective brain cooling during intense exercise and in hostile (hot) ambient conditions in the horse (McConaghy, 1996). Other experiments confirm the thermoregulatory role of the respiratory system in the horse during exercise. Standardbred horses exercised in extremely cold conditions (–25°C) reduce their respiratory frequency during the early stages of exercise and recovery (Dahl et al., 1987). Ponies performing the same test in hot and humid conditions recover with a higher respiratory frequency than in dry and cold conditions (Art and Lekeux, 1988c).

During prolonged exercise in ponies, the bronchial circulation has been shown to increase progressively as core temperature increased with exercise duration (Manohar, 1990c; Manohar et al., 1992). Indeed, the bronchial arteries form a circulatory plexus in the connective tissues along the airways, the role of which is to ensure some heat dissipation. The same study shows a lesser modification in tracheal circulation, suggesting that the heat exchange occurs mainly at the level of the bronchi.

THE RESPIRATORY SYSTEM AND ITS ROLE IN ACID–BASE HOMEOSTASIS

The lung can cause rapid changes in the blood pH by regulating the elimination rate of CO_2. As blood flows through the tissues, CO_2 diffuses into the plasma and the erythrocytes, where carbonic acid forms and then dissociates into hydrogen and bicarbonate ions. This results in a decrease in the blood pH. In the lungs, CO_2 is expelled from blood and the pH increases. This explains why venous blood is more acidic compared with arterial blood.

At rest, under normal conditions, the lung eliminates the CO_2 produced, so pCO2 and the pH of the arterial blood remain relatively constant. In pathologic conditions, alveolar hypoventilation or hyperventilation may occur, resulting

either in respiratory acidosis or alkalosis, respectively (Franklin et al., 2012).

During moderate exercise, an adequate ventilatory response is achieved, and the blood acid–base status is fairly well protected. However, during heavy exercise, the horse retains CO_2, and hypercapnia caused by hypoventilation occurs, with an accompanying respiratory acidosis (Bayly et al., 1989; Franklin et al., 2012).

CONCLUSION

The high muscle mass of horses and the formidable metabolic requirements which result from their activities during heavy exercise involve a very high CO_2 production and O_2 uptake relative to the ventilatory capabilities of this animal. Some researchers indicate that the respiratory system may be the ultimate limiting factor in the racehorse. Indeed, despite the fact that numerous physiologic adjustments are brought into play, such as an enlargement of the surface of the exchanging area, an increase in cardiac output, packed cell volume, and pulmonary ventilation, arterial blood gas homeostasis is impaired in heavy-exercising horses. Although sometimes observed in elite human endurance athletes, the exercise-induced hypoxemia and hypercapnia encountered in healthy horses during strenuous exercise may be considered unique among mammals, at least those already studied.

Some anatomic and physiologic peculiarities in horses may be put forward to explain this specific adjustment to exercise. The impairment of gases diffusion, the compulsory coupling between locomotion and respiration, the high nasal resistance associated to the compulsory nasal breathing, the dramatic increase of the mechanical work of breathing and of the respiratory muscles oxygen uptake, the force–velocity characteristics and limitation of the respiratory muscles, and the fatigue of the respiratory muscles are probably major factors partly responsible for this phenomenon. Actually, ventilation during exercise in horses seems to be a compromise between these anatomic and physiologic "constraints" and the enormous metabolic needs. It probably aims at optimizing rather than at maximizing the gas exchange at the cost of blood gas homeostasis but at the profit of the energy cost of breathing.

If the mechanisms underlying the occurrence of arterial blood gas modifications begin to he progressively elucidated, the final question of knowing whether or not the gas-exchange impairment finally has a real influence on the performance capacity of our horses remains open. The limiting role of the respiratory system is probably more important in horses racing at high speed over middle distances than in horses racing over either very short or very long distances.

Undoubtedly, if heavy exercise induces changes in arterial blood gas tensions in healthy horses, a fortiori, it must dramatically worsen the gas exchange in horses suffering from subclinical respiratory disease. In this case, the resulting important gas-exchange modifications can strongly diminish the performance capability of the horse.

The value of pulmonary function tests is obvious. They help to make an accurate evaluation of respiratory function and consequently are essential in the critical examination of horses suffering from poor performance syndrome. The tests used most commonly for this purpose are arterial blood gases analysis, measurement of mechanics of breathing indices, and endoscopy of the airways at rest, during exercise, or both. Although these tests generally require sophisticated equipment that is only available in specialized units, they are essential for diagnosing subclinical respiratory disorders and for rational decision making regarding therapy.

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CHAPTER

Transport of Horses

10

DESMOND P. LEADON AND DAVID R. HODGSON

Horses have been transported from place to place for thousands of years, with records indicating that this species was moved by sea up to 3500 years ago. Horses were "caged" and usually held below deck. Even in these early times, transport resulted in the increased death rates of those horses transported below deck. In contrast, those shipped on deck generally experienced better health (Hayes, 1902). The difference was ascribed to better air quality on deck than below. By the 1800s horses were transported in horse-drawn vans, followed by rail transport in the mid-late 1800s (Creiger, 1989). This became the major mode of cross-country transport until the 1950s, although it was found that some horses travelled better than others. Therefore, it often has been assumed that transporting horses by road, sea, or air was inherently stressful. Over the recent decades, the stress arising during transport has been a subject that has attracted much more interest than in the past (Creiger, 1982). Appreciating the problems inherent in transport requires "hands on" experience. This can be difficult to obtain because transport companies are highly specialized and have little room in a competitive commercial environment for well-intentioned amateurs. Indeed, these companies retain a highly skilled cadre of individuals (grooms) who routinely participate in the transportation of horses. As a consequence of this, many people (including a significant proportion of those intimately involved with the horse industry for many years) have a very limited understanding of the horse transport industry (Leadon, 1973; Leadon, 1999; Marlin, 2004).

An understanding of this industry and its inherent problems is essential, given the prevalence of national as well as international trade and competition of horses that exists today. For example, it is common for horses in Europe and the United Kingdom to venture to the Breeders Cup in the United States, the Japan Cup, and to Hong Kong and the Magic Millions or Melbourne Cup carnivals (Australia) and other invitational races. Additionally, horses are routinely transported to the Olympic Games and World Equestrian Games and numerous similar sporting events (Leadon, 1999; Marlin, 2004)

This chapter outlines the evolution of the horse transport industry and describes some of its current practices, with particular reference to air transportation. Potential sources of stress within the transport environment are then identified, and methods of quantifying the severity of these stressors are discussed (McCarthy et al., 1990). The transport of horses necessitates their being limited to a restricted space. This confinement brings with it inherent problems in ensuring effective respiratory function (particularly, appropriate functioning of the mucociliary escalator), as well as heat dissipation and ventilation, which, if not dealt with properly, can result in a variety of adverse outcomes for the horse (Copas, 2011; Rackyleft and Love, 1989). One of the most serious is the rapid development of "shipping fever," or pneumonia or pleuropneumonia. Thus, appropriate management and veterinary care prior to, during, and after transport can help reduce the prevalence and severity of shipping fever. Therefore, the chapter concludes with a series of recommendations to horse owners and veterinarians, with the aim of optimizing the health of horses during transport (Leadon, 1999).

HISTORY OF THE TRANSPORT OF PERFORMANCE HORSES

ROAD

Land transport of the horse may have had its origins in the eighteenth century. There are various reports of the carriage of horses in custom-built horseboxes. During the reign of Queen Anne in England (1702–1714), a horse may have been carried in this way, either for a bet or to transport it to a race meeting without "tiring it." This development was accelerated by the transport of Elis, the subsequent winner of the English St. Leger, by road in 1836 (Leaden, 1973). This horse was transported several hundred miles from his home stabling to the racecourse faster than he could have traveled by being led or ridden, which was the usual custom. Massive gambling wagers were undertaken as a result of this innovation. Rail transportation became the vogue from the 1850s till the 1950s, particularly for long-distance movements. Motor vehicles were used between the 1920s and the 1950s for short-distance journeys. This form of transportation replaced rail carriage in the 1950s with the progressive evolution of sophisticated vehicles over the decades (Creiger, 1989).

AIR

The first known shipment of a racehorse by air is thought to have occurred on a biplane in the 1920s (Leaden, 1973). The first trans-Atlantic cargo flight that carried horses is believed to be one that originated, in 1947, from Shannon in Ireland bound for New York (Leaden, 1973).

SIZE OF THE PRESENT-DAY HORSE TRANSPORT INDUSTRY

ROAD

There are limited statistics that reflect the traveling activities of the competition horse industry. In excess of 250 events that represent the elite sector of the equestrian disciplines,

are held in Europe each year under the auspices of the Federation Equestre Internationale (FEI, 2012). Worldwide, the FEI sanctions more than 1200 events on all continents. Thus, on the basis of an average of at least 100 horses entered in the elite FEI-sanctioned events in Europe, this would involve more than 250,000 movements of horses for these purposes alone. It is hard to estimate the number of horse movements related to all shows or competitions for horses on an annual basis, but it appears likely that this number could be in the millions. These estimates reflect the overall extent of these activities, which, by definition, must be accompanied by the movement of horses. The racing industry in the United Kingdom documents over 9500 races per annum with approximately 92,000 starters in these races. Similar figures for Ireland show about 2400 races annually with approximately 30,000 runners. Again, this will represent a very substantial number of horse movements in this sector (Racing Post, 2012).

AIR

The true size of involvement of the air transport industry in the shipment of horses is also impossible to quantify with certainty, but many horses are transported by air. Common routes for horses to travel are from the United States to Europe, the United Kingdom, or Ireland; from South America to the United States or Europe; from the United States, Europe, the United Kingdom, Ireland, or South America to the Middle East, Southeast Asia, and the Antipodes. In the period of the massive increase in investment that occurred in the bloodstock industry in the 1980s, the activities of one Irish-based cargo airline, Aer Turas, were devoted almost entirely to the transport of more than 8000 horses by air annually. These 8000 horses were mostly those traveling between the "tripartite countries," that is, Ireland, the United Kingdom, and France. Currently, major international carriers, such as KLM, Lufthansa, and Polar, to name but three, are carrying more than 10,000 horses each year. For example, KLM cargo has 85 dedicated horse stalls for the transportation of horses in the cargo bay of their "heavy" jets (KLM, 2012). Market forces greatly influence these totals, as may be evidenced by the profound decrease in the importation of horses by air from Europe into Australia totaling more than 800 in the mid-1980s to less than 100 in 1991 (Wallace, personal communication). Similar changes have accompanied the global financial crisis of 2007 with a resultant dramatic change in the profile of horse transportation by air. However, with the progressive increase in sport horses being transported over long distances by air, the effect of the downturn has not been as profound as occurred in the early 1990s.

CURRENT METHODS OF TRANSPORT OF ATHLETIC HORSES

ROAD

Athletic horses may be carried either in trailers (floats) or in modified vans. In Europe, trailers, or "floats," are usually designed to carry two or three horses, but in the United States, similar trailers can carry six, nine, or even more horses. The chassis of heavy-goods trucks are often combined with purpose-built coachwork to provide individual stall accommodations for valuable athletic horses (Figure 10-1). These vehicles may vary considerably in appearance, internal volume, and layout.

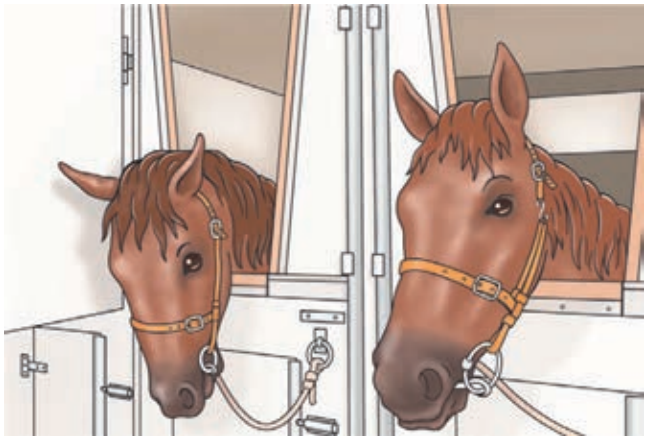

FIGURE 10-1 Horses in a European motor vehicle.

They are usually designed to carry four, six, or nine horses. There is accommodation for "grooms" (trained professional horse handlers) to travel with these horses, usually in the ratio of one groom to up to nine horses. The individual access to horses that is afforded by the vehicle design allows for the provision of food and water for the horses while the vehicle is moving. Food, in the form of hay and water, is often provided at least every 4 hours, with many variations, depending on individual cases. Journey durations can vary from a few hours to several days. The usual practice within this elite sector of the horse transport industry is for overnight rest in stables to be provided after every 24 hours of transport.

AIR

Air transport of horses utilizes either a jet-stall system, in which horses travel in a fully enclosed "air stable," or an open-stall system (Figure 10-2), in which there is a lesser degree of enclosure. The open stall system is usually utilized when the entire aircraft (which would be a freighter type in configuration) or a considerable section of it has been chartered by a horse transport agency. Today, nearly all commercial transportation involves use of jet stalls, as these restrict the movement of horses and also minimize the amount of space taken by

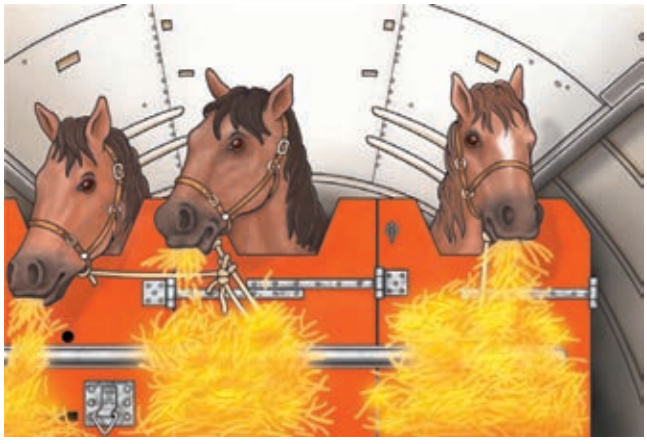

FIGURE 10-2 Horses in an open-stall system in a narrow-bodied aircraft.

each animal in the cargo bay. These stalls measure 294 cm (117") long, 234 cm (93.6") wide, and 232 cm (92.8") high, with the horse floor space being 188 cm (75.2") by 234 cm (93.6"). In a three-horse configuration, each horse has 75 cm (30"), with this being approximately 50% in a two-horse configuration. The numbers of horses that are carried in open-stall systems is determined by the type of aircraft in which they are carried and the sizes of the horses to be transported. Three horses can be accommodated across the width of a narrow-bodied airplane (e.g., a Boeing 727-200) in triple stalls. This number can be extended to four horses, provided they are relatively narrow in conformation, in quad stalls. Wide-bodied jets (e.g., a Douglas MD-11 and Boeing 747, the latter being the most common vehicle for carrying horses by air) can accommodate up to seven horses across their width.

Jet stalls can carry up to three horses, separated by partitions. There is a groom entry door and space for these personnel at the front of the stall. Over the past two decades, an insect-proof jet stall, for the proposed transport of horses through areas of the world in which insect-borne equine diseases are endemic, is in use. Jet stalls are also used in charter arrangements by airlines, but they also can be used in "combi systems," in which passengers are carried in the front of the aircraft, with the freight and the horses in the jet stalls carried in the rear. Passengers are separated from the freight section by a partition. Some transport companies will alter the configuration of the partitions in jet stalls such that their configuration may house three horses (economy class), two horses (business class), or one horse (first class). Naturally, the cost increases substantially with each elevation in class.

The normal practice of the horse air transport industry is that the ideal personnel-to-animal ratio should be one groom for every three horses on the airplane. However, this ideal is not always attainable because of the restricted number of seats available on some aircraft. Horses are usually offered hay ad libitum while the aircraft is in flight, and water is usually offered regularly (maximally every 6 hours) and following landing and during refueling stops (Leadon 1999; Marlin, 2004).

Most horses are transported without being accompanied by a veterinarian. A minority of bloodstock shipping agencies arrange for an experienced equine clinician to travel with valuable horses, for long-haul flights. The authors attest that this practice has much to be recommended. Treatment of clinical disturbances as and when they occur will generally minimize the severity of the problems that many clinicians confront when sick or traumatized horses arrive at their final destinations. Provision of veterinary care is infinitely easier in the aircraft that uses an open-stall system. In contrast, the now almost ubiquitous jet-stall systems are often too restrictive to allow anything other than the most cursory of clinical examinations. However, jet-stall systems are popular with airlines because they fit in well with modern palletized freight loading and unloading practices, thus minimizing aircraft turnaround times at airports and because they provide a rigid structure from which horses cannot escape. Jet stalls are also advantageous, as horses can be moved rapidly around the world without having to wait for specialized horse charters to be organized. Jet-stall movements are now the most common method of horse transport by air that are becoming significantly more popular than charter arrangements. As is so often the case, commercial pressures are ultimately to the detriment of animal welfare. It is usually only in the case of charter shipments of horses that provision of clinical care during transport is a viable financial proposition.

IS THE TRANSPORT OF HORSES STRESSFUL?

POTENTIAL STRESSORS WITHIN THE TRANSPORT ENVIRONMENT

Confinement, movement, noise, lack of previous exposure or experience, and the presence of exhaust or other gases, coupled with changes in air temperature, relative humidity, and the numbers of microorganisms and dust associated endotoxin within the inhaled air, are all potential sources of stress in the transport environment (Leadon, 1999; Marlin, 2004).

There is substantial evidence that the heart and respiratory rates of horses are higher during transport than at rest. The less experienced the horse is in terms of exposure to transport, the higher is the heart rate in general. For example, in horses with no previous experience in a transport vehicle heart rates up to 30 beats per minute (beats/min) higher than at rest may occur. In contrast, seasoned horses often only experience very modest increases in heart rate during transport (e.g., 5–7 beats/min). This is one of the reasons many transport companies require that horses be more mature and experienced travelers prior to air transportation. Elevations in heart rate are most likely to be highest during the initial movements of the vehicle. Similarly, during the takeoff and landing of aircraft, heart rates tend to be greater than those reported in flight (35–55 beats/min in stable flight versus 80–100 beats/min during takeoff and landing). It appears that several factors may result in these elevations in heart rate. These can include release of stress hormones, social pressures (e.g., a horse being next to another that is dominant), and increased energy expenditure. The last is likely caused by the horse having to undertake postural corrections as a result of the movement of the transport vehicle (Leadon, 1999; Marlin, 2004.)

Air temperature and relative humidity will be higher in the aircraft when it is stationary during loading or unloading and during stops for refueling than when it is airborne. The air within the aircraft while at high altitudes flows from the front to the rear of the cargo space. This air is generally much cooler (17°C–18°C) in the cargo hold than the passenger section (21°C) and has a very low relative humidity. Given this pattern of air flow, there is a temperature and relative humidity gradient from the front to the rear of the aircraft. This gradient tends to be maintained even when the aircraft is on the ground during loading and unloading or refueling stops. This occurs because the ground power units and air-conditioning systems used by stationary aircraft also promote a front-to-rear air flow. This variation in temperature and relative humidity reflects the inability of the flight deck crew to provide a uniform environment for horses in transit on fully loaded aircraft. This may be of clinical significance in that variations in environmental conditions can be an additional stressor that horses should be spared, if possible, to prevent the horses succumbing to shipping fever (Leadon, 1999).

The confinement of horses within their stalls will lead to contamination of the environment with microorganisms. Bacterial numbers within the inhaled air can be maintained at levels (while the aircraft is in flight) that are comparable with those normally found in stables. This can be accompanied by increases in the concentrations of dust associated endotoxins

in the air also. Highly significant increases in bacterial numbers and dust associated endotoxins can occur while the aircraft is stationary. These accumulations of bacteria, other microorganisms, and endotoxins will be inhaled and must be cleared from the respiratory system. Improvement of air quality is a gradual process that is achieved by the higher net air flows that occur during flight. However, the airlines may wish to limit total air flow through the cargo hold, given the cost of heating the highly chilled atmospheric air (down to –65°C) surrounding the aircraft at altitudes above 30,000 ft (Leadon, 1999).

The practice of tying horses by their head collars in transport, the presence of a front restraining bar on a jet stall, or both would result in an abnormal "head held high" posture for protracted periods. This practice has been shown to favor the spread of normal nasopharyngeal flora into the deeper respiratory tract and, thus, facilitate the development of respiratory diseases (Copas, 2011; Rackyleft and Love, 1989). This occurs as the mucociliary escalator is compromised, as the horse cannot lower its head. This escalator has been shown to be one of the major mechanisms involved in the clearance of contaminants reaching the lower respiratory tract. The challenge to the respiratory system will be increased when the number of both microorganisms and dust associated endotoxins are increased, as occurs in stationary aircraft. Thereafter, mucociliary clearance of inhaled particles is likely to be reduced as a result of the head being held high by the front bar of the jet stall and the desiccating effects of exposure to dry air and low relative humidity.

These factors have, therefore, the potential to act as stressors of the respiratory system. Similar changes in temperature with net increases in relative humidity and contamination by microorganisms occur in the road transport of horses. It is reasonable to conclude that the environment provided within aircraft and road vehicles for the transport of horses is stressful.

METHODS OF ASSESSMENT OF STRESS

Stress is a term that is easily understood, but it is an entity that is notoriously difficult to define (Fraser et al., 1975; McCarthy et al., 1990). Stress is perhaps more easily identified on the basis of its effects than by definition. Stress has been defined as occurring when an animal is required to make an abnormal or extreme adjustment in its behavior or physiology to cope with adverse effects of the environment or management. This definition is useful because it takes into account the multifactorial nature of the environmental stresses that may occur during transport, as has been described above.

Stress in horses also has been divided into "psychological stress," which is usually quantified in terms of heart rate; responses to adrenocorticotropic hormone (ACTH), cortisol, and β-endorphin; and physical or physiologic stress, which can reflect trauma, disease, or both (Fraser et al., 1975; McCarthy et al., 1990). It is important to note that different stressors invoke different responses in animals and that these responses will vary with age, physiologic status, and previous experiences.

The ultimate result of extreme stress is death. A literature search has failed to reveal any reliable mortality statistics for the road transport of athletic horses. This may be because fatality can often, but not always, be prevented in the road transport of athletic horses by stopping the vehicle and unloading, if necessary. Other methods used to estimate stress involve measurement of heart rate (as described above) and, on occasion, circulating hormone activities, particularly cortisol. In general, transportation is associated with increases in the concentration of circulating cortisol activities, although this is highly variable. Very minor changes have been reported following both road travel and air travel, with other studies showing increases of up to fourfold resting values (Marlin, 2004).

There are occasional reports of mortality resulting from frenzy in horses transported by air. The outcome of these accidents in terms of injury, rather than death, may be determined by the experience of the grooms on board, the presence of an experienced and properly equipped equine veterinarian, and the availability of suitable tranquilizers or sedatives and other forms of restraint (e.g., "cow collars"). Mortality, then, would appear to be an insufficiently sensitive indicator of transport stress in horses (Leadon, 1999).

MANIFESTATIONS OF STRESS

Behavior and Orientation in Relation to the Direction of Movement during Road Transport

It has been suggested that positioning of horses to face the rear in two-horse trailers and in horse trucks or vans may result in more relaxed behavior than when placed to face forward. However, more recent evidence indicates that this may not be the case, with a broad interpretation being that orientation had no major effects on physiology; despite this, body orientation during transport may be an important stressor for some, although not all, horses (Clark et al., 1991).

Changes in Laboratory Measurements in Horses Transported by Road

It has been reported that road transportation over distances of 130 to 350 km resulted in significant elevations in serum creatinine and creatine kinase (CK,) aspartate amino transferase (AST), lactate dehydrogenase (LDH), alanine aminotransferase (AAT), and serum alkaline phosphatase (SAP). These changes appear, however, to be dependent on the experience of the horses, as other studies have shown no changes in these muscle related enzymes in response to 14 hours of road transport. Total white blood cell count has been reported to occur in response to road transport, although this is by no means routine. Similarly, increases in packed cell volume (PCV) and total plasma protein (TPP) concentration may occur in response to road transport. PCV is likely to rise in response to "stress" with concurrent splenic contraction. In contrast, TPP is likely to increase in direct relationship to the degree of dehydration present. As a result, horses that have regular access to food and water during transport are usually able to maintain body fluid homeostasis. Thus, in summary, the reported changes in laboratory measurements in horses that are transported by road are generally unlikely to be of clinical significance (Marlin, 2004).

Changes in Laboratory Measurements in Horses Transported by Air

Post-flight elevations in total white cell counts, particularly due to neutrophilia and fibrinogen concentrations have been variably reported by 2 days following prolonged air transport. These changes may be reflective of otherwise inapparent responses to confinement and transport. Although other evidence indicates that these changes are by no means ubiquitous following all air transportation. There is, however, clear evidence that

horses showing clinical signs of postflight pneumonic processes routinely demonstrate elevations in these two variables—a direct reflection of an acute inflammatory response (Leadon, 1999; Marlin, 2004).

Effects on Respiratory and Enteric Health Status

Stress as a result of transport may be a predisposing factor in the development of respiratory and enteric disease. Certainly, these body systems are the most frequently affected in response to transport. Predisposition to respiratory disease after transport is likely caused by a variety of factors. Prolonged head elevation, which is common in jet stalls or in horses with the head tied up, is a major contributing factor. This probably results from diminution of the efficacy of the mucociliary escalator and increased concentrations of irritants in the inspired air. This may be compounded by impaired nonspecific immune function of the lungs (e.g., alveolar macrophage downregulation) but is by no means a reproducible finding (Copas, 2011; Leadon, 1999).

Jet Lag

Humans have a propensity to anthropomorphize, and as such, there are various lay references to jet lag in horses. This is not surprising as jet lag is a common problem in elite human athletes who cross many time zones in the lead up to major competition. Thus, horses are ascribed this diurnal disturbance also. There is no clear evidence of jet lag occurring in horses; however, equally, there is no evidence definitively precluding its occurrence. One study showed a reversal of the normal diurnal body temperature pattern in horses that crossed six time zones in 9 hours (Marlin, 2004). Another study showed improvement of athletic performance in horses subjected to experimental jet lag (Tortonese et al., 2011). Of course, these are preliminary and limited studies, which, in the authors' opinions, are in no way conclusive of the presence of jet lag.

Effects on Subsequent Performance

Short-term (up to 4 hours) transport in trailers or vans has been found to have relatively little effect on performance. This is highlighted by this method of transport being used routinely in the racing industries, with no evidence of reductions in performance. Conversely, the effects of air transport are extremely difficult to quantify. Dehydration occurs as a routine outcome of air transport, particularly if food and water are not offered regularly. Horses that do not eat or drink sufficiently may lose >25 kg in response to transport. Certainly, there is some evidence to indicate that those racehorses that have been successful in their target race on arrival after long-distance air transport have tended to lose less body weight (<10 kg) in transit than those which have raced poorly (>20 kg weight loss) (Leadon, unpublished data).

MANAGEMENT AND VETERINARY CARE OF HORSES IN TRANSIT

PRIOR TO TRANSPORT

The horse transport industry does not usually make provision for the veterinary care of horses in transit. Most professional horse transport companies do have protocols they follow to minimize health problems in horses being transported. However, veterinarians are occasionally given the opportunity to provide this care for individual horses or groups of horses of high value. Where this care or just general advice is sought, a number of measures are appropriate. The health status of horses should be checked prior to and on the day of transport, with special reference to the identification of subclinical respiratory disease. This measure is advisable because it can help identify horses that would be more likely to succumb to shipping fever compared with their healthy counterparts. Appropriate therapy can then be initiated and the response to this therapy can be identified prior to departure. Clinical judgment on maintenance of therapy during transport, if appropriate, can then be exercised. The shipment of horses with existing clinical respiratory disease is contraindicated unless warranted by a need for emergency hospitalization because of other considerations such as limb fracture and so on. Unnecessary medication such as the administration of "prophylactic antimicrobial therapy" without any evidence of respiratory disease and the unjustified use of sedatives should be avoided (Leadon, 1999).

Special dietary provisions are not required for horses being transported short distances. It is the usual practice of the horse transport industry to provide horses with a light laxative diet, for example, a series of bran mashes, prior to medium- or long-haul journeys. Care should be exercised in the administration of laxatives such as liquid mineral oil (paraffin) prior to these longer journeys. Excessive fluid loss through feces is clearly contraindicated where dehydration in transit can occur easily. Generally, during air transportation, horses are only fed high-quality hay. Naturally, a period of acclimation to this diet prior to transport is strongly indicated. Some professional transportation companies and their grooms will restrict feed for several hours prior to taxiing and take off. Hay will be offered immediately prior to these activities in an attempt to distract the horses during this more stressful period of the flight.

Efforts should be made to preserve the best attainable standard of air hygiene for horses in transit. Moldy hay should not be provided. Even good-quality hay should be well shaken or passed through a vacuum-driven particle remover to minimize the workload of the horse's mucociliary clearance mechanisms in the confined transport environment. Preparation of hay in hay steamers prior to transport is also likely to be of benefit, as these machines lower the dust load (and, thus, dust-associated endotoxin) in hay and kill nearly all the microorganisms in the hay.

DURING TRANSPORT

Figure 10-3 illustrates the factors involved in the development of shipping fever. Hay should be provided on an ad-libitum basis throughout the journey, and water should be offered at least every few hours. Delays in transport should be avoided, and on road journeys, overnight rest away from the vehicle should be provided to enable the horses to lower their heads to facilitate mucociliary clearance.

Clinicians who accept responsibility for the health care of horses in transit by accompanying them on a journey must make adequate preparations to fulfill this responsibility. Road transport vehicles can be diverted to the nearest veterinary hospital and clinic if the need should arise, but the available facilities may not always be either sufficient or appropriate for the needs of athletic horses. It is, therefore, advisable for clinicians to carry adequate veterinary supplies when accompanying horses transported by road or air (Leadon, 1999).

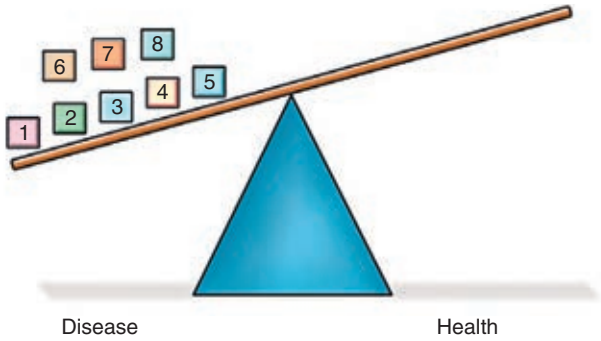

FIGURE 10-3 Schematic illustration of some of the factors that are involved in the development of shipping fever. 1 = pre-existing respiratory disease; 2 = surges in T° and RH %; 3 = increasing numbers CPU in air; 4 = immunocompromise: 5 = dehydration; 6 = delays; 7 = prolonged exposure; 8 = other unknown factors.

Provision should be made to deal with the occurrence of frenzy on repeated occasions among the horses included in the shipment. Supplies for the repair of injury and treatment of respiratory disease and colic are also essential. Although there is a popular perspective that a gun should be used in emergency situations at altitude, few airline captains or transportation authorities will endorse the use of a free bullet within the airframe at any time. The advent of modern, potent equine tranquilizers, sedatives, analgesics, and anesthetics has, in the authors' experience, obviated the requirement for the use of a firearm.

The clinician must be aware of the fact that as well as having a responsibility for the horses, there is also, particularly at altitude, a responsibility for the welfare and safety of all the personnel on board. Heroic attempts at manual restraint of the occasional cases of frenzy that occur in horses in transit by lay staff devoted to their horses should be discouraged, even if the administration of a tranquilizer may result in the disqualification of the horse in subsequent competition.

AFTER TRANSPORT

Shipping fever is not just restricted to long-distance travel; it can occur after short journeys, although less frequently. In ideal circumstances, clinical evaluation should be carried out for all horses after medium- or long-haul journeys. Horses should be bright and alert, and they should drink and exhibit interest in palatable feed soon after arrival. Signs of depression and inappetence with the presence of a soft cough, shallow frequent respirations, and a febrile response are classical signs of shipping fever (Copas, 2011). However, the febrile response can be variable, with a relatively high proportion of horses affected until 2 days or sometimes even 3 days after arrival (Leadon, 1999). It is, therefore, advisable to record rectal temperature morning and evening for at least 3 days after arrival following long journeys. Veterinary advice should be sought at once by horse owners or managers if any of the above signs are noted within this period after transport.

TREATMENT OF SHIPPING FEVER

This section provides a brief outline of strategies available for the management of shipping fever. Prompt, appropriate treatment will result, in many cases, in a full recovery from this disorder (Copas, 2011). However, this condition should always be considered a life threatening one, as fatalities do occur. The more rapidly the disease is diagnosed and treatment instituted, the more likely it is that there will be a successful outcome. The disease results from the lung defense mechanisms being overwhelmed by aspiration of oropharyngeal bacteria. Clinical signs are often subtle but very helpful in identifying the disorder, as mentioned above. Hematology and blood biochemistry can be useful diagnostic and prognostic aids in cases of pleuropneumonia. Thoracic ultrasonography, with particular focus on pleural surfaces, the pleural space, and the lung parenchyma, is regarded as a routine diagnostic method. A tracheal aspirate using a guarded catheter system via endoscope for cytological, and possibly bacterial, culture is ideal but not always practical. Thoracocentesis is indicated where there is marked pleural effusion. Acquisition of fluid also enables cytologic, biochemical, and microbial examination or culture to be performed. Some clinicians prefer to repeatedly drain the chest, whereas others insert drains that remain in place for days. Both approaches appear to be well tolerated in most cases. Mixed infections are common, involving both aerobes and anaerobes. It is, therefore, reasonable for clinicians to initiate a regimen of β-lactamase and aminoglycoside antimicrobial therapy (while awaiting culture and sensitivity results). Simultaneous anti-inflammatory therapy can be very helpful, and metronidazole should be incorporated into the therapeutic regimen where involvement of anaerobic organisms is suspected or confirmed.

POST-TRANSPORT RECOVERY PERIOD

Objective monitoring of the post-transport recovery period is a difficult task. There is considerable individual variation among horses in terms of responses to transport (Leadon, 1999; Marlin, 2004). Although it may be thought that laboratory based assessments are the most useful criteria for monitoring recovery from a journey, this is not the case in practice. Research and experimental studies in which horses are transported from their place of origin and returned to it after journeys of variable duration permit use of standardized methods of laboratory evaluation. In the real world, laboratory tests carried out prior to departure are often not comparable with those available on arrival at some distant final destination.

The subjective judgment of experienced personnel who are familiar with the horses in their care is often the best method of evaluating recovery after a journey in normal, healthy horses. However, horses should, if possible, be weighed prior to transport. The measurement and recording of body weight gain after the initial loss that is associated with transport is a useful adjunct to monitoring recovery in both healthy horses and (when combined with rectal temperature, clinical examination, laboratory data, and ultrasonography) in those that have been affected by shipping fever. Dehydration is common after transport, with some affected animals benefiting greatly from intravenous administration of 15 to 30 L of polyionic replacement fluids over several hours. Fluids may also be given via nasogastric tube, but this presumes normal gastrointestinal motility (Marlin, 2004).

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CHAPTER

11 The Cardiovascular System: Anatomy, Physiology, and Adaptations to Exercise and Training

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The cardiovascular system is a transport system consisting of a muscular pump, the heart, and a network of blood vessels that contain blood. Its principal function is transport of water, oxygen, carbon dioxide, fuels for energy production, electrolytes, hormones, and metabolic products. The cardiovascular system of the horse is specifically designed for exceptional transport of oxygen from the lungs to body tissues.

Horses have a high maximal oxygen consumption ($\dot{V}O_{2max}$) relative to body weight compared with most other mammals. The superior oxygen transport of the horse is attributed to its specialized spleen, which is able to add an extra volume of red blood cells (RBCs) to the circulation when it contracts after the stimuli of fear, excitement or exercise. This infusion of erythrocytes increases the oxygen-transport capacity of arterial blood and enables horses to greatly increase $\dot{V}O_{2max}$ during exercise. The stroke volume (SV) of blood pumped with each cardiac contraction is over 1 liter (L) in trained horses, and maximal rates of blood flow during exercise are likely to be up to approximately 400 liters per minute (L/min) in the most exceptional equine athletes. The structure and function of the equine cardiovascular system are, therefore, fundamental to the superior athletic performance of the horse.

ANATOMY AND BASIC PHYSIOLOGY

The heart's role is to pump sufficient blood to maintain blood pressure and oxygen flow to tissues. The anatomy of the equine heart is similar to that found in other mammals (Figure 11-1). The layout of the heart and circulatory system is illustrated in Figure 11-2. The thick-walled left ventricle pumps a stroke volume of blood into the aorta with each contraction. This establishes a wave of accelerated blood flow through the systemic arteries. Blood flow in arteries is continuous because relaxation of the elastic arterial walls compresses the blood in the vessel, forcing it away from the heart toward tissues.

The pressure of blood in the arteries depends on the rate of blood flow, or cardiac output, and the amount of resistance to the flow. The main regulator of resistance is the degree of constriction or dilation of arterioles. These vessels also regulate the rate of blood flow through the downstream capillaries, where oxygen diffuses from the hemoglobin in RBCs to the mitochondria in tissue cells for the support of aerobic metabolism. Blood returns to the right atrium and ventricle via the venous system. Venous blood flow depends greatly on muscular contractions, which compress the thin-walled veins resulting in a pumping mechanism. Changes in air pressures within the abdomen and thorax during breathing also assist venous return. Blood is then pumped to the lungs by the right ventricle via pulmonary arteries. This enables removal of carbon dioxide (CO_2) and reoxygenation of blood for transport to working muscles.

The heart mass in Thoroughbred horses averages about 4 to 5 kilograms (kg), or 1% of body weight. Trained horses have slightly higher relative heart masses (1.1%) compared with untrained horses (0.94%), which seems to suggest that training causes hypertrophy of cardiac muscle (Evans, 2007; Kubo et al., 1974; Marr and Burton, 2010). These changes appear to occur soon after the onset of training, and this is highlighted by the finding that there is no difference in heart mass between horses in training for 2 months and those in training for 19 months. Heart mass to body weight ratio is also a function of breed. Racing horses have a relative heart mass of 0.86, compared with 0.76 in Arabian horses and 0.62 in draft horses (Kline and Foreman, 1991).

CARDIAC CYCLE

The cardiac cycle is the sequence of events occurring in the heart during every contraction (systole) and relaxation (diastole). The sequence and timing of blood pressures and valvular events in the equine heart and major arteries at rest are illustrated in Figure 11-3. The cyclic nature of cardiac activity depends on normal conduction of electrical impulses from the sinoatrial (SA) node, or pacemaker, through the atrial and ventricular myocardium. The conduction of impulses in the equine heart is illustrated in Figure 11-4. The diffuse distribution of Purkinje fibers through the left ventricular wall enables rapid depolarization and development of muscular tension. However, right ventricular contraction slightly precedes left ventricular contraction. This is so because pulmonary arterial pressure is lower than aortic pressure (Holmes, 1982; Poole and Erickson, 2004).

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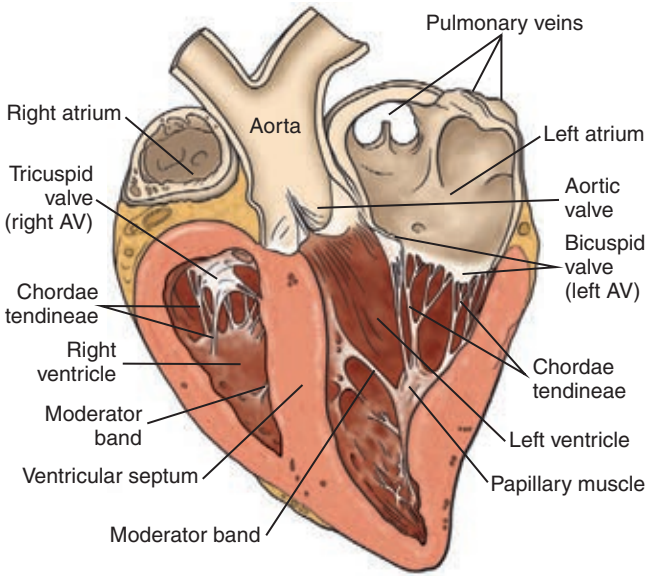

FIGURE 11-1 Cross-section of the equine heart showing the cardiac chambers and valves and direction of blood flow.

Abnormal blood flow through the heart can cause murmurs and possibly poor performance. Further discussion on this can be found in publications on equine cardiology (e.g., Marr and Burton, 2010).

CARDIOVASCULAR ADAPTATIONS TO EXERCISE

HEART RATE

Heart Rate in the Resting Horse

Heart rate in the resting horse depends mainly on the degree of relaxation of the individual horse. In relaxed horses, resting heart rate is usually in the range 25 to 40 beats per minute (beats/min). At night, when horses are relaxed or sleeping, heart rates tend toward the lower end of this range. Sudden excitement, fear, or anticipation of exercise can elevate heart rate rapidly to over 100 beats/min. Rapid heart rate changes in the range of 20 to 110 beats/min in resting horses can be explained entirely by alterations in parasympathetic nerve activity (Evans, 2007; Hamlin et al., 1972).

It has been suggested that resting heart rate is lower in fit horses than in unfit horses (Evans, 2007; Littlejohn, 1987;

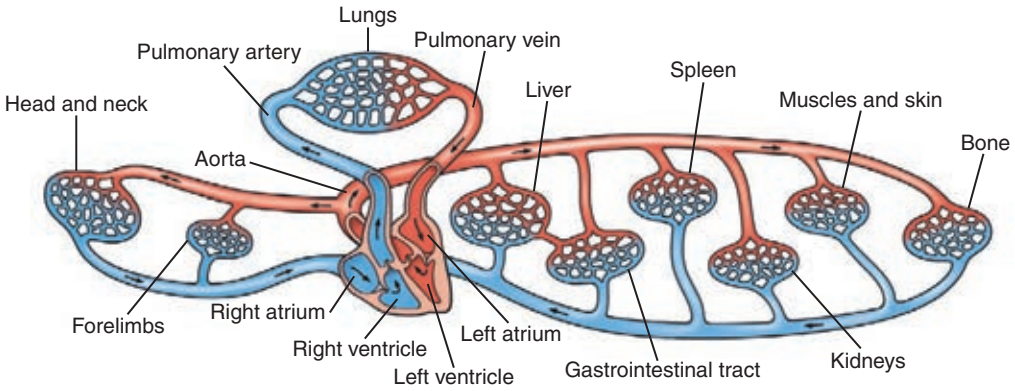

FIGURE 11-2 The cardiovascular system illustrating the blood flow to and from the main body systems.

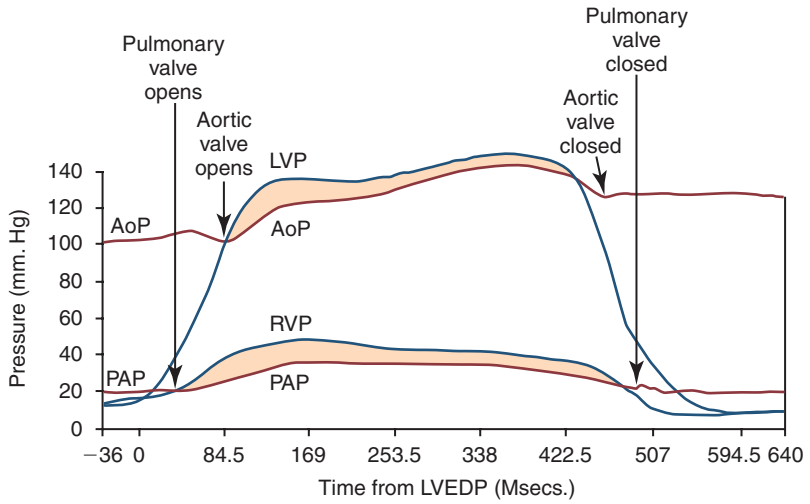

FIGURE 11-3 Aortic pressure (AoP), left ventricular pressure (LVP), pulmonary artery pressure (PAP), and right ventricular pressure (RVP) recorded simultaneously with intravascular transducers (*LVEDP*, left ventricular end-diastolic pressure). Ejection begins earlier on the right side and lasts longer than on the left. The shaded areas represent the pressure gradients from the ventricles to the arterial systems.

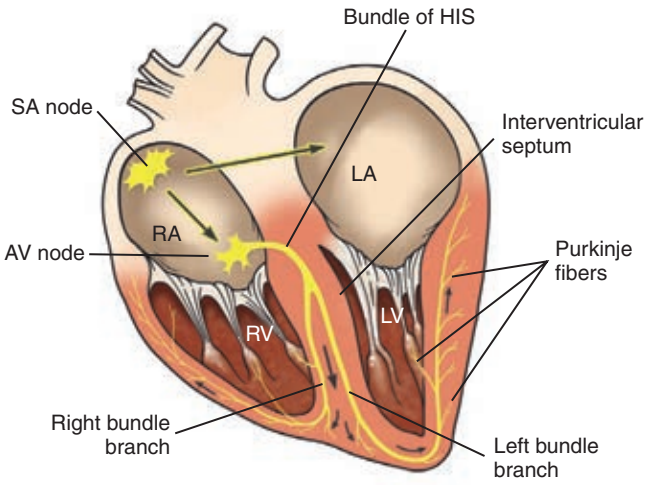

FIGURE 11-4 Conduction pathway of the equine heart.

Marr and Burton, 2010). However, resting heart rate in the horse generally does not decrease after training, as in human athletes (Bayly et al., 1983; Marr and Burton, 2010; Milne et al., 1977; Poole and Erickson, 2004; Skarda et al., 1976; Thomas et al., 1983). Use of heart rate measurements to monitor fitness is, therefore, restricted to measurements during or after exercise.

Measurement of Heart Rate during Exercise

Heart rate measurements during exercise in athletic horses have been used to describe the intensity of work, to measure fitness, and to study the effects of training and detraining. There are several suitable commercial heart rate meters designed for use in exercising horses (Courouce et al. 2002; Evans, 2007; Evans and Rose, 1986; Foreman and Rabin, 1984; Marr and Burton, 2010; Physic-Sheard et al., 1987). Heart rate during exercise also can be monitored electrocardiographically or digitally. The electrocardiogram (ECG) can be obtained by wiring the horse directly to the recorder, recording the ECG digitally for examination at a later time, or by radio telemetry (Evans, 2007; Marr and Burton, 2010; Patterson, 1996; Young, 2004).

Commercial heart rate meters usually employ two or three electrodes incorporated into a belt or placed on the chest beneath the saddle. Such heart rate meters enable exercise testing to be performed under racetrack conditions, facilitating assessment of the response to exercise and prescription of specific exercise loads during exercise. Care must be taken to ensure that there is no faulty contact of the surface electrodes with the horse's skin. However, under ideal conditions, the repeatability of heart rate measurements obtained during treadmill exercise is very high (Evans and Rose, 1988a; Seeherman and Morris, 1990; Evans, 2007; Young, 2004; Marr and Burton, 2010).

Heart Rate at the Start of Exercise

At the onset of exercise, heart rate quickly increases and reaches a steady state in 2 to 3 minutes. This increase is associated with increased sympathetic nerve activity, catecholamine release, or both (Evans, 2007; Marr and Burton, 2010; Poole and Erickson, 2004; Young, 2004). Steady-state heart rate remains constant during submaximal workloads (Engelhardt, 1977; Evans 2007; Marr and Burton, 2010; Young, 2004). An overshooting of heart rate to values above the submaximal steady-state heart rate may occur at the commencement of exercise (Persson, 1967; Persson and Lydin, 1973; Young 2004). Mean time taken to reach maximal heart rates after onset of exercise in Thoroughbreds was 22 seconds (Krzywanek et al., 1970; Young 2004). In Standardbreds trotting at speeds of 12 to 12.5 meters per second (m/s), heart rates were not maximal until at least 700 m had been run (Courouce et al., 2002; Lindholm and Saltin, 1974).

The kinetics of heart rate at the commencement of exercise without prior warmup is also dependent on the intensity of exercise (Evans 2007; Poole and Erickson, 2004). In six Standardbred horses, the typical overshoot was found at the start of exercise at 50% of $\dot{V}O_{2max}$, but at 100% percent $\dot{V}O_{2max}$, heart rate gradually increased during a 5-minute period of exercise (Evans, 2007; Evans and Rose, 1988b; Poole and Erickson, 2004) (Figure 11-5). These data emphasize the importance of a suitable warmup prior to competition in horses, since oxygen consumption also increases more rapidly at the commencement of exercise if there has been prior warmup (Evans 2007; Rose and Evans, 1987; Young 2004).

Heart Rate during Submaximal Exercise

A linear relationship between heart rate and submaximal work effort has been observed in horses trotting, galloping, and swimming (Ehrlein, Hornicke, Engelhardt, 1973; Lindholm and Saltin, 1974; Maier-Bock and Ehrlein, 1978; Marr and Burton, 2010; Persson, 1967; Persson and Lydin, 1973; Poole and Erickson, 2004; Senta, Smetzer, Smith, 1970; Thomas, Fregin, Gerber, 1980; Thomas and Fregin, 1981; Young 2004;). Many factors influence the position of the regression line of heart rate on work speed. These include gait, (Littlejohn et al., 1977), length of the exercise track (Ehrlein et al., 1973), and treadmill slope (Sexton and Erickson, 1990), and the position is also influenced by the presence of a breathing mask (Persson, 1983). The heart rate at a specific working velocity can vary markedly between individual horses, but if standardized

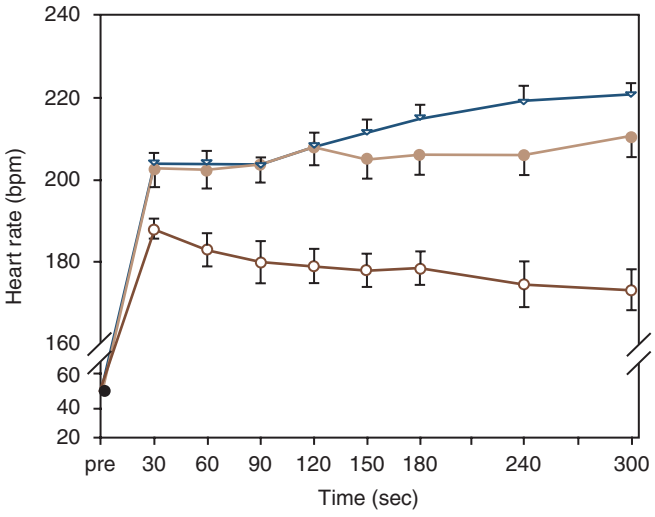

FIGURE 11-5 Heart rate at the commencement of three different intensities of exercise without prior warmup in Standardbred racehorses (*0*, 50%; •, 75%; ▼, 100%).

treadmill exercise tests are used, the relationship between heart rate and work is very precise and reproducible for individual horses at heart rates between 120 and 210 beats/min (Courouce et al., 2002; Ehrlein et al., 1973; Evans, 2007; Evans and Rose, 1988a; Marr and Burton, 2010; Poole and Erickson, 2004; Young 2004).

This relationship is usually defined by the use of treadmill tests that involve increasing the speed or, less commonly, treadmill angle and measuring heart rate at the completion of 1 to 2 minutes of exercise at each speed. After a suitable warmup, for example, 3 minutes of trotting, heart rate is stable after 1 minute of further exercise at higher speeds. Figure 11-6 illustrates heart rates recorded during the last 15 seconds at each speed in a fit Thoroughbred racehorse during a treadmill exercise test. More recently, information relating heart rate responses in field conditions have been obtained by the use of onboard heart rate monitors with speed of exercise determined by GPS (global positioning system) tracking (Evans, 2007).

The heart rate–workload relationship is also affected by disease states because horses with recurrent airway obstruction (RAO) have submaximal exercise heart rates that are significantly higher than those found in normal horses (Littlejohn et al., 1977; Littlejohn et al., 1983; Young, 2004). Heart rate during standardized submaximal exercise is also higher in horses with cardiac disease such as atrial fibrillation (Evans, 2007; Young, 2004).

The time to fatigue during treadmill exercise is dependent on the intensity of exercise. At heart rates of 170 to 180 beats/min, seven partially trained Thoroughbreds were able to exercise for an average of 24 minutes, whereas at a heart rate of 208 beats/min, equivalent to 100% $\dot{V}O_{2max}$, the horses fatigued in 4 minutes (Hodgson et al., 1990). This relationship translates to field conditions (Courouce et al., 2002; Evans, 2007).

Heart rates during swimming vary greatly between horses and are usually in the range 130 to 180 beats/min (Murakami et al., 1976; Patterson, 1996; Poole and Erickson, 2004; Thomas Fregin and Gerber, 1980; Young 2004). The mean highest heart rate recorded in nine horses during show jumping was 191 ± 3 beats/min (Art et al., 1990; Marr and Burton, 2010; Young, 2004).

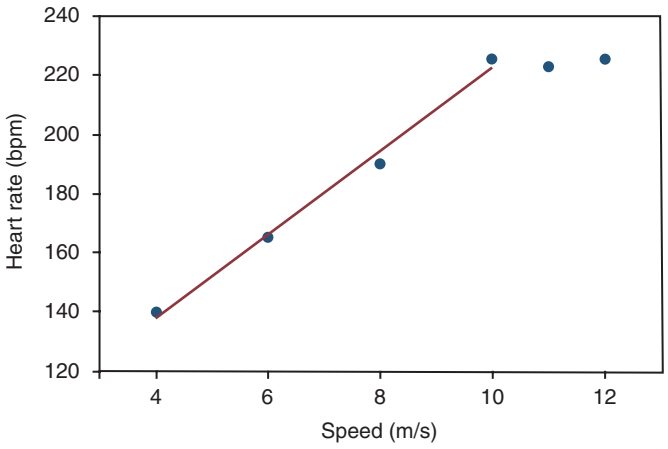

FIGURE 11-6 Heart rates during a stepwise incremental treadmill exercise test in a race fit Thoroughbred racehorse. The horse exercised for 3 minutes at 4 meters per second(m/s), 2 minutes at 6 m/s, and 1 minute at 10, 11, and 12 m/s. Heart rates were recorded by telemetry electrocardiography during the last 15 seconds of each speed. The horse was unable to keep pace with the treadmill when the speed was increased to 13 m/s indicating fatigue and completion of the exercise test.

During prolonged strenuous submaximal exercise at a constant work rate, a gradual increase in heart rate, or cardiovascular drift, can occur. For example, during 30 minutes of constant load exercise, mean heart rate increased from 154 to 173 beats/min. This drift was accompanied by increases in minute ventilation and cardiac output, while stroke volume was unchanged (Evans, 2007; Poole and Erickson, 2004; Thomas and Fregin, 1990). In another study, horses exercising at 55% to 60% of individual maximal heart rate (HR_{max}) for 60 minutes had minimal changes in heart rate (Hinchcliff et al., 1990). Heart rate during prolonged exercise in horses probably depends on the intensity of exercise, environmental conditions, and possibly fitness (Evans, 2007; Young, 2004).

Maximal Heart Rate

A loss of linearity of the heart rate on velocity regression line is typical at high work speeds (see Figure 11-6). HR_{max} is defined as the highest heart rate measured in an incremental-speed treadmill test, which results in a plateau of heart rate. If a plateau is not demonstrated, the highest heart rate recorded in an incremental-speed treadmill exercise test is referred to as the *peak heart rate*. Alternatively, HR_{max} can be measured in horses after 1 minute of maximal exercise after suitable warmup (Courouce et al., 2002; Evans, 2007).

HR_{max} can vary considerably between horses. Maximal heart rates recorded during racing in 19 Thoroughbreds averaged 223 beats/min, with a range of 204 to 241 beats/min (Evans, 2007; Krzywanek et al., 1970; Patterson, 1996; Poole and Erickson, 2004; Young, 2004). High individual variability in heart rates while racing also have been recorded in Standardbreds, ranging from 210 to 238 beats/min, with a mean of 221 beats/min (Åsheim et al., 1970; Courouce et al., 2002; Evans, 2007).

In humans, HR_{max} declines with age (Åstrand and Rodahl, 1977). There is no predictable relationship between age and HR_{max} in horses. Mean peak heart rate in eight yearling Thoroughbreds was approximately 240 beats/min, compared with 220 to 230 beats/min in 2- to 4-year-old horses (Rose et al., 1990). Likewise, yearling, 2-year-old, and adult Thoroughbreds had similar means (229 to 231 beats/min) and ranges (215 to 254 beats/min) of peak heart rates during an incremental treadmill exercise test (Seeherman and Morris, 1991). Evans (2007) and Courouce et al. (2002) reported similar values for horses working in the field.

The individual HR_{max} is a highly repeatable measurement in individual horses (Courouce et al., 2002; Evans, 2007; Evans and Rose, 1988a), but it is not an important measure of fitness, since it is not affected by training, despite increases in $\dot{V}O_{2max}$. (Courouce et al., 2002; Evans and Rose, 1988a; Evans, 2007; Seeherman and Morris, 1991).

The treadmill speed at which HR_{max} is achieved (V-HR_{max}) during a stepwise test is significantly correlated with $\dot{V}O_{2max}$ and is, therefore, a suitable measurement of fitness in treadmill tests that do not measure oxygen consumption (Evans and Rose, 1987). This correlation occurs under field conditions, as is well described by Evans (2007), Courouce et al. (2002), Marr and Burton (2010). In addition, the relative heart rate (as a percentage of HR_{max}) is highly correlated with relative

oxygen consumption in horses during treadmill and field exercises (Evans, 2007; Evans and Rose, 1987) (Figure 11-7).

Submaximal Exercise Heart Rates and Fitness Measurements

Heart rate measurements during submaximal treadmill and field exercise have been expressed relative to treadmill speed in some studies for measurement of fitness. For example, the treadmill velocities that result in heart rates of 140 (V_{140}) or 200 beats/min (V_{200}) have been used. The V_{200} is calculated by measuring the heart rate at the end of three to four treadmill or racetrack runs, each of which results in heart rates between 120 and 210 beats/min (Courouce et al., 2002; Evans 2007; Persson, 1983; Persson and Ullberg, 1974). Evans (2007) described the application, over the past decade, of these measurements under field conditions by using onboard heart rate meters and telemetric respiratory analysis, with ground speed determined by GPS technologies.

As previously mentioned, V-HR_{max} also may be a useful measure of fitness. This value is obtained by substituting HR_{max} in the regression equation describing the linear heart rate on velocity relationship during submaximal exercise. Figure 11-8 shows the method of calculating V_{200} and V-HR_{max}. Evans (2007) demonstrated the direct application of this technology in the training of horses for athletic pursuits.

The first reports of using trained Standardbred racehorses exercise-tested on a racetrack to calculate V_{200} were published in the 1980s (Evans, 2007). Rest periods after each step of a test or in response to a bout of exercise allow for determination of venous blood lactate and for calculation of HR_4, another suggested index of fitness. This measurement refers to the heart rate at which blood lactate is 4 millimoles per liter (mmol/L). It is generally higher in fitter horses. Evans (2007) demonstrated how modern technologies can be adapted to in-field testing of horses for determination of many of these variables.

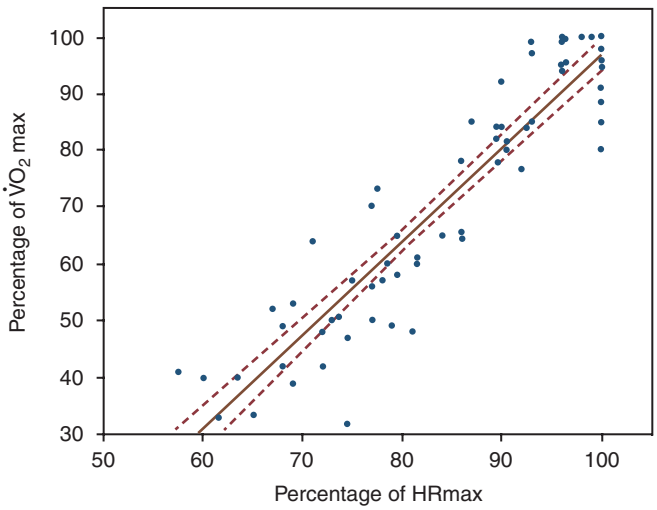

FIGURE 11-7 Relationship between percentage of maximal heart rate and maximal oxygen consumption in fit and unfit horses. (*Evans DL, Rose RJ: Maximal oxygen consumption in racehorses: changes with training state and prediction from submaximal indices of cardiorespiratory function. In Gillespie JR, Robinson NE, editors:* Equine exercise physiology 2, *Davis, CA, 1987, ICEEP Publications, p 52.*)

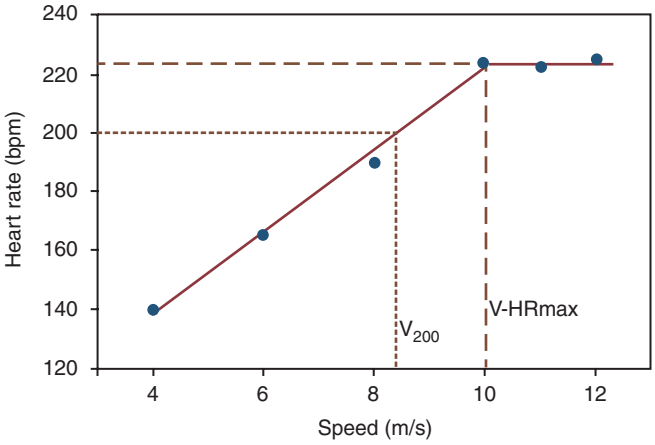

FIGURE 11-8 Calculation of maximal heart rate (V-HR_{max}) from the results of a stepwise incremental-speed exercise test.

A similar racetrack exercise test was used to investigate the relationship between several measures of fitness and maximal trotting velocity in Swedish trotters. Both V_{200} and the heart rate after 4000 m trotting at 10 m/s (HR_{10}) were significantly correlated with maximal trotting velocity over 1000 m. Respective correlations were 0.6 and 0.74 (Persson and Ullberg, 1974). Courouce et al. (2002) and Evans (2007) demonstrated similar relationships in French Standardbred trotters and Thoroughbred racehorses, respectively.

A decrease in indices such as V_{140} or V_{200} indicates that the heart rate is abnormally elevated during submaximal exercise such as trotting and slow cantering (Courouce et al., 2002; Evans, 2007). This finding in a horse in training could suggest loss of cardiovascular fitness, cardiac or pulmonary disease, lameness, or overtraining.

Measurements such as V_{200} are most useful for comparing an individual or group of horses with itself over time (Evans, 2007). Caution should be exercised if V_{200} is used to compare different horses, since the HR_{max} can vary greatly between individuals. At a heart rate of 200 beats/min, horses with HR_{max} of 215 and 245 beats/min are exercising at 93% and 82% of their respective maximal heart rates (Evans, 2007). The relative work rates are, therefore, quite dissimilar. In addition, there is no evidence that V_{200} is superior to measurement of one heart rate at a set treadmill or ground speed. For example, the heart rate response to treadmill exercise at 8 m/s on a treadmill after a standardized warmup would probably give the same information as V_{200}, obviating the need for multiple exercise steps and interpolation of a regression line to the heart rate of 200 beats/min. Similar correlations exist in horses working on the track (Courouce et al., 2002; Evans, 2007).

A racetrack fitness test based on telemetric heart rate measurements for racing Standardbred horses was described in the 1960s (Marsland, 1968). This test is easier to conduct, and the results have correlated with racetrack performance. It does not require measurement of heart rate during three to four runs and subsequent calculation of velocity at a set heart rate. The horses were jogged 3 miles, then exercised for 1 mile in 170 seconds as a warmup, and then after 60 minutes of rest were exercised over 1 mile in 150 ± 1 second. The mean heart rate in 22 horses thus tested was 202 beats/min after one

quarter of a mile and 212 beats/min at the end of the test run. The heart rate during the last quarter mile was highly correlated with fastest winning time ($r = 0.9$; $p < 0.01$).

A racetrack exercise test to measure V_{170} in ridden horses also has been described (Cikrytova et al., 1991). Horses were exercised over 800 m at constant speeds of 220, 270, 360, 450, and 540 m/min, and heart rates were recorded telemetrically. V_{170} differed significantly between breeds and was highly reproducible, but there was no relationship between this measurement and a subjectively derived assessment of the performance of 339 Czech Warmbloods in cross-country races.

Heart Rate Recovery after Exercise

Heart rate recovery is usually very rapid in the first minute after exercise stops (Banister and Purvis, 1968; Evans, 2007; Hall et al., 1976; Marsland, 1968; Persson, 1967; Young 2004). It then decreases more gradually toward normal resting values. It is, therefore, not possible to determine the heart rate during exercise by evaluating heart rate after exercise in horses.

Recovery heart rates after a standardized exercise test on a track were only moderately correlated with the fastest winning time in Standardbred horses ($r = 0.34$–0.51), with the highest correlation found at 5 minutes after exercise (Marsland, 1968). The author of this study also points out that the test should be carried out as part of the normal training routine and in familiar surroundings and that recovery heart rates are notoriously susceptible to rapid increases because of excitement.

Recovery heart rates are used to assess the fitness of horses competing in endurance rides. Endurance riders should, therefore, ensure that horses to be used in competition are accustomed to the approach of strangers and to cardiac auscultation. Poorly performing endurance horses have higher postexercise heart rates compared with better performers (Cardinet et al., 1963). Horses with heart rates less than 60 beats/min 30 minutes after exercise were found to show less evidence of dehydration and myopathy (Rose et al., 1977). Horses with heart rates greater than 65 to 70 beats/min at the 30-minute recovery time at the mid-point of an endurance ride often develop severe dehydration and exhaustion if they are allowed to continue (Rose, 1983). These results have been adapted to modern endurance racing with strict heart rate recovery required for horses undertaking sanctioned events. Readers are referred to Chapter 22 in which training the Endurance horse is discussed.

It has been suggested that recovery heart rates are a suitable index of recovery between heats when using interval training. For example, it has been proposed that a recovery heart rate of 120 beats/min indicates that a horse has sufficiently recovered to undertake further heats. However, there is no evidence to support this claim, and it cannot be assumed that a horse is ready for further exercise on the basis of recovery heart rates. However, abnormally delayed recovery heart rates in the individual horse compared with normal should alert trainers to the possibility of illness or lameness.

Heart Rate and Training

In many studies, heart rates during submaximal exercise are lower after training. Heart rates during submaximal exercise may, therefore, provide a means of monitoring the adaptation of the cardiovascular system to training. Thoroughbreds exercise tested at submaximal workloads on a treadmill had lower exercise heart rates after a conventional training program (Foreman et al., 1990). Exercise bradycardia, evident as a mean increase in V_{200} of 0.57 m/s, occurred after 5 weeks of treadmill training (Thornton et al., 1983).

However, not all studies concluded that significant changes occur in heart rate during submaximal exercise. Heart rates during submaximal exercise were not significantly different in Standardbreds after either racetrack training (Milne et al., 1977) or treadmill training (Rose et al., 1983). In two treadmill training studies that demonstrated significant increases in $\dot{V}O_{2max}$, no significant changes were found in heart rate during submaximal exercise (Evans and Rose, 1988a; Knight et al., 1991). Decreasing heart rate during submaximal exercise is, therefore, an unreliable index of fitness in horses. The decrease in heart rate with training is often only 10 to 20 beats/min at any submaximal speed (Thomas et al., 1983) (Figure 11-9).

The use of heart rates during submaximal exercise to monitor increasing fitness also may be complicated by higher heart rates in horses at the start of training due to inadequate acclimation to the testing procedures (Thomas et al., 1983), which often include venipuncture, vascular catheterization, and wearing a mask for simultaneous measurements of respiratory function.

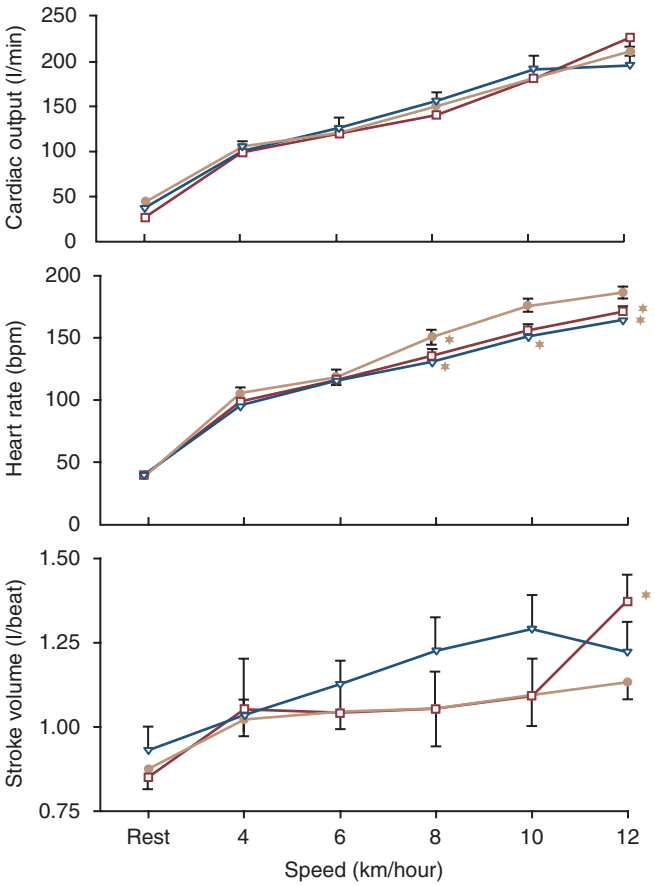

FIGURE 11-9 Effect of 5 and 10 weeks of training on cardiac output (Q), heart rate (HR), and stroke volume (SV) at rest and during treadmill exercise (means ± SE). Significant differences from pretraining (*$p < 0.05$; **$p < 0.01$).10 (•, untrained; Δ, partially trained; □, trained).

There is now clear evidence to suggest that HR_{max} is not altered by training (Evans, 2007). This investigator also reported that in Thoroughbred horses, the faster the speed at which V-HR_{max} is achieved generally the greater the $\dot{V}O_{2max}$. For example, horses achieving V-HR_{max} at speeds >15.3 m/s generally have $\dot{V}O_{2max}$ values >150 mL/kg/min, whereas those reaching V-HR_{max} at speeds below 13.6 m/s have $\dot{V}O_{2max}$ values <120 mL/kg/min.

OXYGEN PULSE

Oxygen pulse is defined as the ratio of oxygen consumption to heart rate and expresses the volume of oxygen ejected from the ventricles with each cardiac contraction. In humans, oxygen pulse during exercise reflects the maximal aerobic capacity (Wasserman et al., 1967). It is likely that this relationship also generally holds true for horses because HR_{max} varies only by about ± 5% in the population. The maximal aerobic capacity in untrained and trained horses can range from 90 to 180 mL/kg/min (possibly even higher than this in elite performers), a difference of at least 100%. Oxygen pulse during maximal exercise could, therefore, be expected to range from 0.4 to 0.8 mL/kg/beat, or 180 to 400 mL/beat, in horses. Obviously, this large range must be caused by individual differences in maximal stroke volume and arteriovenous oxygen content difference. Oxygen pulse increases as heart rate increases during exercise (Waugh et al., 1980), and it is, therefore, not possible to predict maximal aerobic capacity from oxygen pulse measurements during exercise at low heart rates. However, the close linear relationship between oxygen consumption and heart rate in horses during exercise could enable an estimation of maximal aerobic capacity, since the slope of the line would reflect the individual's oxygen pulse (for further discussion, refer to Evans, 2007).

STROKE VOLUME AND CARDIAC OUTPUT

Cardiac output is the product of heart rate and stroke volume. Stroke volume is the volume of blood ejected from each cardiac ventricle with each contraction. Cardiac output is, therefore, the volume of blood ejected from the left or right ventricle each minute, expressed as liters per minute (L/min) or milliliters per kilogram per minute (mL/kg/min). Increasing cardiac output is the principal means of increasing oxygen uptake during exercise.

Oxygen uptake is the product of cardiac output and the amount of oxygen extracted from blood in the exercising muscles. It has been estimated that increases in cardiac output contribute about two thirds of the increase in oxygen uptake that occurs during submaximal exercise (Poole and Erickson, 2004; Thornton et al., 1983). These relationships are expressed by the Fick equation:

$$\text{Oxygen uptake (L/min)} = \text{cardiac output (L/min)} \times C(a - v)O_2 \text{ (L/L)}$$

where $C(a - v)O_2$ is the difference in oxygen concentration between arterial and mixed venous blood.

Cardiac output at rest and during exercise has been measured in horses by using a variety of techniques. During submaximal exercise, cardiac output increases linearly with increases in work load, principally due to tachycardia (Evans, 2007) (see Figure 11-9).

Stroke volume in the resting horse is approximately 800 to 900 mL, or about 2 to 2.5 mL/kg (Poole and Erickson, 2004). Stroke volumes of 3.8 ± 0.4 mL/kg (approximately 1700 mL) have been reported in fit Thoroughbreds undertaking intense exercise (Butler et al., 1991; Poole and Erickson, 2004). Stroke volume increases by about 20% to 50% in the transition from rest to submaximal exercise (Poole and Erickson, 2004) (see Figure 11-9). It does not change as intensity of exercise increases from approximately 40% to 100% $\dot{V}O_{2max}$ (Poole and Erickson, 2004). This is true despite the limited time available for ventricular filling at high heart rates during exercise. Increased venous return during exercise and the increased blood volume must increase ventricular filling pressure sufficiently to prevent compromise of stroke volume.

During tethered swimming at low workloads, stroke volume was shown to decrease from 2.06 mL/kg at rest to about 1.5 mL/kg. This response may be related to decreased venous return secondary to the alterations in breathing pattern during swimming (Thomas et al., 1980). Values reported for cardiac output in fit Thoroughbreds during treadmill exercise are reported to vary from about 275 to 400 L/min (Poole and Erickson, 2004).

There have been few studies of the effect of training on cardiac output and stroke volume in horses. Several studies have found that training does not affect cardiac output or stroke volume measured at rest or after exercise, with others suggesting some small increase in these variables (Poole and Erickson, 2004). In one study, a 23% increase in $\dot{V}O_{2max}$ was accompanied by a significant increase in stroke volume during maximal exercise (Evans and Rose, 1988a). In another study that used an open-flow system for measuring oxygen consumption, stroke volume during exercise at 100% $\dot{V}O_{2max}$ did not change significantly with training but decreased significantly by 11% from 1426 ± 50 mL to 1271 ± 68 mL after 6 weeks of detraining (Knight et al., 1991). Studies of the effects of training on stroke volume and cardiac output during maximal exercise may be complicated by the variability of the initial state of training, as in humans (Blomquist and Saltin, 1983). Other factors that could influence the cardiovascular response to training include training frequency, intensity, and duration (Evans, 2007).

BLOOD PRESSURE AND VASCULAR RESISTANCE

Blood pressure is the product of cardiac output and total peripheral resistance (TPR). TPR is primarily dependent on the diameter of the arterioles but is also influenced by blood viscosity. Regulation of blood pressure at the start of and during exercise is not fully understood but probably involves reflexes that originate in the brain ("central command") and the working muscle (Mitchell et al., 1983). In muscle, receptors respond to mechanical and chemical disturbances. Chemical disturbances include factors such as the decrease in arterial PO_2 and the increased local concentrations of metabolites such as CO_2, H^+, and lactate, which modulate "central command" signals that originate in the central nervous system. Arterial baroreceptor reflexes are also postulated to have a role in blood pressure regulation during exercise, possibly involving responses to "error" signals that necessitate the establishment of a higher blood pressure (Rowell and O'Leary, 1990; Patterson, 1996).

Systemic Circulation

Mean systemic arterial blood pressure found in resting horses is in the range 113 to 138 mm Hg (Poole and Erickson, 2004). Light treadmill exercise has no significant effect on mean

pressures in the carotid artery. During more strenuous treadmill exercise, however, significant increases in mean systemic arterial pressure were observed (Poole and Erickson, 2004) (Figure 11-10). At light exercise loads, falls in arterial diastolic pressure also have been reported (Poole and Erickson, 2004). A fall in mean arterial pressure has been recorded in some horses as they commence galloping exercise, and it then increases within 2 minutes. At a speed of 548 m/min and mean heart rate of 184 beats/min, galloping Thoroughbreds recorded the following systemic arterial pressures (resting values in parentheses): systolic 205 (115) mm Hg, diastolic 116 (83) mm Hg, mean 160 (97) mm Hg, and pulse pressure 89 (32) mm Hg (Hornicke et al., 1977; Poole and Erickson, 2004).

Carotid artery pressure was linearly related to work effort (Thomas et al., 1980). Linear relationships between mean arterial and pulse pressures with speed on a racetrack also have been reported (Hornicke et al., 1977). However, tethered swimming causes higher blood pressures than galloping. The large increases in both systemic and pulmonic blood pressures during swimming are partly attributable to the increased hydrostatic pressure on the submerged chest (Thomas et al., 1980). During forced swimming with heart rates of approximately 200 beats/min, systolic carotid artery pressures routinely reached 300 mm Hg, and mean arterial blood pressure doubled from 113 ± 2 mm Hg at rest.

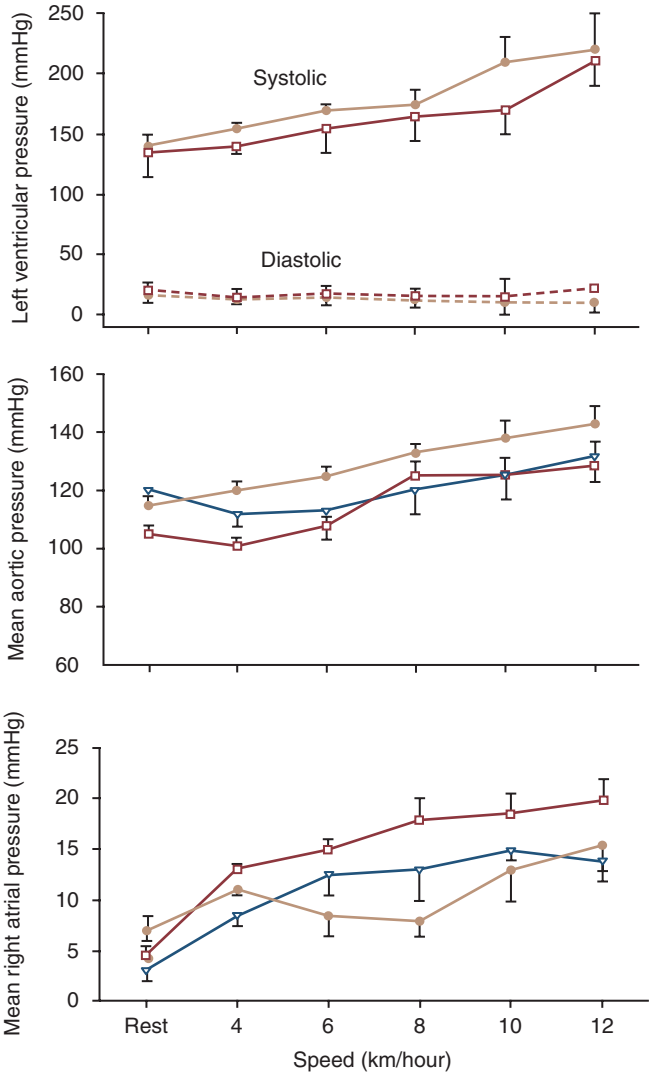

FIGURE 11-10 Effect of 5 and 10 weeks of training on left ventricular (LVP), aortic (AoP), and mean right atrial (RAP) pressures at rest and during treadmill exercise (means ± SE). Significant differences from pretraining 0.05; <0.01. (•untrained; Δ: partially trained; □ , trained). *(Adapted from Thomas DP, Fregin GF, Gerber NH, et al: Effects of training on cardiorespiratory function in the horse,* Am J Physiol *245:R160, 1983. With permission.)*

Significant elevations in systolic left ventricular and mean right atrial pressures have been recorded during exercise (Poole and Erickson, 2004; Thomas and Fregin, 1981; Thomas et al., 1983). The maximal rate of rise in left ventricular pressure has been used to assess left ventricular function at rest (Miller and Holmes, 1984) and during treadmill exercise (Manohar and Parks, 1983; Thomas and Fregin, 1981; Thomas et al., 1983). During exercise, myocardial contractility (expressed as peak time derivative of left ventricular pressure, LV dP/dt) increased with each increase in treadmill speed. In untrained adult ponies, treadmill exercise increased left ventricular end-diastolic pressure and right ventricular systolic and end-diastolic pressures (Parks and Manohar, 1983a; Poole and Erickson, 2004).

The ratio of mean arterial pressure to heart rate has been used to assess total peripheral resistance during exercise (Hornicke et al., 1977). Assuming constant stroke volume during the exercise test, large and significant falls in total peripheral resistance appear to occur (Poole and Erickson, 2004). Measurement of mean pulmonary and carotid artery pressures and cardiac output during treadmill exercise at 4 m/s also demonstrated large decreases in both pulmonary and peripheral vascular resistances. These results were presumed to be caused by expansion of the vascular bed in the lungs and working skeletal muscles (Bergsten, 1974). Similar results were reported in ponies and Standardbreds in treadmill studies (Bayly et al., 1983; Parks and Manohar, 1983a). In view of the large increases in cardiac output during exercise in horses, a fall in peripheral vascular resistance is an important means of maintaining mean blood pressures within narrow limits (Poole and Erickson, 2004).

Pulmonary Circulation

Pressures in the pulmonary artery are, generally, systolic 45 mm Hg, diastolic 20 mm Hg, and mean 25 mm Hg at rest (Poole and Erickson, 2004). During submaximal treadmill exercise, an increase of about 30% in mean pulmonary artery pressure occurred with increasing speed until a plateau was reached at about 100% increase over control values (Poole and Erickson, 2004; Rose et al., 1983). During more intense treadmill exercise, mean pulmonary artery pressure increased from 28 mm Hg at rest to 80 mm Hg at a heart rate of 202 beats/min (Erickson et al., 1992). These changes in pulmonary artery pressure during exercise are closely correlated with heart rate. Mean pulmonary artery pressure increased from 24 ± 1 mm Hg at rest to 40 to 100 mm Hg during tethered swimming (Thomas et al., 1980). The relatively high pulmonary artery pressures in exercising horses are probably not attributable to hypoxic pulmonary vasoconstriction (Pelletier and Leith, 1991; Poole and Erickson, 2004).

In resting horses, blood flow is well matched to alveolar ventilation, and strenuous exercise generally has little effect on this homogeneity (Wagner et al., 1989). Inequalities between

blood flow and pulmonary alveolar ventilation have been suggested to occur. Intrapulmonary shunting of blood appears to be negligible both at rest and during exercise. It was concluded that neither shunting nor ventilation–perfusion mismatch explained the hypoxemia of intense exercise in horses (Poole and Erickson, 2004).

The pulmonary and systemic arterial blood pressures and vascular resistances are all lower in splenectomized ponies than in normal ponies during exercise (Davis and Manohar, 1988; Poole and Erickson, 2004). This occurs despite similar cardiac outputs in the two conditions. It was concluded that increases in blood viscosity because of hemoconcentration in normal ponies may contribute substantially to the pulmonary and systemic hypertension of exercise.

Blood Pressure and Training

Values for resting arterial blood pressures measured indirectly in race-conditioned Thoroughbreds have been reported (Johnson et al., 1976). Mean, systolic, and diastolic aortic blood pressures were lower during exercise after treadmill training (Thomas et al., 1983) (see Figure 11-10). Treadmill training also has resulted in higher mean right atrial pressure and a tendency to lower systolic left ventricular pressure during exercise (Poole and Erickson, 2004; Thomas et al., 1983). Training also caused lower LV dP/dt_{max} at rest and during exercise. Racetrack training resulted in a decrease in mean arterial blood pressure during an exercise test (Hornicke et al., 1977; Poole and Erickson, 2004).

In humans, training is usually associated with a fall in peripheral vascular resistance (Blomquist and Saltin, 1983). A reduction in total peripheral resistance in the arterial circulation after training also has been reported in horses (Bayly et al., 1983; Poole and Erickson, 2004; Thomas et al., 1983).

BLOOD VOLUME

The total blood volume consists of the combined volume of the plasma and the cells in blood. In resting horses, blood volume is about 9% of body weight. Approximately 20% of the blood volume is found in the pulmonary circulation and 80% in the systemic circulation (Holmes, 1982). Of the blood in the systemic circulation, 60% is present in veins and venules and only 15% in arteries.

Blood volume can vary greatly during excitement and exercise. The contraction of the spleen in such circumstances adds RBCs to the circulation; and this addition of erythrocytes can increase hematocrit from values of 35% to 45% at rest to 50% to 70% after exercise. The extent of the increase in total blood volume during exercise is dependent on the work intensity, age, sex, and training state of the horse (Persson, 1967; Poole and Erickson, 2004). The weight of the spleen varies by over 200% in various breeds, and this variation may explain the differences in total blood volume between breeds of horse (Kline and Foreman, 1991; Poole and Erickson, 2004).

Measurement of total blood volume in the horse, therefore, necessitates measurement of the plasma volume and postexercise hematocrit (Persson, 1967). Mean total blood volume in 10 untrained Thoroughbred horses was 53.3 L, of which only 40% comprised plasma (Knight et al., 1991). In a study involving 32 weeks of treadmill training, no changes in blood volume were found in 12 Standardbred horses (Golland et al., 2003).

Plasma Volume

Plasma volume is measured in horses by injecting a known mass of a dye or other marker and subsequently measuring its concentration in plasma after a suitable time for mixing. Evans blue dye (11824) is frequently used. The plasma volume in the resting horse ranges from 16 to 31 L, or 38 to 64 mL/kg of body weight (Golland et al., 2003; Knight et al., 1991; Kohn et al., 1978; Masri et al., 1990; McKeever et al., 1987).

Effect of Exercise on Plasma Volume

Exercise usually results in a decrease in plasma volume. This change has been attributed to water movement from the intravascular to extravascular compartment (Kohn et al., 1978). A 13% decrease in plasma volume was measured within 10 minutes of completion of a 1000-m maximal gallop in six fit Thoroughbred horses (Masri et al., 1990). The decrease in plasma volume is accompanied by an increase in total plasma protein (TPP) concentration. Total plasma protein returns to prerace values 1 hour after racing (Keenan, 1979). However, changes in TPP after exercise are not an accurate assessment of exercise-induced changes in plasma volume, since protein is added to the circulation during exercise, after exercise, or at both times. McKeever et al. (1993) reported similar findings in horses subjected to an incremental submaximal exercise test. These authors compared splenectomized and intact horses and found that the plasma volume was lower at rest in the splenectomized horses but that both intact and splenectomized horses experienced similar decreases in plasma volume in response to exercise. Changes in plasma osmolality may be a better indicator of changes in plasma volume after brief exercise (Masri et al., 1990).

Maximal exercise over 600m did not alter plasma volume or extracellular fluid volume significantly in six trained Standardbreds (Kohn et al., 1978). Detection of any changes in plasma volume after maximal exercise may depend on duration of exercise and the experimental techniques used. In addition, decreases in plasma volume after exercise are quite variable, ranging from 3% to 27% (Masri et al., 1990; Poole and Erickson, 2004).

Effect of Training on Plasma Volume

Plasma volume in the resting horse is reported to increase after training (McKeever et al., 1987), whereas Golland et al. (2003) found a decrease in this variable after 32 weeks of training. If, indeed, there is an increase in plasma volume, it is associated with increased right atrial pressure and stroke volume. An increase in plasma volume after training may augment ventricular filling and thus contribute to the increased stroke volume found with training. An expanded plasma volume is also likely to be an important mechanism in increased thermoregulatory capacity during exercise, enabling increased blood flow to skin.

Plasma Volume and Diet

Plasma volume can change during the day, depending on the feeding regimen adopted (Clarke et al., 1988; Kerr and Snow, 1982). When horses were fed small portions at 4-hour intervals, there were only small changes in total plasma protein concentration and hematocrit, whereas these variables increased greatly after a single large meal. This suggests that plasma volume decreases by 10% to 15% for at least 1 hour after a large meal. Horses, therefore, should not be fed a large meal 1 to 2 hours before competition.

Plasma Volume and Athletic Performance

Plasma volume is often decreased in endurance horses during competition because of sodium and water losses in sweat (Carlson, 1975; McKeever, 2004). Many studies in human athletes have illustrated the importance of maintenance of plasma volume and body water for performance of endurance exercise (Convertino, 1987; Nadel, 1987).

Total Red Blood Cell Volume

The total RBC volume refers to the volume of RBCs in the circulation after exercise or after an injection of epinephrine. Whether training results in an increase of the total RBC volume in horses is still being debated (Golland et al., 2003; McKeever, 2004; Poole and Erickson, 2004). The potential for increasing oxygen-transport capacity by splenic emptying during exercise is, therefore, augmented by training. Total RBC volume relative to body weight is also related to racing performance in Swedish trotters (Persson and Ullberg, 1974). A correlation of 0.68 ($p < 0.001$) was found between this measurement and maximal trotting speed over 1000 m in 35 horses. This correlation was as high as found for other measurements of fitness, such as the blood lactate and heart rate response to submaximal exercise for 4000 m at 10 m/s in the same group of horses.

Unfortunately, the total RBC volume is not a simple measurement. It is unlikely to become a routine method of evaluating the athletic horse until its relevance to performance and relationship to whole-body $\dot{V}O_{2max}$ have been confirmed in other studies.

DISTRIBUTION OF CARDIAC OUTPUT

During exercise, there is redirection of blood flow to the working muscles without compromising blood flow to the central nervous system. Vasodilation in skeletal muscles and skin and vasoconstriction in the splanchnic region and in the nonworking muscles are characteristic responses (Evans, 2007; Manohar, 1987; Poole and Erickson 2004).

The effect of treadmill exercise on blood flow distribution during moderate and strenuous exercises in untrained ponies has been investigated. Blood flow to the exercising muscles increased by 31-fold to 38-fold during moderate exercise and by 70-fold to 76-fold during intense exercise. Blood flow to the cerebellum and diaphragm also increased. Large increases in blood flow to both the right and left ventricles and decreased renal blood flow also were recorded (Parks and Manohar, 1983a; Poole and Erickson, 2004). Renal vasoconstriction occurs during maximal exercise in ponies, and blood flow to the kidney is only about 20% of that measured in the resting horse (Manohar, 1991). However, no changes in renal blood flow, creatinine clearances, or filtration fraction were found during prolonged exercise at heart rates of only 55% to 60% of individual HR_{max} (Hinchcliff et al., 1990). A reduction occurs in blood flow to gastrointestinal organs and the spleen, but blood flow to the adrenal glands is more than doubled during exercise (Manohar, 1987). The respiratory muscles in resting ponies receive about 10% of the cardiac output, and this increases to about 15% during strenuous exercise (Manohar, 1991).

The distribution of tracer microspheres has been used to study blood flow in the walls of the right and left cardiac ventricles at rest and after exercise. Total ventricular myocardial blood flow commands approximately 3% of the cardiac output in resting ponies, and this increases to 4% during maximal exercise (Parks and Manohar, 1983b) (Figure 11-11). Left ventricular myocardial blood flow increases homogeneously and by approximately fivefold above resting values during maximal exercise, resulting in a 470% increase in cardiac output. This study also demonstrated that the myocardium of ponies possesses considerable vasodilator reserve, since injection of adenosine, a myocardial vasodilator, resulted in greatly augmented coronary blood flow (Manohar and Parks, 1983b).

An interaction between blood flow during submaximal exercise and feeding has been described in ponies trotting at 28 km/hr on a treadmill inclined at 7% (Duren et al., 1992). Eight ponies that had been fasted for 24 hours before exercise had lower heart rates, stroke volume, cardiac output, and arterial blood pressure compared with ponies given a pelleted grain concentrate and ad lib alfalfa hay over the same period. Blood flow to the locomotor muscles, respiratory muscles, and longissimus dorsi was higher in fed ponies during exercise. These results may have implications for dietary strategies before prolonged exercise.

Long-term training has a significant effect on the vascularity of skeletal muscle. A 6-month training program of young Standardbred trotters resulted in increased capillary supply to skeletal muscle fibers (Henckel, 1983). In contrast, no increase in muscle capillarity was observed in a shorter training program in Thoroughbreds (Nimmo et al., 1982).

CONCLUSION

The cardiovascular system of the horse has remarkable reserves of RBC volume and heart rate during exercise. These reserves are the basis of a comparatively superior oxygen-transport capacity

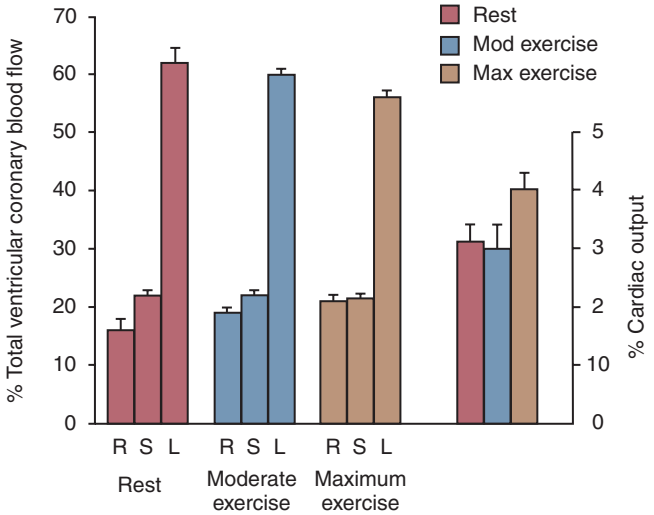

FIGURE 11-11 Changes in blood flow (milliliter per minute per 100 grams [mL/min/100 g]) to the ventricular myocardium, kidneys, and the muscular portion of the diaphragm brought about by graded treadmill exercise in ponies. The percentage of cardiac output received by the ventricles is shown on the right-hand side of the figure (*significantly different from rest) (*R*, right ventricle; *S*, septum; *L*, left ventricle). (*Parks CM, Manohar M: Distribution of blood flow during moderate and strenuous exercise in ponies* (Equus caballus), Am J Vet Res *44:1861, 1983.*)

during maximal exercise. The assessment of the cardiovascular system of equine athletes during standardized exercise tests, on treadmills and more so now "in the field," is now routine at many centers. Such tests enable assessment of the likely influence of cardiac abnormalities such as murmurs and dysrhythmias on future athletic performance. Many of the normal responses to exercise and training in horses have been described, but there are still many unresolved issues (Marr and Burton, 2010; Young, 2004). These include an understanding of the possible role of normal as well as abnormal cardiovascular physiology in the pathophysiology of exercise-induced pulmonary hemorrhage (EIPH), the physiologic mechanisms of responses to training and overtraining, and the relevance of assessments of heart rate during exercise to performance in athletic competition.

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CHAPTER

12 Muscle Anatomy, Physiology, and Adaptations to Exercise and Training

STEPHANIE J. VALBERG

As illustrated throughout this book, the horse has evolved into the supreme athlete, being capable of both high speeds and endurance. Fine neuromuscular coordination, strength, and stamina allow a Thoroughbred to attain speeds in excess of 18 meters per second (m/s) or 65 kilometers per hour (km/h), which can be maintained for about 1 minute with a stride length of up to almost 7.5 m. This feat is possible because the muscular system of the horse has evolved to produce powerful, efficient movement at high speeds. In most mammalian species, muscle mass makes up about 40% to 45% of body weight; however, in the Thoroughbred, muscle mass can comprise up to 55% of body weight (Gunn, 1979). In addition to increased muscle bulk, elite performance is achieved by adaptations of muscle at many different levels: the gross arrangement of muscle groups, the architecture of muscle fibers and connective tissue within muscles, and the highly specialized ultrastructure of the muscle fibers themselves. Our understanding of muscle anatomy in the horse can be traced back through centuries of study of the organization and attachment of muscles and their tendons. The details of the cellular organization of muscle fibers in the horse, however, have only been characterized during the last few decades. On an ultrastructural level, much of our current understanding of the horse still relies on parallels drawn from other animal species.

This chapter is divided into three parts: (1) a general consideration of muscle structure and function, (2) muscle fiber composition, muscular responses to exercise and adaptations with growth and training programs, and (3) insights gained from studying inherited metabolic disturbances.

MUSCLE STRUCTURE AND FUNCTION

MUSCLE ANATOMY

Locomotor muscles in the horse are strategically located proximally on the skeleton, creating a pendulum-like effect that decreases the energy necessary to swing the limb. The arrangement of spindle-shaped muscle cells (muscle fibers) within the muscle also maximizes efficiency and power output during locomotion. In many limb muscles, muscle fibers are arranged in a "pennate" shape whereby they join one or more tendinous insertions at an angle to the direction of force. This maximizes the cross-sectional area and power output of the muscle in relation to the limited space available on the upper limb. Other muscles, strap muscles, maximize their range of movement by a parallel organization of muscle fibers along the direction of force. Several perpendicular tendinous insertions are usually present within this type of muscle, since most muscle fibers are only about 5 to 10 centimeters (cm) in length, with a cross-sectional area of 2000 to 6000 micrometers squared (μm^2) (Karlstrom et al., 1994). For efficiency, muscle fibers are grouped within the muscle such that the slower-contracting fibers commonly used for postural support are frequently located deeper in the muscle and the faster-contracting fibers used for higher speeds are located more superficially.

Muscles are surrounded by a loose layer of connective tissue, the *epimysium*, which lies below the external fascia and extends internally around groups of muscle fibers (fascicles) as the *perimysium*. A delicate layer of reticular fibers, the *endomysium*, envelops each individual muscle fiber. Arteries course within the perimysium and supply an average of one to three capillaries per fiber within the endomysium. Nerve fibers containing both sensory and motor neurons are also present in the perimysium. Motor neurons innervate muscle fibers at a specialized site on the muscle cell membrane called the *motor end plate*. In limb muscles, one motor neuron probably innervates between 1000 and 2000 muscle fibers scattered throughout numerous fascicles.

Embryology

The components of limb muscle are derived from paired embryonic somites (muscle fibers) and somatopleure (connective tissue elements). In the embryo, primitive muscle cells migrate to their position in the limb and separate into dorsal and ventral muscle masses, where they proliferate and differentiate into postmitotic muscle fibers. The immature muscle cells, called *myoblasts*, fuse to form myotubes, and motor neurons establish primitive neuromuscular junctions with myotubes. Elongation of muscle fibers occurs gradually and results eventually in continuity with independently established tendons attached to the skeleton.

The eventual mosaic of contractile fiber types within a muscle will be determined by innate developmental directives, temporal and positional factors, neural innervations, and activation of specific signal transduction pathways. Positional factors dictate that those portions of muscles that are primarily postural have a higher percentage of slow twitch fibers, whereas neural innervations dictate that all fibers supplied by the same nerve branch have the same muscle fiber type. In the

equine fetus, the perinatal form of contractile protein within the myosin heavy chain (MHC) is initially expressed. However, differentiation of slow-contracting and fast-contracting muscle fibers becomes well established at 158 days of gestation and evident in newborn foal muscle. Fast twitch fibers may continue to coexpress perinatal MHC in foals up to 10 weeks of age, and many slow twitch fibers may coexpress a cardiac MHC isoform up to 22 weeks of age (Dingboom et al., 1999). Development of subtypes of fast twitch fibers (2a, 2ax, or 2x) occurs in concert with the emergence of thyroid function (Russell et al., 1988). Muscle fiber composition remains plastic well into adulthood, affected by growth and training.

Muscle Ultrastructure

Muscle fibers possess a number of structural adaptations that confer the ability to generate force through contraction. These include a precise alignment of *contractile proteins*, a cell membrane capable of propagating an electrical potential, and internal membrane structures and energy-generating pathways that can regulate the amount of calcium and adenosine triphosphate (ATP) available for excitation–contraction coupling.

Contractile Proteins

Myofibrils comprise a highly repeating arrangement of filaments of noncovalently associated proteins connected in series within the length of each muscle fiber (Figure 12-1). The number of myofibrils in a muscle fiber varies with its contractile type and cross-sectional area. Each myofibril is 1 to 3 µm in diameter and has a polygonal shape. The organization of numerous myofibrils in register within a cell gives skeletal muscle fibers a striated appearance under the light microscope.

Sarcomere

The repeating unit of myofilaments within the myofibril is referred to as a *sarcomere*, the fundamental unit of contraction (see Figure 12-1). Filaments of actin (molecular weight 43 kilodaltons [kDa]) traverse the sarcomere at each end to form the Z-line. The width of the Z-line varies from 50 nanometers (nm) in slow-contracting muscle fibers to 65 nm in fast-contracting muscle fibers. Thin myofilaments extend axially from either side of the Z-line and form a hexagonal array around overlapping thick myofilaments. Muscle contractions occur when, within each sarcomere, thin myofilaments slide over the thick myofilaments bringing consecutive Z-lines closer together. This complex arrangement of overlapping filaments permits several distinctive areas to be identified with electron microscopy. The I-band contains the Z-line centrally and extends to include the adjoining area of nonoverlapping thin myofilaments. Centrally, in the sarcomere, the A-band is defined by the full extent of the thick myofilaments. Within the A-band, the H-band is defined by the central area where thick myofilaments do not overlap with thin myofilaments. In the middle of the H-band, a dark line is formed by three to five M-line filaments traversing the sarcomere (see Figure 12-1).

Thick Myofilaments

The thick myofilaments are bipolar, spindle-shaped structures 1.6 µm in length and 15 nm in diameter. Myosin is the primary protein in the thick myofilament with a molecular weight of 460,000 kDa, and it possesses both structural and enzymatic properties. Myosin is composed of two identical heavy chains

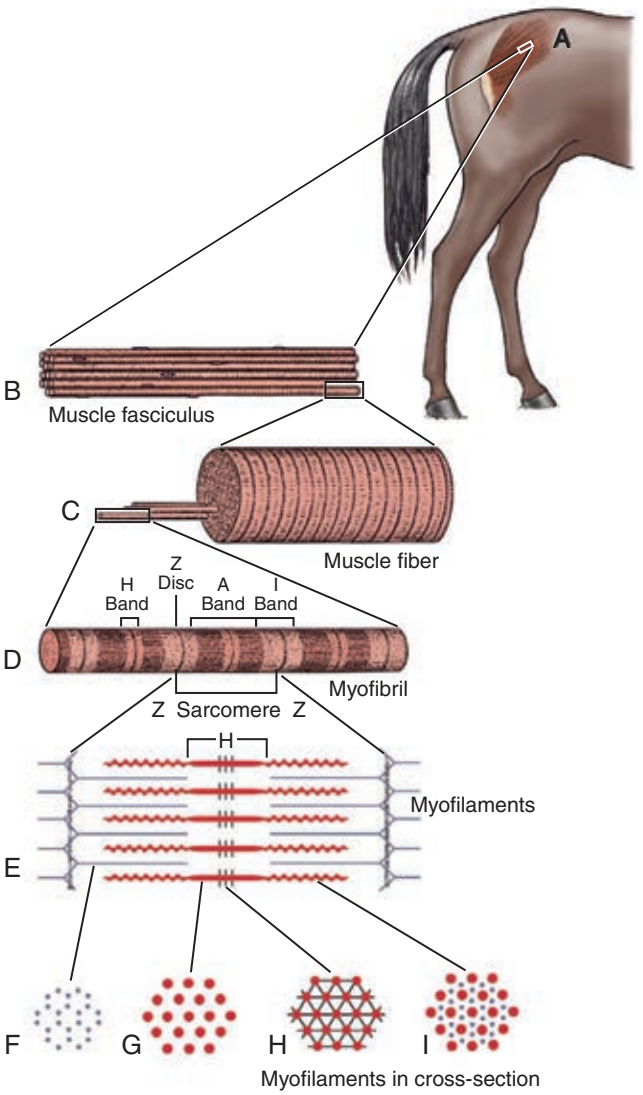

FIGURE 12-1 Skeletal muscle. The organization of skeletal muscle contractile proteins from the gross to the molecular level. *(Adapted with permission from Bloom W, Fawcett DW:* A textbook of histology, *Philadelphia, PA, 1986, WB Saunders Co, p 282.)*

(polypeptide chains with an approximate molecular mass of 200 kDa) and two pairs of light chains (polypeptide chains with molecular masses ranging from 16 to 27 kDa) (Figure 12-2, *A*). The two myosin heavy chains are arranged in a double helix to form a long stable tail at one end. At the opposite end, each heavy chain is folded to form one globular, pear-shaped head. Four myosin light chains are contained within the globular heads (two per head) near the junction of the head and neck domains.

The composition of MHCs within sarcomeres varies between individual muscles and between individual muscle cells. Equine skeletal muscle cells may express the following distinct heavy chains: perinatal (or neonatal), slow, fast type 2a, fast type 2x (or 2d) or a hybrid of 2a/2x (Dingboom et al., 1999; Eizema et al., 2005). The speed of contraction of these myosin heavy chain isoforms increases in the order listed above.

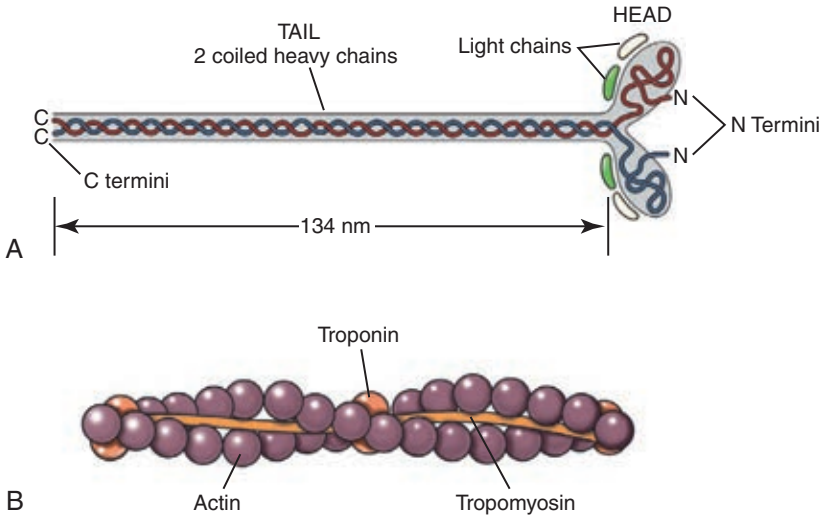

FIGURE 12-2 **A,** Myosin. A model of the myosin molecule showing the arrangement of two heavy chains and two light chains on each globular head. **B,** Thin filament. A model of the thin filament showing the interrelationships among actin, troponin, and tropomyosin. *(Adapted with permission from Bloom W, Fawcett DW:* A textbook of histology, *Philadelphia, PA, 1986, WB Saunders Co, p 287.)*

The tails of approximately 300 myosin molecules aggregate together to form the backbone of one thick myofilament. Myosin globular heads extend outward in a three-dimensional helical array from this backbone, leaving a central bare area without any globular heads. The globular heads contain binding sites for both actin and ATP, as well as the enzyme adenosine triphosphatase (ATPase) and, as such, are a central component of the contractile process.

Thin Myofilaments

Thin myofilaments are 1 µm long and 8 nm in diameter, consisting primarily of actin. *Actin* is a globular protein with a molecular weight of 43 kDa. Two F-actin strands twisted in a double helix form the backbone of the thin myofilament and possess a complementary binding site for the myosin globular head. Actin has identical isoforms in fast-contracting and slow-contracting fibers. The interaction between myosin globular heads and actin is regulated by tropomyosin and troponin (see Figure 12-2, *B*). Tropomyosin is a two-stranded α-helix with a molecular weight of about 70 kDa, and it lies in the helical grooves formed by actin strands. Troponin is a complex of three noncovalently linked subunits, with molecular weights ranging from 18 to 35 kDa, which attaches at regular intervals to tropomyosin along the thin filament. Each subunit has a distinct physiologic function. The subunit TN-T, the tropomyosin-binding component, attaches the complex to tropomyosin at intervals along the thin myofilaments. The inhibitory subunit (TN-I), when bound to actin, acts together with tropomyosin to inhibit actin–myosin interaction. TN-C is the calcium-binding subunit of troponin, which, in the presence of calcium, can remove the inhibitory effects of TN-I and facilitate actin–myosin binding.

Cytoskeleton

Both the organization of myofilaments within sarcomeres and the organization of myofibrils in myofibers are supported by a complex cytoskeletal network of intermediate filaments (Wang and Ramirez-Mitchell, 1983). Intermediate filaments, as well as a number of accessory proteins that form fine filaments, function to (1) maintain the alignment of myofilaments and sarcomeres, (2) attach and maintain alignment of adjacent myofibrils, (3) attach and transfer forces from the sarcomeres of peripheral myofibrils to the sarcolemma, and (4) connect terminal sarcomeres to the sarcolemma at myotendinous junctions. In addition, cytoskeletal proteins serve a vital role in intracellular signaling and in maintaining the shape of organelles such as mitochondria and nuclei.

Titin is one of the largest cytoskeletal proteins, which attaches thick myofilaments to Z-lines, and in the I-band region this attachment imparts a passive elasticity to sarcomeres (Maruyama, 1999). An additional cytoskeletal protein, *nebulin*, forms small filaments that run the length of thin myofilaments, which may regulate the length of thin myofilaments. Both M-protein and creatine kinase are cytoskeletal elements located at the M-line that may serve as structural support for the thick filaments by linking neighboring filaments to each other, in addition to providing a source of ATP from creatine phosphate.

A-actinin and *vinculin*, among other cytoskeletal proteins at myotendinous junctions, attach the thin myofilaments of the last sarcomere to the sarcolemma. At the periphery of myofibrils, adjacent Z-lines within the same sarcomere are connected by intermediate filaments of *desmin*. Also, intermediate filaments of desmin encircle the circumference of Z-lines and appear to form linkages with Z-lines of adjacent myofibrils to aid in the alignment of sarcomeres in register. Desmin filaments are also anchored to the sarcolemma by a number of adhesion proteins such as vinculin. The cytoskeletal elements that connect the sarcomere to the basement membrane are called *costameres*. They serve to laterally transmit contractile forces from sarcomeres across the sarcolemma to the basement membrane and potentially to convert mechanical stimuli to alterations in cell signaling and gene expression. To date, the distribution of only one cytoskeletal protein, *tubulin*, has been studied in the horse (Horak et al., 1991).

Sarcolemma and Sarcoplasmic Reticulum

Muscle Basement Membrane

The basement membrane surrounds skeletal muscle fibers, is directly linked to the sarcolemma, and plays a critical role in myogenesis, muscle fiber development, regeneration, structure, and function. In addition, it is required for the proper assembly of the neuromuscular and myotendinous junctions. Transsarcolemma complexes provide critical mechanical links among the basement membrane, the cytoskeleton, and the myoplasm. Two such sarcolemma protein complexes include (1) the dystrophin–glycoprotein complex and (2) integrins (Michele and Campbell, 2003). The dystrophin–glycoprotein complex likely plays a key role in protecting the sarcolemma from mechanical damage during muscle contraction. It is grouped into three subcomplexes: (1) dystroglycan (α- and β-dystroglycan), (2) the sarcoglycan–sarcospan subcomplex, and (3) the cytoskeletal components dystrophin, syntrophin, and dystrobrevin (Ervasti and Sonnemann, 2008). These proteins are well characterized in other species and their absence causes various forms of muscular dystrophy; however, there are no specific studies of these complexes in horses.

Sarcolemma

The *sarcolemma* maintains the intracellular milieu, actively transports substrates into the muscle cell, serves as a docking location for proteins originating in the basement membrane and cytoskeleton, and also transmits neural excitatory impulses that lead to muscle contraction. Facilitated diffusion of glucose across the sarcolemma occurs via glucose transporters (GLUT). GLUT-1 is constitutively present in the sarcolemma and provides basal amounts of glucose uptake, whereas GLUT-4 is present in the endosomes in the sarcoplasma, which migrate to and then dock and fuse with the sarcolemma when stimulated by insulin and contraction-dependent processes. Long-chain fats are transported across the sarcolemma by fatty acid translocase.

The sarcolemmal properties of excitation and conduction are largely due to the presence of membrane-spanning ion conducting pathways and channel gates within the sarcolemmal lipid bilayer that regulate the selective and nonselective conductance of sodium, potassium, calcium, and chloride. They activate (open) in response to ligands, transmitters, or changes in voltage and inactivate (close) by intrinsic regulatory processes. Voltage-gated channels contain additional voltage-sensing transmembrane domains and are essential for the generation and modification of action potentials. Ligand-gated ion channels are essential for setting myoplasmic calcium concentrations and establishing signal transduction pathways.

The sarcolemma forms tubular invaginations, *t-tubules*, at regular intervals along its length which traverse perpendicularly across the myofibrils at the junction of the A-bands and I-bands (Figure 12-3). The t-tubule membranes have a lower protein content but similar lipid content as the sarcolemma and contain numerous voltage-gated calcium channels called *dihydropyridine receptors (DHPR)*. The t-tubules serve to transmit electrical impulses into the interior of the muscle fiber, where, through association with the intracellular membranous system, they can almost simultaneously initiate myofibrillar contraction.

Neuromuscular Junctions

The neuromuscular junction is a specialized region on the sarcolemma, where a motor neuron axon terminal rests. The synaptic cleft is the space between the axon terminal and the postsynaptic sarcolemma (motor end plate), and it is filled with basal lamina containing acetylcholinesterase. Depolarization of the motor neuron results in calcium-dependent exocytosis of the chemical transmitter acetylcholine (ACh) from the small vesicles in the presynaptic axon terminal. The ACh that is released diffuses across the synaptic cleft to bind with acetylcholine receptors (AChRs) in the postsynaptic sarcolemma. Excitation of the myofiber is initiated by the reversible binding of ACh with AChRs. The binding of ACh to AChRs is transient, and its effects are abolished by the diffusion of ACh away from the receptors and its hydrolysis by acetylcholinesterase. The binding of ACh with AChR results in a local depolarization of the postsynaptic membrane caused by the transient increased conductance of sodium. When a threshold is reached, voltage-gated sodium ion channels in the synaptic clefts and along the sarcolemma are activated, and a wave of depolarization extends into the myofiber via contiguous t-tubules.

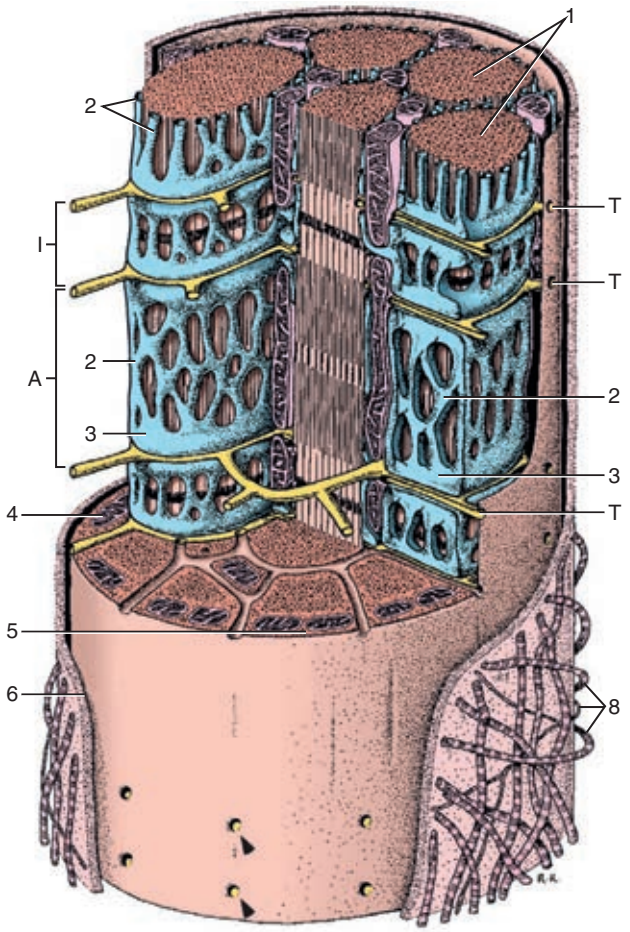

FIGURE 12-3 A three-dimensional view of the internal structure of a muscle fiber. Every myofibril (*1*) is surrounded by a parallel arrangement of the sarcoplasmic reticulum (*2*), which converges to form terminal cisternae (*3*). Between each terminal cisterna at the Al junction is an invagination of sarcolemma called the t-tubule (*T*). Mitochondria (*4*) are present between myofibrils. The sarcolemma (*5*) is surrounded by a basement membrane (*6*), and a network of reticular and collagen fibrils (*7*) surrounds each muscle fiber. *(Adapted with permission from Krstic RV:* General histology of the mammal, *New York, 1984, Springer-Verlag, p 265.)*

Sarcoplasmic Reticulum

The intracellular membranous system of skeletal muscle, or *sarcoplasmic reticulum (SR)*, is physically separate from the sarcolemma and surrounds each myofibril in a highly repetitive pattern. The SR membranes contain a high concentration of calcium ATPase, the protein calsequestrin, and the calcium release channel called the *ryanodine receptor (RYR)*. This system of membranes sequesters calcium in the relaxed muscle fiber, leaving extremely low concentrations in the sarcoplasm surrounding the myofibrils. Over the A-bands and I-bands, the SR runs parallel to the myofibrils. At the A–I junction, the SR tubules change their membrane composition and converge to form terminal cisternae (see Figure 12-3). The terminal cisternae run perpendicular to the myofibril on either side of the t-tubules. The t-tubules, along with the two neighboring terminal cisternae, form a functional association called a *triad*. A triad occurs twice in each sarcomere. The gap between the terminal cisternae and t-tubules is bridged by a tetragonal arrangement of foot processes called *junctional feet*. These feet act structurally to maintain the architecture of the triad during contraction and to mediate the coupling of sarcolemmal excitation with the release of calcium for muscle contraction. They do this by maintaining a proximal relationship between the voltage-gated dihydropyridine receptor (DHPR) in the sarcolemma and the RYR located in the terminal cisternae of the sarcoplasm.

Excitation–Contraction Coupling

Excitation–contraction coupling is the transformation of depolarizing events in the sarcolemma into the initiation of mechanical shortening of the myofibrils. The action potential that is propagated into the depths of the myofiber via transverse t tubules triggers the voltage-gated DHPR located within the triads. Activation of the DHPR triggers the release of calcium ions from the terminal cisternae into the sarcoplasm by opening the RYR in the SR membrane. This elevates the calcium ion concentration surrounding the myofilaments in the sarcoplasm from 0.1 micromolar (10-7 moles/L), to more than 10 micromolar (10-5 moles/L). Accessory proteins that regulate RYR function include calmodulin and the FK-506–binding protein.

Calcium released into the sarcoplasm binds to the troponin-C subunit of troponin and removes the inhibitory effect of TN-I, resulting in tropomyosin moving deeper into the groove of the actin helix and exposing the myosin binding site. Once revealed, the globular head of myosin forms a cross-bridge with actin at this binding site, which activates myosin ATPase and releases ATP. The actin filaments are displaced toward the center of the A-band, and further binding of the primed myosin globular heads to actin occurs. Several cycles of cross-bridge formation and cross-bridge breaking are repeated per contraction, which shortens each half of the sarcomere in a ratchet-like fashion.

Relaxation of myofibrils occurs through active transport of calcium ions into the lumen of the SR by the SR calcium–ATPase (SERCA). Lower sarcoplasmic calcium concentrations allow tropomyosin to once again cover the myosin-binding site on the actin filament. The activity of SERCA increases at higher myoplasmic calcium concentrations and is inhibited by phospholamban at low sarcoplasmic calcium concentrations.

Other Organelles

Skeletal muscle fibers contain hundreds to thousands of post-mitotic nuclei, which are located directly underneath the sarcolemma. The number of myonuclei per fiber is established at birth. Regeneration of myofibers is dependent on small satellite cells, which are situated between the basement membrane and the sarcolemma. In light microscopy, satellite cell nuclei could be easily confused with myonuclei. A varying complement of smooth and rough endoplasmic reticulum, Golgi apparatuses, and lysosomes is usually found near myonuclei. Numerous proteins, including myoglobin and the enzymes involved in glycolysis, are distributed in the sarcoplasm. Enzymes involved in oxidative metabolism are located within the mitochondrial membranes. Mitochondria in horse muscle are concentrated in subsarcolemmal locations, particularly in association with capillaries. A lesser distribution of mitochondria is found between myofibrils. The volume density of mitochondria varies in different horse muscles and in different breeds from 2% to 24% (Kayar et al., 1988). Equine mitochondria are generally cylindrical in shape but may produce transverse extensions at the level of the I-band.

Glycogen granules and, to a varying extent, lipid droplets are also distributed throughout the sarcoplasm between the myofilaments and under the sarcolemma. Glycogen is stored in intracellular granules composed of self-glycosylating protein primer called *glycogenin* and variable amounts of glucose joined by straight α-1, 4 or branched α-1, 6 glycosyl linkages. This structure is treelike in its branching and allows glycogen granules to be potentially regulated individually and regionally within a cell for metabolism. Glycogen synthesis of new granules is catalyzed by glycogenin. It generates an oligosaccharide primer of 7 to 11 glycosyl units, which serves as a substrate for glycogen synthase. In combination with glycogen-branching enzyme, glycogen synthase forms glycogen granules, which, in the initial stages, have a low molecular weight and a high protein-to-carbohydrate ratio and can precipitate in acid (Brojer et al., 2002). These glycogen granules are termed *proglycogen* and have a molecular weight of up to approximately 400 kDa. When proglycogen granules grow larger by the addition of glucose residues, they are termed *macroglycogen*. These macroglycogen granules are acid soluble and range in size from 400 to 10,000 kDa. Studies in horses have revealed that these two fractions of glycogen granules respond differently during times of glycogen catabolism and anabolism (Brojer et al., 2002; Brojer et al., 2006).

EQUINE MUSCLE COMPOSITION: RESPONSE TO EXERCISE AND ADAPTATIONS WITH TRAINING

MUSCLE COMPOSITION

The development of the muscle biopsy technique brought many new insights into skeletal muscle form and function in the horse (Lindholm and Piehl, 1974; Snow and Guy, 1976). Collected muscle samples were analyzed with a range of physiologic, biochemical, histochemical, and molecular techniques to understand the influences of growth, training, diet, and genetics on equine muscle function.

Open surgical techniques provide large superficially located muscle specimens for study, and the percutaneous needle muscle biopsy technique provides insight into a variety of muscles, sample depths, and longitudinal studies of muscle responses to exercise and training. The muscle most frequently examined in

the horse by percutaneous biopsy is the gluteus medius, or the middle gluteal muscle. This muscle is easily sampled in the unsedated horse, is very active during exercise at a range of speeds, and demonstrates a metabolic response to exercise and training adaptation. Biopsies of the semimembranosus or the semitendinosus are also used in metabolic and training studies, but these can be more difficult or dangerous to obtain in unsedated horses. Although the quadriceps muscles are the muscle of choice in human studies, in horses, this muscle does not show nearly the same degree of training adaptation (Essen et al., 1980). This may be because the quadriceps in horses serves more of a role in the stay apparatus to lock the patella in place at rest rather than to advance the limb during locomotion. Unless otherwise stated, the metabolic responses and training effects reviewed in this chapter refer to the gluteal or the semitendinosus or membranosus muscles.

Percutaneous Muscle Biopsy

Percutaneous needle muscle biopsy safely and relatively atraumatically provides specimens satisfactory for histochemical, biochemical, ultrastructural, and molecular studies (Figure 12-4). In addition, repeated sampling can be carried out during and following exercise bouts without any adverse effect on performance. Following removal of the core of the muscle, the tissue is replaced by regeneration of myofibers rather than by fibrous tissue. Samples ranging from 200 to 500 milligrams (mg) are often obtained using the needle technique. If larger samples are required for investigation, an open excision biopsy of the semitendinosus or semimembranosus is often the most expedient choice. Muscle fiber types vary among different muscles in the same horses as well as across horses and breeds (Essen et al., 1980; Snow and Guy, 1980). Standardization of the site of the muscle biopsy is imperative because of the heterogeneous distribution of muscle fiber types both within the depth and along the length of the same muscle. Deeper regions within locomotor muscles have contractile and metabolic characteristics similar to postural muscles (Rivero et al., 1993a). When sampling site and depth are consistent, repeatable results are obtained (Rivero et al., 1993a). For gluteal muscle biopsies, a site along a straight line from the most dorsal point of the tuber coxae to the head of the tail is often used. A specific length along that line is selected within a given study, usually 15 to 17 cm from the dorsal aspect of the tuber coxae and a specific depth is selected, usually 4 to 8 cm in an adult horse. The depth and length need to be reduced for studies of younger animals to reflect the same relative part of the gluteal muscle as examined in adults.

Prior to obtaining a biopsy, an area of skin of approximately 2.5 cm^2 is shaved, washed, and cleaned with surgical

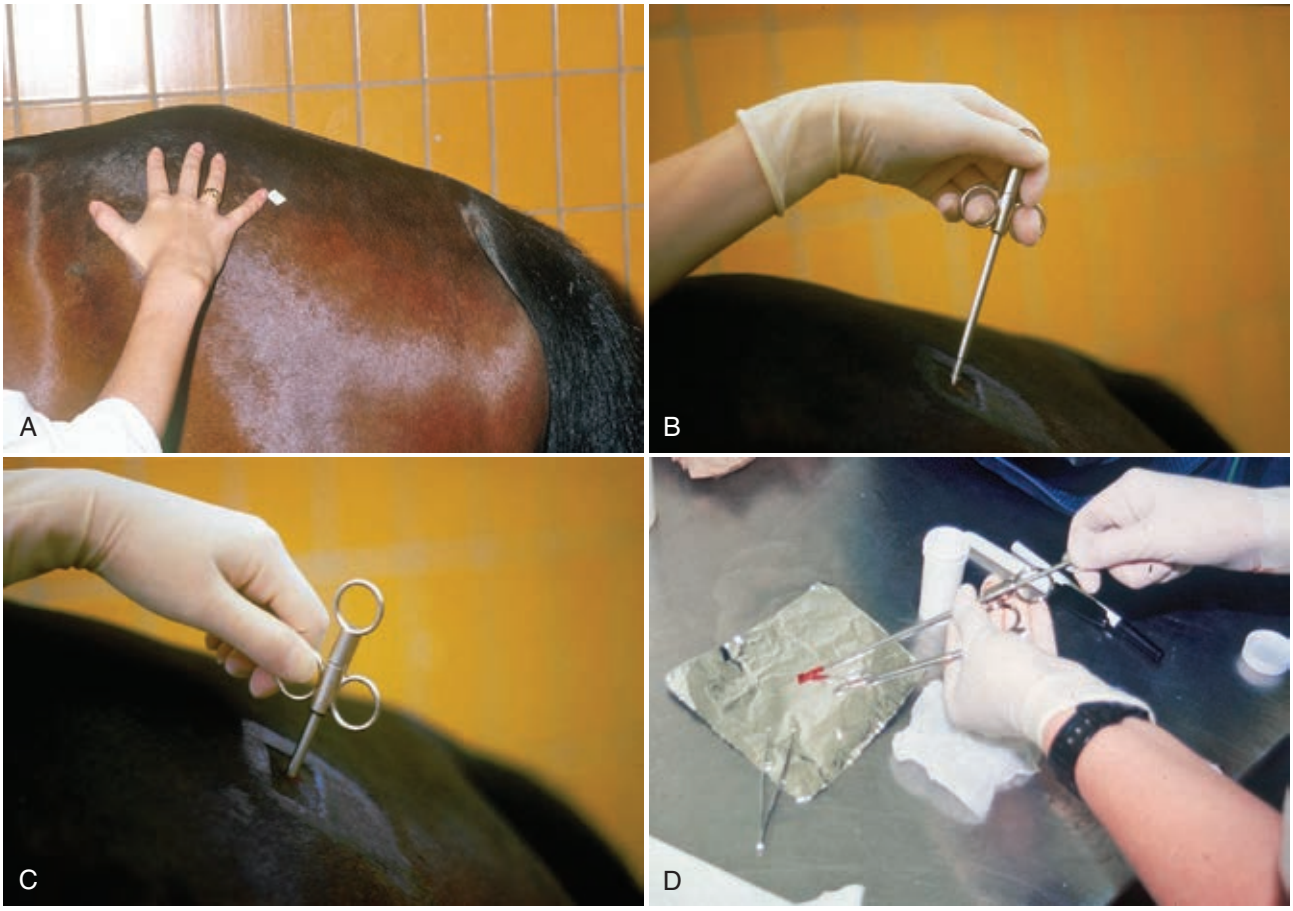

FIGURE 12-4 **A,** Standardized site for obtaining a muscle biopsy 8 inches along a line from the top of the tuber coxae to the tail head shown by the tip of little finger. **B,** Modified Bergstrom needle prior to insertion. **C,** Needle inserted to a depth of 3 inches. **D,** Expressing a muscle biopsy core from the biopsy needle.

spirits or equivalent. Three milliliters (mL) of local anesthetic is then injected subcutaneously at the site of the proposed incision and into the fascia overlaying the muscle, but not into the muscle itself. An incision about 1 cm long is made through the skin and, where necessary, the fascia. The needle together with the cutting cylinder is then inserted into the muscle. Once within the muscle, the cutting cylinder is partially withdrawn, so that the window is opened up. The window is positioned such that it is upward or sideward and pressed firmly against the muscle to catch a small piece of muscle within the needle. Finally, the cutting cylinder is pushed down to detach the trapped muscle. This can be repeated several times so that between 50 and 250 mg of muscle is obtained. The needle is carefully withdrawn, and the excised muscle is removed with the stylet. Good technique allows a 1-cm to 2.5-cm cylindrical-shaped piece to be obtained. The incision is allowed to heal without suturing or with one nonabsorbable suture if a gap exists (see Figure 12-4). This procedure usually can be carried out without using tranquillizers and with minimal restraint. The author has carried out thousands of biopsies with negligible adverse effects. Rarely, a hematoma may result if a larger blood vessel is accidentally cut during the biopsy.

Specimens for histochemical analysis are usually oriented in cross-section and rapidly frozen in isopentane chilled to the appropriate temperature in liquid nitrogen. This technique avoids the formation of *freeze artifacts*, which are large vacuoles that form if water within the cell is allowed to form ice crystals. In the field, histochemical samples may be prepared by coating with talc and dropping the sample into vials containing liquid nitrogen. Samples for biochemistry or isolation of messenger ribonucleic acid (mRNA) should be frozen immediately in liquid nitrogen. Samples for electron microscopy (EM) require appropriate fixation in glutaraldehyde preparations. Ideally, thin sections of muscle for EM should be clamped in vivo to maintain fibers at a resting length before they are excised. However, if the alignment of thick and thin myofilaments is not the subject of interest, small 2-mm to 4-mm muscle pieces can be sectioned from a needle biopsy sample that has been left in room air for 5 minutes and pieces placed directly in an appropriate EM fixative.

Fiber Types

Equine muscle usually has a mosaic of fiber types within a given muscle that can be identified using histochemical methods. This diversity in muscle fiber composition allows a graded response to demands ranging from posture maintenance to rapid movement. A plethora of classification systems have been developed for fiber types; the nomenclature varies, depending on the methods used, number of characteristics examined, and the subdivisions used within each characteristic. Currently, in the horse, the most commonly used nomenclatures for classification are those dependent on the examination of the contractile properties of fibers or one in which this is combined with oxidative capacity.

Myosin–ATPase Differentiation

Histochemical methods based on the sensitivity of myosin ATPase to acid and alkaline preincubation usually identify three contractile fiber types in the gluteal and semitendinosus muscles from horses. Following preincubation at pH 10.4, type I fibers have low myosin ATPase activity, and type II fibers have high activity, whereas this activity is reversed following incubation at pH 4.3. These reactions reflect slow- and fast-contracting fibers, respectively, and therefore are also referred to as *slow (type I)* and *fast (type II) twitch fibers*. Type I fibers also have a slower relaxation time and are more fatigue resistant than are type II fibers.

Type II fibers can be further divided into the subtypes IIA, IIB, and IIC, according to the lability of myosin ATPase following preincubation at either acidic or alkaline pH (Brooke and Kaiser, 1970) (Figure 12-5). A continuum of staining intensity usually exists between the type IIA and IIB fibers (Linnane et al., 1999) (see Figure 12-5). Type IIC fibers do not reverse staining activity in alkaline as well as acid pH and likely represent fibers containing both slow and fast myosin. They can be found in muscles of very young animals but are rare in mature animals unless the fibers are regenerating after an injury.

Unfortunately, due to unknown technical problems, reliable differentiation following preincubation often can be difficult in the horse, since optimal conditions can vary both within and between laboratories.

Immunohistochemical Differentiation

Monoclonal antibodies for specific myosin isoforms provide a more accurate means to discern fiber types in equine skeletal muscle (Linnane et al., 1999). MHC fiber types are based on three adult MHC isoforms that define five fiber types: pure type 1, 2a, and 2x fibers and the hybrids (i.e., coexistence of two MHC isoforms) 1+2a and 2a+2x fibers (Pette and Staron, 1997; Pette and Staron, 2000). Thus, a continuum of activity exists in the hybrid fibers. In situ hybridization with RNA probes specific for each MHC isoform show that the majority of fibers express identical mRNA and protein isoform, whereas hybrid fibers present a mismatch between coexpression at the protein level rather than at the mRNA level (Eizema et al., 2005).

The nomenclature for contractile fiber types differs between myosin ATPase histochemistry and MHC immunohistochemistry. Type IIB fibers were originally named because their myosin ATPase staining properties resembled those of rodent type IIB fibers. However, MHC immunohistochemistry revealed that equine type IIB fibers do not correspond to 2b MHC found in the fastest-contracting rodent muscle fibers. Further, no complementary deoxyribonucleic acid (cDNA) encoding the *2b* gene has been identified in horses. Rather, fibers originally identified as IIB by ATPase stains in horses actually most closely correspond to type 2x or type 2a/x MHC (Linnane et al., 1999).

For the purposes of this chapter, studies in which fiber typing is conducted by immunohistochemistry will use the original histochemical fiber typing of I, IIA, and IIB, and studies using MHC isoforms will use the new fiber typing 1, 2a, and 2x. This distinction is necessary because there is no perfect correspondence between fiber types using these two different methods.

Speed of Contraction

Using skinned single muscle fibers from the equine soleus, which contains three fiber types in horses, the maximum velocity ($\dot{V}_{max}$) of shortening was determined (Rome et al., 1990). In contrast to small animals, where a three- to fivefold difference in $\dot{V}_{max}$ exists between fiber types, the horse has a 10-fold difference, the type IIB fibers shortening more rapidly than would be expected from normal scaling. $\dot{V}_{max}$ was found primarily to be associated with fiber type rather than diameter

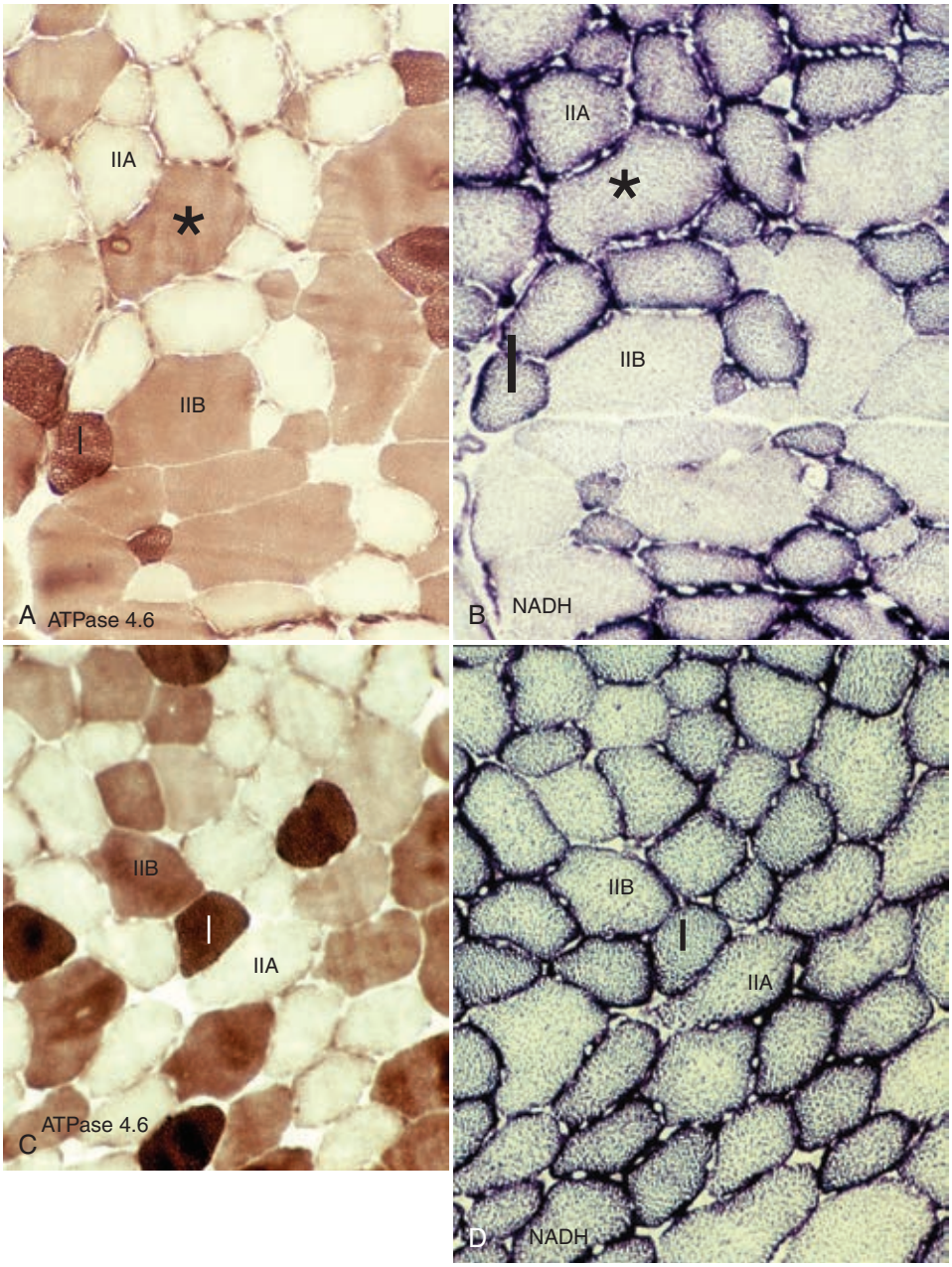

FIGURE 12-5 Histochemical stains of myosin adenosine triphosphatase (ATPase) activity following pH 4.6 acid preincubation illustrating type I, IIA, and IIB fibers in gluteus medius muscle from a conventionally trained Thoroughbred (*A*) and a conventionally trained Standardbred racehorse (*B*). Nicotinamide adenine dinucleotide tetrazolium reductase (NADH) activity in serial sections is shown from the same biopsy showing low-oxidative and high-oxidative fibers. Corresponding type I and IIA fibers are high oxidative in NADH stains whereas corresponding type IIB fibers can be low oxidative (IIB) or high oxidative (*). Note the smaller and more similar cross-sectional areas of muscle fibers in the Standardbred racehorse compared with the relatively large areas for type IIB fibers in the Thoroughbred racehorse.

or force generation. This higher $\dot{V}_{max}$ for type IIB fibers indicates that there is a sacrifice of efficiency for increased mechanical power, since power is higher in fibers with a high $\dot{V}_{max}$ than in those with a lower $\dot{V}_{max}$.

Metabolic Differentiation

In addition to fiber typing solely on the basis of contractile speed, divisions have been made in the horse solely on the basis of metabolic properties or, more commonly, in combination with myosin ATPase activity at pH 9.4. Usually, only oxidative capacity is determined as a metabolic marker, since it is accepted that type I fibers have relatively low glycolytic capacity and all type II fibers have high glycolytic activity. For the assessment of oxidative capacity, fibers are incubated for determination of either succinate dehydrogenase (SDH) or nicotinamide adenine dinucleotide tetrazolium reductase (NADH) diaphorase activity and classed as either having high or low activity (see Figure 12-5). In combination with

myosin-ATPase activity at pH 9.4, this allows fibers to be classified into three categories: (1) slow-twitch high-oxidative (ST), (2) fast-twitch high-oxidative (FTH), and (3) fast-twitch low-oxidative (FT) fibers (see Figure 12-5).

A fairly clear distinction can be made between high- and low-oxidative fibers in the untrained horse, but as training progresses, differentiation becomes more difficult because there is a continuum of activity similar to that seen for myosin-ATPase activity of type II fibers or hybrid 2a+2x fibers. Because type IIB (2x) fibers show the greatest change in oxidative staining with training, some researchers using a type I, IIA, and IIB classification subdivide the IIB fibers into low- and high-oxidative categories (see Figure 12-5). The oxidative differences shown histochemically also have been supported by biochemical studies on pools of single fibers (Valberg et al., 1988). The histochemical reactions for oxidative capacity only provide a relative difference between fibers in the same sample. Biochemical studies have shown that oxidative markers such as citrate synthase (CS) activity could vary as much as twofold in type I and IIA fibers within a horse, whereas histochemical staining showed a similar high-oxidative staining intensity in type I and IIA fibers. Because of the ability of type IIB fibers to have varying oxidative status depending on training status, it is important to realize that the subdivision of type II fibers using metabolic or contractile properties do not correlate exactly. The properties of type I and type II fibers are outlined in Table 12-1.

The glycolytic capacity of muscle fibers can be evaluated using stains for phosphorylase or phosphofructokinase (PFK) enzyme activity. The phosphorylase stain has the disadvantage of being dependent on in situ glycogen, whereas stains coupled to PFK enzyme activity are hampered by the lability of PFK.

TABLE 12–1

Characteristics of Contractile Fiber Types of Skeletal Muscle in Untrained Horses

	Type 1	Type 2a	Type 2x
Fiber size (cross-sectional area/ standard depth)	Smallest	Intermediate	Largest
Specific ATP activity (alkaline pH)	Low	High	High
Specific ATP activity (pH 4.6)	High	Low	Intermediate
Speed for contraction	Low	Intermediate	High
Power output	Low	Intermediate	High
Muscle fibers per motor unit	Low	High	High
Resistance to fatigue	High	Intermediate	Low
Capillary bed	High	Intermediate	Low
Oxidative capacity	Low	Intermediate	High
Lipid content	High	Intermediate	Low
Glycolytic capacity	High	Intermediate	Low
Glycogen content	Intermediate	High	High

ATP, adenosine triphosphate.

Other Features

The periodic acid Schiff's (PAS) reaction shows equine muscle to be rich in glycogen and staining may be slightly less in type I than in type II fibers, whereas oil red O-stain for lipid content is relatively low in horses with intracellular lipid concentrated in the most highly oxidative fibers. Capillarization of fibers has been studied using the PAS reaction following glycogen removal with amylase (Karlstrom et al., 1994). Capillary number can then be expressed either in terms of per fiber or per unit area of fiber type. High-oxidative fibers have a greater capillarization than low-oxidative fibers. Not surprisingly, a relationship exists between the capillary supply and the functional and dimensional capacities of the cardiocirculatory system as well as the structural and biochemical properties of the muscle (Kayar et al., 1989)

Immunofluorescent and Immunohistochemical Stains

Detailed identification of structures within myofibers can be accomplished using monoclonal antibodies coupled to fluorescent tags. The location of insulin-sensitive glucose transporters, GLUT4, in equine muscle either within intracellular storage pools and when actively inserted in the sarcolemma has been characterized by this method (Annandale et al., 2004). Furthermore, localization of isoforms of the calcium (Ca^{2+})–ATPase (SERCA) within equine skeletal muscle has been accomplished by SERCA immunohistochemistry (Eizema et al., 2007; Quiroz-Rothe and Rivero, 2001).

Fiber Areas

Fiber areas can be determined from histochemical preparations. Care has to be taken in selecting sections for this determination, since uneven muscle fiber contraction may occur within samples collected by needle biopsy. A more accurate indication of fiber size can be obtained by measurement of the minimum diameter, since this is not affected by any oblique orientation of the section. Measurement of fiber areas now can he carried out rapidly by using digitized planimetry and specialized software packages. Muscle biopsy investigations generally have found that fiber areas vary according to type, with the type 1 fibers being the smallest and the type 2x low-oxidative the largest, although this can vary between muscles and even within the depth of a muscle (Table 12-2). Fiber area measurements also depend on the histochemical preparation used, since sections reacted for myosin–ATPase activity result in measurements 10% to 20% less than those reacted for SDH (Snow and Guy, 1981).

Ultrastructural Differentiation

High-oxidative fibers have many more subsarcolemmal and intermyofibrillar mitochondria than low-oxidative fibers. Although histochemically type I fibers generally appear more highly aerobic than type IIA fibers, an examination of the semitendinosus revealed that the type IIA fibers had slightly higher mean volume density of mitochondria compared with type I fibers (Hoppeler et al., 1983). It also has been shown that horse muscle has a high mitochondrial content, helping explain the exceptionally high $\dot{V}O_{2max}$ (Hoppeler et al., 1987). Type I fibers have an abundant supply of lipid droplets, whereas fewer are seen in the type II high-oxidative fibers, with low-oxidative fibers not surprisingly having negligible amounts. Glycogen is found as discrete granules throughout

TABLE 12–2

Mean Cross-Sectional Area and Fiber Type Composition*

Horse	Fiber Type	Area (μm^2)	Fiber Type Composition (%)	CS (μmol/g/min)
Standardbred 1	I	1900 ± 8	34	44
	IIA	3900 ± 15	52	40
	IIB	5100 ± 11	14	22
Standardbred 2	I	2100 ± 10	22	34
	IIA	2500 ± 16	50	24
	IIB	7000 ± 26	28	14
Thoroughbred 1	I	2900 ± 8	13	42
	IIA	3300 ± 10	45	31
	IIB	3900 ± 14	42	25
Thoroughbred 2	I	2300 ± 8	16	20
	IIA	3800 ± 9	42	14
	IIB	5300 ± 12	42	10

*Determined from histochemical analysis and activity of the oxidative marker citrate synthase (CS) in pools of fibers of identified fiber type from two Standardbred and two Thoroughbred racehorses. Note the wide variation in oxidative capacity of all fiber types between horses.

the sarcoplasm. With respect to the contractile properties, type II fibers, which have a greater requirement for rapid Ca^{2+} turnover, have a more prominent sarcoplasmic reticulum. Z- and M-band width also can vary between types. In some species, fiber typing has been carried out on the basis of these ultrastructural differences.

Biochemical Assays

A more quantitative means to assess metabolic capacity of skeletal muscle is to measure the activity of enzymes or substrates in whole muscle homogenates or on pools of fibers or single fibers of identified type. Frequently used markers of oxidative capacity include assays of citrate synthase (CS) or succinate dehydrogenase (SDH) activity within the citric acid cycle or 3-OH-acyl-CoA dehydrogenase (HAD) in free fatty acid (FFA) oxidation. Glycolytic capacity is often assessed by determining lactate dehydrogenase (LDH) or phosphofructokinase (PFK) activities. Hexokinase (HK) activity is used to evaluate the capacity for phosphorylation of glucose. Type I fibers in untrained horses have higher activities of CS and HAD and lower activity of LDH compared with type II fibers. Type IIB fibers usually have the lowest oxidative and highest glycolytic activities. However, training can markedly influence these activities such that the CS activity in type IIB fibers of a trained racehorse can be greater than the CS activity of a type I fiber in an untrained horse (see Table 12-2) (Valberg and Essen-Gustavsson, 1987).

Further assessment of the concentrations of triglycerides, glycogen, glucose-6-phosphate, pyruvate, lactate, and adenine nucleotides can be made by fluorometric or high-performance liquid chromatography (HPLC) assays of rapidly frozen muscle samples. Metabolite analyses on whole muscle must be evaluated with caution as this represents only a mean value for the metabolic responses in different fiber types. In some cases, analysis of single fibers may prove most elucidating (Essen-Gustavsson et al., 1997).

Gene Transcription

A rapidly developing application for equine muscle biopsies is the use of real-time reverse transcriptase–polymerase chain reaction (RT-PCR) which provides a means to evaluate gene transcription within muscle under varying metabolic stimuli. Gene sequence and transcription of lactate dehydrogenase, pyruvate kinase, myosin heavy chains, GLUT-4, sodium/potassium ATPase, myosin isoforms glycogen synthase, branching, and debranching enzymes have all been evaluated in horses. Microarrays for gene expression studies in horses are currently under development and should provide further insights into genes that are active in the muscle's acute and chronic responses to exercise and their relationship to performance.

Fiber Type Populations within Muscles

Although very difficult to accurately measure, the number of fibers found in a particular muscle can vary considerably between breeds (Gunn, 1979). Animals bred for speed, such as the greyhound and Thoroughbred racehorse, have a greater number of fibers within the semitendinosus compared with "slower" breeds of these species. The difference is present in early life and becomes more pronounced in the mature animal. There is no reason not to suspect that similar differences occur in other key locomotor, if not all, muscles. Difference in fiber number may be largely under genetic control, although the possibility exists for training-induced hyperplasia.

The fiber type composition of a muscle is expressed as the percentage of fibers of different types examined in a biopsy (out of at least 250 fibers examined) (see Table 12-2). Varying functional requirements result in muscle fiber type composition differing between muscles. However, the fiber type composition will also vary along the length and depth of a muscle due to compartmentalization. This effect on muscle fiber composition in horses has been studied in the horse in greatest detail in the middle gluteal muscle. The ease of sampling and its importance in locomotion make the middle gluteal the most frequently biopsied muscle in equine exercise physiology studies (see Figure 12-4).

The middle gluteal muscle is divided into dorsal and ventral parts by a tendon that runs from the crest of the ilium to the greater trochanter (Bruce et al., 1993). The two compartments have separate points of origin and insertion. The ventral compartment is innervated by the cranial gluteal nerve and the dorsal by the caudal gluteal nerve. The dorsal region is visually considerably darker than the ventral region. Within each compartment, there is generally an increasing proportion of type I fibers with increasing depth, and almost complete disappearance of type IIB fibers occurs as the proportion of high-oxidative fibers increases (Table 12-3). In addition to an increasing proportion of type I and oxidative fibers, a change in fiber size occurs. In superficial sites, type I fibers have the smallest area, with type IIB being the largest, whereas in deeper sites, the predominant type I fibers are the largest. Biochemical variation also occurs through the muscle. Not surprisingly, the deeper within the muscle one goes, the higher are the CS and HAD activities and the lower are the phosphorylase and LDH activities. This diversity within the middle gluteal muscle suggests that the deeper portions have a more postural function, whereas the more superficial portions are recruited with increasing workloads. This nonhomogeneity also has been described in a number of other muscles.

TABLE 12–3

Mean ± Standard Deviation Percentage of Each Fiber Type at Different Depths within the Middle Gluteal Muscle (3 Horses, 10 Sample Sites per Horse at Each Depth)

Sampling Depth	Type I	Type IIA	Type IIB
2 cm	21.6 ± 8	36.5 ± 5	42.0 ± 9
4 cm	35.5 ± 9	35.0 ± 4	29.5 ± 8
6 cm	49.5 ± 10	34 ± 7	16.35 ± 9
8 cm	61.5 ± 12	34 ± 10	4.5 ± 6
F values			
F depth	88.18*	0.96	12.14*
F site	3.67†	1.11	4.43‡

Note: Variance ratios of a two-way ANOVA testing variation in fiber types attributable to different sampling depths (F depth) and different sample site (F site):
*p <0.001; †p <0.01; ‡p <0.05.
Adapted from Lopez-Rivero JL, Serrano AL, Diz AM, et al: Variability of muscle fiber composition and fiber sizes in the horse gluteus medius: an enzyme-histochemical and morphometric study, *J Anat* 181:1, 1992 with permission.

TABLE 12–4

Fiber Composition in the Middle Gluteal of Different Breeds of Horses

	n	ST	FTH	FT
Quarterhorse	28*, ‡	8.7 ± 0.8	51.0 ± 1.6	40.3 ± 1.6
Thoroughbred	22*, †	7.3 ± 0.9	61.2 ± 1.5	28.8 ± 1.5
Thoroughbred	50*, ‡	11.0 ± 0.7	57.1 ± 1.3	32.0 ± 1.3
Arab	6*	14.4 ± 2.5	47.8 ± 3.2	37.8 ± 2.8
Standardbred	9*, †	18.1 ± 1.6	55.4 ± 2.2	26.6 ± 2.0
Standardbred	8	24.0 ± 3.6	49.0 ± 3.1	27.0 ± 3.3
Shetland Pony	4*	21.0 ± 1.2	38.8 ± 1.9	40.2 ± 2.7
Pony	8*	22.5 ± 2.6	40.4 ± 2.3	37.1 ± 2.8
Donkey	5*	24.0 ± 3.0	38.2 ± 3.0	32.1 ± 3.4
Heavy Hunter	7*	30.8 ± 3.1	37.1 ± 3.3	37.8 ± 2.8

*Out of work.
†Elite stallions at stud.
‡Elite broodmares.
Note: % Fiber Type is expressed as slow twitch (ST), fast-twitch high-oxidative (FTH) and fast-twitch low-oxidative (FT) based on ATPase and NADH stains; (Mean ± SEM).
Adapted, with permission, from Snow DH: Skeletal muscle adaptations: a review. In Snow DH, Persson SGB, Rose RJ, editors: *Equine exercise physiology,* Cambridge, UK, 1983, Granta Editions, p 160.

Nonuniformity of fiber types within a muscle is an important consideration when trying to compare fiber composition in longitudinal and cross-sectional studies. If a constant site has to be sampled, allowance has to be made for the overall size of the muscle. When comparing results in a foal with those in an adult, for example, sampling depth at the same site should be approximately 2.5 cm in the foal and approximately 5 cm in the adult. In addition, frequent sampling from the same animal also requires confidence that a similar site can be sampled. During biopsy of the middle gluteal muscle in the adult horse, most workers have adopted a site 10 to 15 cm caudodorsal from the tuber coxae and at an angle of 45 degrees at a depth of 5 to 10 cm in the adult (see Figure 12-4). Samples taken at a uniform depth and within about a 5-cm radius of this point give reproducible results with slight variation in fiber types (Snow, 1983). Although this difference may require caution when fiber typing is done in an attempt to evaluate performance potential, a relatively small difference does allow repeated sampling in short time frames, for example, in recovery studies following bouts of exercise. At a similar site, no difference exists between samples taken from the right or left middle gluteal of normal muscle.

The standardized fiber type composition of the gluteal muscle varies between breeds and between individual horses and is impacted by training (Table 12-4 and Table 12-5). In addition, gender also has a significant effect on fiber composition. A higher proportion of type I fibers is found in the gluteal muscle of Andalusian stallions versus mares but this is not so in Arabians (Rivero et al., 1995). Large-scale studies of Standardbreds and Thoroughbreds have found no difference between stallions and mares in the proportion of type I fibers, but stallions have a higher ratio of type IIA to type IIB fibers beginning at 1 year of age (Roneus, 1993; Roneus et al., 1991; Roneus et al., 1992). In general, the higher the composition of type IIB fibers in a muscle biopsy, the lower is the oxidative capacity in muscle and the more is the lactate that is produced with maximal exertion such as racing (Figure 12-6 and Figure 12-7).

Muscle Fiber Recruitment

For smooth, coordinated locomotion, muscles are recruited in an orderly manner, with both extensors and flexors being involved during each stride cycle. Within a particular muscle, only certain portions may operate, having different and often complex functions due to functional compartmentalization. Even within a recruited muscle, not all fibers are stimulated, since it is not generally necessary for muscle to generate maximum tension. Fibers are selectively recruited in a specific pattern that varies according to the gait, speed, and duration of exercise. This occurs through the differential stimulation of α–motor neurons in line with the size principle. The smallest-diameter motor neurons, which have the lowest threshold, innervate the type 1 fibers, whereas the largest innervate the type 2x fibers. One of the techniques used to study fiber recruitment patterns has involved the examination of glycogen depletion patterns using either semiquantitative or quantitative analysis (Valberg, 1986). For the maintenance of posture and for exercise at low speeds, it is only generally necessary to recruit type 1 fibers. As the speed of movement increases, the development of more tension to generate the required torque is necessary, and type 2a and then 2ax fibers are recruited. The very forceful contractions required for rapid acceleration and maintenance of high speeds or for jumping result in the additional recruitment of first the type 2x high oxidative and then the 2x low oxidative fibers.

RESPONSE TO EXERCISE

The energy systems required to fuel muscle contraction, and hence performance, have been described in detail in Chapter 3. A review of that chapter may be necessary prior to reading this section on the mechanisms involved in fatigue of skeletal muscle during aerobic and anaerobic exercise. The duration and intensity of the exercise are central to the process by which fatigue occurs. A reduction in performance from the required

TABLE 12–5

Percent Fiber Type Composition of Various Horse Breeds Based on Immunohistochemical Staining for Contractile Fiber Type

Breed	Type 1	Type 2a	Type 2ax	Type 2x	Muscle
Thoroughbred	11.7 ± 5.0	47.3 ± 5.1	No data	41.0 ± 5.1	gluteal
Thoroughbreds	12.3 ± 4.8	43.5 ± 5.5	15.6 ± 6.4%	34.5 ± 4.1	gluteal
Quarter horses	10.7 ± 1.8	32.9 ± 1.5	No data	56.4 ± 1.1	semimembranosus
Standardbreds	17 ± 12.2	46 ± 1.4	10 ± 1.4	27 ± 2.8	gluteal
Andalusians	41 ± 2	35 ± 3	15 ± 1	7 ± 1	gluteal

From Karlström K, Essén-Gustavsson B: Myosin heavy chain-based fibre types in red cell hyper- and normovolaemic Standardbred trotters, *Equine Vet J* 34(Suppl):279, 2002; Rivero JL, Ruz A, Martí-Korff S, et al: Effects of intensity and duration of exercise on muscular responses to training of thoroughbred racehorses, *J Appl Physiol* 102:1871, 2007; Serrano AL, Quiroz-Rothe E, Rivero JL: Early and long-term changes of equine skeletal muscle in response to endurance training and detraining, *Pflugers Arch* 441:263, 2000; Yamano S, Eto D, Sugiura T, et al: Effect of growth and training on muscle adaptation in Thoroughbred horses, *Am J Vet Res* 63:1408, 2002.

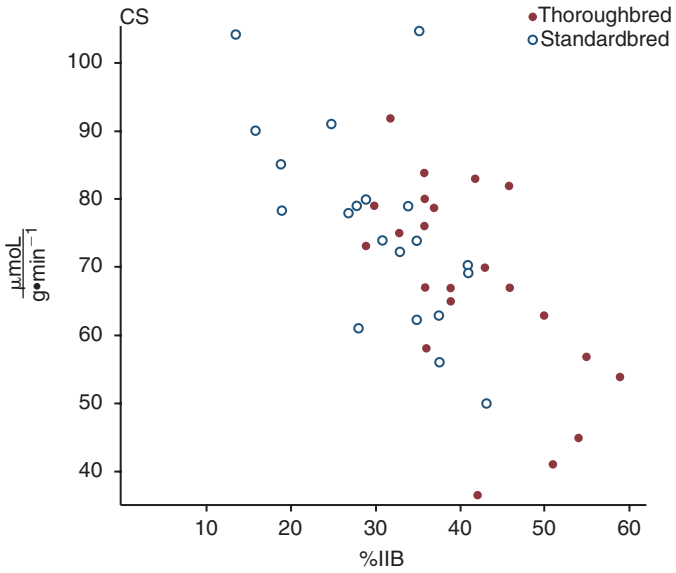

FIGURE 12-6 The relationship between oxidative capacity, measured as citrate synthase activity, and percentage of type IIB fibers in muscle biopsies from competitive Standardbred and Thoroughbred racehorses. Note the negative linear relationship between oxidative capacity and the percentage of type IIB fibers. Although there is a trend for Thoroughbreds to have a higher percentage of type IIB fibers than Standardbreds, considerable overlap exists between breeds. *(From Valberg SJ: Metabolic response to racing and fiber properties of skeletal muscle in Standardbred and Thoroughbred horses,* Equine Vet Sci *1:6, 1987.)*

level is an indicator of the onset of fatigue. The inability of skeletal muscle to maintain power and speed is generally not due to sudden cessation of contractile activity in all muscle fibers but rather due to a selective impairment of muscle cell function, depending on the pattern of fiber recruitment. It could occur for a variety of reasons, including the following:

- Central nervous system impairment of muscle fiber recruitment
- Depletion of a substrate necessary for energy production
- Accumulation of a metabolite in the internal milieu, resulting in impaired energy flux or contraction
- Changes in electrolyte gradients that alter neuromuscular excitability
- Alterations in Ca^{2+} uptake or release by the sarcoplasmic reticulum interfering with the contractile process
- Excessive increase in muscle temperature
- Inadequate blood flow or oxygen delivery

The degree to which these factors are responsible for fatigue depends on the intensity and duration of exercise, the level of training, and a number of environmental factors. In many cases, it is likely that a combination of these factors operate. To date, understanding of the likely contributing factors to muscle fatigue in the horse has come from the collection of biopsy samples following different intensities and durations of exercise.

Fatigue with High Intensities of Exercise

The effects of high-intensity exercise on muscle have been studied by exercising horses on a treadmill, running on a track, or doing draught work. Overall, the metabolic changes seen with the highest-intensity exercise are greater than those reported in humans and other species and are a further indication of the horse as an elite athlete. These changes can be associated with the disproportionally high $\dot{V}O_{2max}$ and muscle enzyme activities in this species.

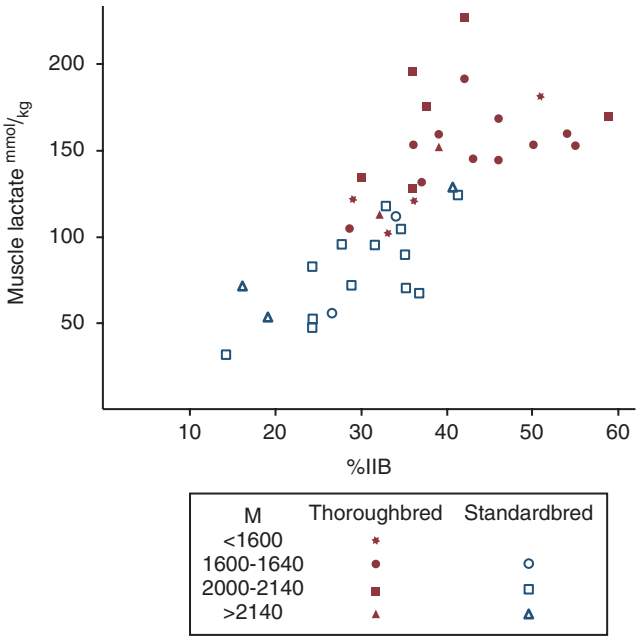

FIGURE 12-7 The relationship between the accumulation of lactate in the gluteal muscle after racing and the percentage of type IIB fibers in Standardbred and Thoroughbred racehorses competing over a variety of distances. Thoroughbred horses with a higher percentage of type IIB fibers had higher lactate concentrations than those horses with fewer type IIB fibers but there is no relationship between muscle lactate concentrations and placement in a race. *(From Valberg SJ: Metabolic response to racing and fiber properties of skeletal muscle in Standardbred and Thoroughbred horses,* Equine Vet Sci *1:6, 1987.)*

Lactate Accumulation

It is now accepted that with almost all intensities of exercise a degree of anaerobic metabolism and production of lactate occurs (Table 12-6). Production of lactate is accompanied by hydrogen ions, which can alter cellular pH. Once produced, lactate is transported across the sarcolemma into the bloodstream by monocarboxylate transporters. At lower intensities, very little or no change is seen in blood lactate concentration, since removal keeps pace with production. However, as the intensity of exercise increases and progressively more type 2 fibers and then especially type 2x low-oxidative fibers are recruited, energy production becomes increasingly dependent on anaerobic metabolism and the consequent formation of lactate (see Figure 12-7). With repeated bouts of exercise or after racing, muscle lactate concentrations in excess of 200 millimoles per kilogram (mmol/kg) of dry weight have been recorded (Sewell and Harris, 1992), with the associated proton accumulation leading to a marked decline in muscle pH. Single bouts of exercise lead to lower muscle lactate concentrations, which are related to both intensity and, at higher workloads, the duration of exercise. Galloping, compared with trotting races, results in greater anaerobic demands because of faster speeds. Mean muscle lactate concentrations of 82 and 148 mmol/kg of dry weight were found after Standardbred and Thoroughbred races, respectively (see Figure 12-7) (Valberg, 1987). A high correlation exists between lactate accumulation and the percentage of type IIB (2x) fibers in the muscle (Valberg, 1987).

A point is reached where lactate efflux mechanisms from muscle fibers to the bloodstream are probably saturated, and rapid accumulation of intracellular lactate and its consequent effects on pH commence. In horses, saturation of lactate removal occurs at concentrations of 10 mmol and 15 mmol for whole blood and plasma, respectively (Marlin et al., 1987). Blood lactate concentrations on the order of 25 to 30 millimoles per liter (mmol/L) are seen in Thoroughbreds and Standardbreds after racing, which corresponds to venous blood pH declining to about 7.0. Following cessation of high-intensity exercise, lactate disappearance normally occurs at a linear rate, which can be hastened by submaximal exercise (Marlin et al., 1987). Training also may increase the rate of removal of lactate.

Acidosis in muscle can lead to impairment of glycolysis and the respiratory capacity of mitochondria, and both may be associated with a decline in muscle ATP concentration. However, although lactate accumulation may contribute to muscle fatigue, it is not a simple relationship. For example, there is

TABLE 12–6

Muscle and Blood Metabolites before and after 2 Minutes of Exercise at Varying Intensities on a Treadmill at 5% Incline (Thoroughbreds, Mean ± SD, n = 6)

	Speed (m/s)							
	Pre-exercise	6	7	8	9	10	11	12
Muscle ATP (mmol/kg/dm)	22.8 ± 1.3	22.4 ± 1.8	22.2 ± 1.7	21.8 ± 1.7	21.6 ± 2.0	20.4 ± 2.1	18.0 ± 2.9	13.8 ± 1.9
Muscle lactate (mmol/kg/dm)	9.7 ± 1.3	19.1 ± 7.5	23.9 ± 11.1	43.7 ± 13.9	57.3 ± 13.1	76.5 ± 31.0	97.0 ± 28.7	132.9 ± 19.5
Muscle pyruvate (mmol/kg/dm)	0.4 ± 0.1	0.5 ± 0.2	0.3 ± 0.2	0.5 ± 0.2	0.6 ± 0.3	0.8 ± 0.4	0.7 ± 0.4	1.0 ± 0.5
Blood lactate (mmol/L)	0.5 ± 0.2	3.5 ± 1.4	4.9 ± 2.1	7.9 ± 2.9	11.1 ± 2.2	13.6 ± 3.8	17.8 ± 6.2	23.8 ± 3.3
Plasma ammonia (μmol/L)	75 ± 24	110 ± 40	114 ± 14	145 ± 35	178 ± 72	315 ± 92	536 ± 215	792 ± 397

ATP, adenosine triphosphate; *m/s,* meters per second, *kg/dm,* kg/dry matter

Adapted, with permission, from Harris RC, Martin DJ, Snow DH, et al: Muscle ATP loss and lactate accumulation at different work intensities in the exercising thoroughbred horse, *Eur J Appl Physiol* 62:235, 1991.

not a direct correlation between muscle lactate concentrations and placement in a race (Valberg, 1987) or between postrace plasma lactate concentrations and performance index (Roneus et al., 1993; Roneus et al., 1994).

Nucleotide Depletion

A decline in muscle ATP after maximal exercise also has been shown in many investigations in conjunction with the expected high muscle lactate concentrations (see Table 12-6) (Essen-Gustavsson et al., 1997; Harris et al., 1991; Valberg and Essen-Gustavsson, 1987). ATP provides energy in the form of inorganic phosphate (Pi) and results in the production of adenosine diphosphate (ADP). The myokinase reaction coupled with the need to maintain a high ATP:ADP ratio during maximal exercise leads to a series of enzymatic reactions, where ADP is converted to adenosine monophosphate (AMP), which is then deaminated by AMP deaminase to form inosine monophosphate (IMP). AMP deaminase is activated at low intramuscular pH (6.5) and has high activity in equine muscle. Recovery of normal muscle ATP content does not occur rapidly after the cessation of high-intensity exercise, taking over 1 hour, depending on the extent of the initial depletion. Restoration is dependent on the purine nucleotide cycle and reamination of IMP.

The importance of nucleotide depletion has been shown by measuring a decline in muscle ATP concentration ranging from 14% to 50% after both trotting and galloping races, and greater depletion occurs with 2000 m of maximal exercise compared with 800 m (Harris et al., 1987). Muscle ATP concentration begins to decrease after muscle lactate exceeds 40 mmol/kg to 80 mmol/kg of dry muscle and muscle pH falls below 6.8. Repeated bouts of exercise have indicated that a relationship may exist between running time and muscle ATP content (Harris and Snow et al., 1985). However, ATP concentrations in homogenates of muscle biopsies have not been shown to be directly related to finishing position in a race (Valberg, 1987). One explanation for this may be that fatigue may not be as closely related to the average ATP concentration in a homogenized muscle sample as to the ATP concentration in single muscle fibers. A study on pooled single fibers dissected from biopsy samples collected after racing found greatest ATP depletion in type IIB fibers with little change in type I fibers (Valberg and Essen-Gustavsson, 1987). Additionally, another study of single fibers obtained from Standardbred trotters after racing found ATP concentration as low as 1 to 5 mmol/kg dry muscle in individual type IIB fibers (Figure 12-8), whereas whole muscle samples showed a mean ATP concentration of 18.3 mmol/kg after the race (Essen-Gustavsson et al., 1997). Thus, ATP concentration in individual fibers may have the greatest impact on fatigue. The importance of ATP concentration relative to fatigue is underscored by the negative correlation between stride frequency at the time of fatigue and accumulation of ADP in muscle (Schuback et al., 1999). Whether the decline in ATP concentration is a major contributory factor in fatigue is still uncertain, but it does seem probable that the very low levels in some fibers would impair the optimal functioning of many of the ATP-dependent processes such as contraction, reuptake of Ca^{2+} by SR, and the sodium–potassium (Na^{+}– K^{+}) exchange.

A marked decline in phosphocreatine (PCr) to approximately 30% of baseline values also has been measured in a number of the previously described studies. The exact decline in PCr is difficult to determine, since rapid recovery occurs after the cessation of exercise.

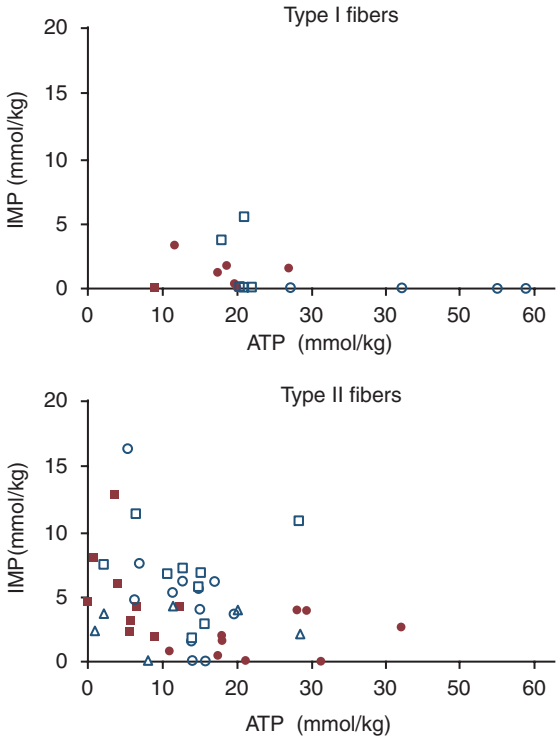

FIGURE 12-8 The concentration of inosine monophosphate (IMP) in relation to adenosine triphosphate (ATP) in portions of individual muscle fibers from the gluteus medius muscle of five Standardbred horses (five different symbols) after a race. Note the very low ATP concentrations in some type II muscle fibers and correspondingly high IMP concentrations indicating adenine nucleotide degradation.

Potassium

Alterations in plasma potassium indicate that losses occur from recruited muscle fibers during high-intensity exercise (Harris and Snow, 1988). This is supported by atriovenous differences in potassium concentrations in horses performing strenuous exercise which also show that potassium released from contracting muscle tissue is taken up by noncontracting muscle (Schott et al., 2002). With increasing intensity of exercise and progressive recruitment of muscle fibers, there is a progressive increase in plasma potassium concentration, and for short-duration maximal activity, this is highly correlated with lactate production. During maximal efforts leading to plasma lactate concentrations in excess of 30 mmol/L, plasma potassium concentrations above 10 mmol/L occur (Harris and Snow, 1988). With duration of exercise greater than 2 minutes, a plateau for plasma potassium concentration is reached. This is thought to reflect an equilibrium between release from active muscle and reuptake by inactive fibers which are under β_2-adrenergic receptor control. However, at the very highest workloads (lasting less than a few minutes), there is a continual rise until the onset of fatigue, since reuptake cannot match release (Figure 12-9). This efflux from muscle is solely due to electrical activity of the exercising muscle independent of acidosis or glycogen breakdown. Immediately on the cessation of exercise, there is a rapid decline in plasma potassium as almost immediate reuptake into now inactive muscle

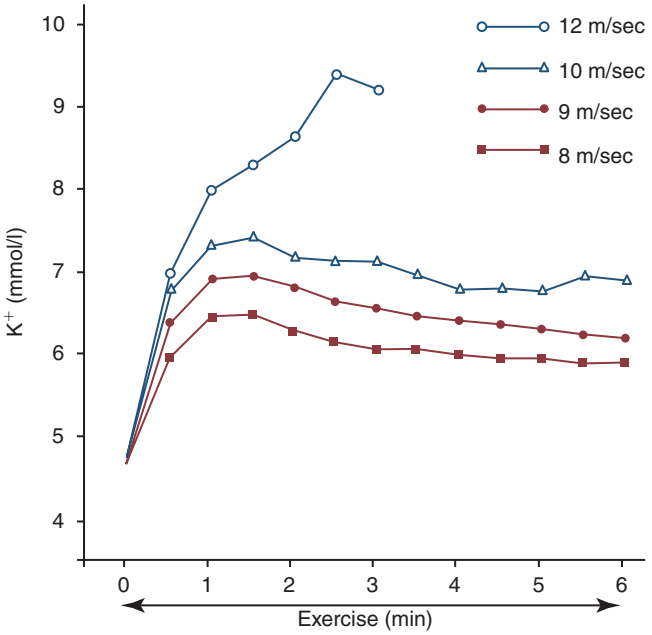

FIGURE 12-9 Estimated mean plasma potassium concentrations in horses in response to exercise. *(With permission from Harris P, Snow DH: Plasma potassium and lactate concentrations in thoroughbred horses during exercise of varying intensity,* Equine Vet J *23:220, 1992.)*

occurs. The large changes in intramuscular potassium concentration could result in altered sarcoplasmic membrane potential and could be a contributory factor to fatigue in maximal exercise.

In addition to the preceding major changes which may contribute to fatigue, there are numerous other metabolic changes occurring within muscle, possibly resulting in reduced performance capacity.

Glycogen

Although a decline in muscle glycogen concentration (both pro- and macro-glycogen) occurs rapidly with maximal exercise, this is not thought to be a contributory factor in fatigue for a single bout of exercise (Brojer et al., 2006; Davie et al., 1999). Run time to fatigue at 115% of $\dot{V}O_{2max}$ was not affected by a 22% depletion of glycogen (Davie et al., 1999). Not surprisingly, the extent of glycogen utilization is related to the amount of lactate produced. Single bouts of exercise at maximal intensity only cause a reduction in whole-muscle glycogen in the order of 30%, whereas with repeated bouts, reductions of up to 50% may occur. From studies using both single bouts of exercise of varying duration and repeated bouts of high-intensity exercise, the highest proportion of glycogen is utilized during the initial stages or first bout of exercise. This is probably due to the higher anaerobic metabolism earlier in exercise and before complete circulatory adjustment and potential to utilize bloodborne glucose as a substrate. In the early stages of maximal exercise, glycogen utilization rates may reach 160 mmol glycosyl units per kilogram dry muscle per minute (Snow et al., 1985). Glycogen depletion may play a role in fatigue in horses that perform repeated bouts of exercise prior to an anaerobic exercise session because glycogen repletion rates are so low in horses (Lacombe et al., 1999; Lacombe et al., 2001). Three-day event horses, for example, may have a 60% reduction in muscle glycogen concentrations prior to the stadium jumping effort (Hodgson et al., 1985). Under these circumstances, glycogen depletion could play a role in fatigue. In an experimental protocol, glycogen depletion of 78% before maximal exercise was associated with decreased run time to fatigue and decreased anaerobic capacity (Lacombe et al., 1999).

Histochemical studies of glycogen depletion have shown that after both racing and repeated bouts of exercise leading to ATP depletion, glycogen was still present in even the fibers with highest anaerobic capacity (IIB) (Figure 12-10). If exercise intensity is high enough to recruit all fibers, glycogen utilization and hence depletion will occur most rapidly in the type IIB low-oxidative fibers as they use this substrate to produce ATP with an associated formation of lactate. However, since type I fibers contain the lowest glycogen stores, they often appear to have less glycogen in PAS stains of muscle biopsies obtained from horses after maximal exercise (see Figure 12-10).

Muscle Temperature

A progressive rise in muscle temperature is associated with increasing intensity of exercise and increasing metabolic activity. Temperatures in excess of 43°C have been recorded. Although moderate increases in temperatures are considered favorable for metabolic activity, the very high levels seen at highest intensities of exercise may be a contributory factor to fatigue, not only by altering Ca^2+ uptake by SR but also in hot environments by redistribution of cardiac output away from contracting muscle.

Effect of Warmup

Oxidative substrate utilization during high-intensity sprint exercise can be improved in horses if it is preceded by either a high- or low-intensity exercise warmup (McCutcheon et al., 1999a).

Fatigue with Prolonged Low-Intensity (Submaximal) Exercise

The cause of fatigue during submaximal exercise likely varies depending on the intensity and duration of exercise, initial stores of liver and muscle glycogen, environmental temperature, as well as the motivation of the horse to continue exercise. Although small amounts of lactate may be produced with aerobic exercise, it appears to be taken up and used as a substrate by other muscle fibers as concentrations decline over time in muscle at low exercise intensities. Adenine nucleotide degradation has been demonstrated in skeletal muscle of horses exercised to exhaustion at 7 m/s (48–58 min) and 10 m/s (10–115.5 min) (Essen-Gustavsson et al., 1999). Concentrations of ATP, ADP, and AMP were unchanged, but IMP concentrations increased substantially, indicating fatigue could be related to adenine nucleotide degradation potentially due to substrate depletion.

Substrate Depletion

As exercise is initiated at submaximal speeds metabolism shifts from reliance on intramuscular glycogen and blood glucose to metabolism of circulating FFAs and intramuscular triglyceride stores (Figure 12-11). Amino acids may contribute up to 10% of energy during prolonged submaximal exercise

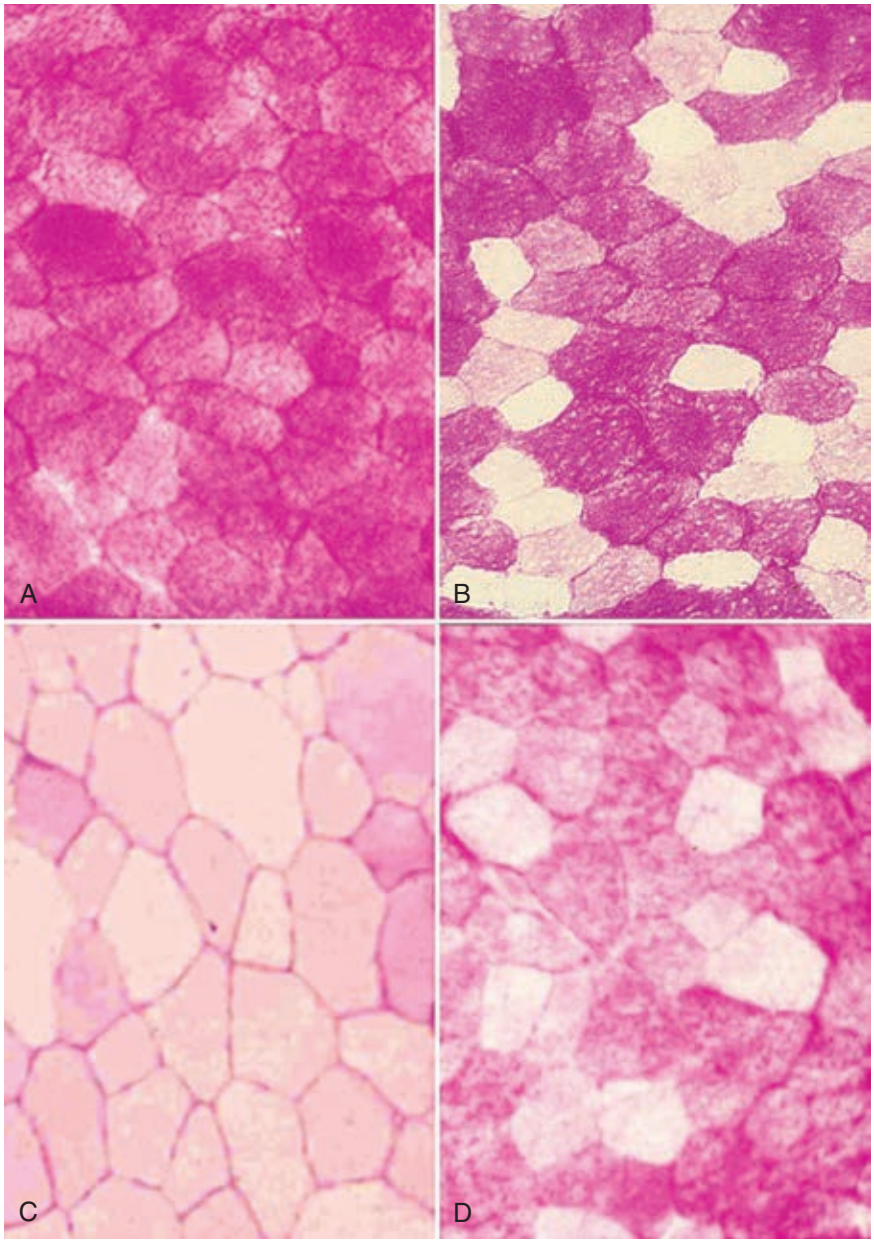

FIGURE 12-10 Periodic acid Schiff's (PAS) stains showing muscle glycogen depletion patterns in a Standardbred trotter at rest (*A*), the same horse after trotting at 5 meters per second (m/s) for 100 minutes (*B*), another horse that completed a 100-km endurance race (*C*), and a Standardbred trotter after an 1800 m race (*D*). Note the selective depletion of glycogen in some fibers (type 1 and IIA) after 100 minutes of trotting and complete depletion of glycogen in all fiber types after the endurance race. In contrast, a shorter duration high-intensity effort such as racing results in a degree of glycogen depletion in all fiber types with type I fibers showing the most depletion. Adenosine triphosphatase (ATPase) stains are not shown.

(Essen-Gustavsson et al., 2002). At 35% $\dot{V}O_{2max}$ intramuscular glycogen provides about 50% of energy for the first 30 minutes of exercise, which declines to less than 20% of energy utilized after 1 hour of exercise (Geor et al., 2000). Compared with glycogen, plasma glucose makes a smaller contribution to energy expenditure during submaximal exercise, supplying approximately 10% of oxidized carbohydrate (Geor et al., 2000).

Fat

Circulating FFA concentrations increase within 15 minutes of low-intensity exercise, and assume increasing importance as a substrate for muscular work as exercise continues. Uptake of circulating FFA into muscle is aided by high circulating concentrations enhancing diffusion of short-chain and medium-chain FFA across muscle membranes. In contrast, long-chain fats are translocated across the sarcolemma by fatty acid transporters. Transport of FFA into mitochondria for β-oxidation is facilitated by an acylcarnitine transport system. Significant increases in short-chain, medium-chain, and long-chain acylcarnitines occur in plasma of horses during prolonged submaximal exercise (McCue et al., 2009). It would be expected that intramuscular triglyceride depots also would be used

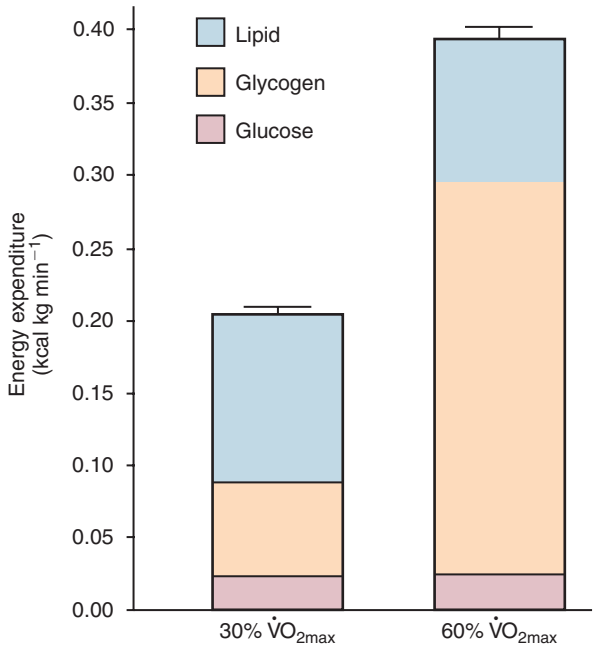

FIGURE 12-11 The contribution of various substrates for energy generation during 20 to 30 minutes at 30% of maximum oxygen uptake and 35 to 45 minutes at 60% of maximum oxygen uptake for six horses. With increasing work intensity, there is an increase in muscle glycogen utilization, with a concomitant reduction in the rate of lipid oxidation in equine skeletal muscle. *(Modified from Geor R, et al: Glucose infusion attenuates endogenous glucose production and enhances glucose use of horses during exercise,* J Appl Physiol *88:1765, 2000.)*

during submaximal exercise in horses. This has been shown biochemically (Essen-Gustavsson et al., 1984), although the extent is quite variable between animals. The body contains abundant lipid stores to maintain energy supplies for days but fatigue sets in with prolonged endurance exercise, before complete utilization of lipid depots has occurred. This may be because glycogen depletion has occurred within the liver and active muscle, and oxidation of FFA cannot provide sufficient ATP without a source of pyruvate. Glycogen provides pyruvate which, through conversion to oxaloacetate, replenishes intermediates in the citric acid cycle allowing acetyl coenzyme A (CoA) derived from β-oxidation of FFA to enter the first step of the citric acid cycle. A continuous supply of pyruvate from glucose or glycogen is, therefore, essential for metabolism of FFA.

Muscle Glycogen

Continuous utilization of muscle glycogen stores is vital during prolonged submaximal exercise and macroglycogen appears to be used to a greater extent than proglycogen during endurance exercise (Essen-Gustavsson et al., 2002). Glycogen provides pyruvate, which can be metabolized to acetyl CoA and enter the citric acid cycle. During submaximal exercise, glycogen depletion patterns have shown a progressive recruitment of muscle fiber types from type I to IIA to IIB (see Figure 12-10). Because of the relative low intensity of exercise, initial activity only requires recruitment of type I and some type IIA fibers, but as they become fatigued and depleted of glycogen, recruitment of new fibers occurs. At the point of fatigue in elite endurance events, both biochemical and histochemical studies have shown negligible muscle glycogen even in type IIB fibers (see Figure 12-10) (Essen-Gustavsson et al., 1984). At this stage, only very low-intensity exercise can be maintained, since energy is dependent on utilization of FFA by oxidative fibers. In horses, complete repletion of glycogen is remarkably slow, taking up to 72 hours despite consumption of starch and sugar (Jose-Cunilleras et al., 2006). Following glycogen depletion, repletion occurs in the reverse order to depletion, that is, initially in type IIB, then in type IIA, and finally in type I fibers. For horses competing in prolonged endurance events or strenuous competition over several days, glycogen depletion may play a role in the onset of fatigue (Lacombe et al., 2003). In other cases, fatigue develops during submaximal exercise prior to the onset of depletion of muscle glycogen (Farris et al., 1998).

Blood Glucose

Serum insulin concentrations decline during exercise and blood glucose concentrations are maintained within a normal range by continuous release of glucose from liver glycogen stores. Although dependent on dietary carbohydrate content, depletion of liver glycogen normally requires prolonged exercise of 72 km or more (Lindholm et al., 1974). Therefore, under most circumstances liver glycogen depletion may not have a primary role in fatigue.

Hyperthermia and Electrolyte Depletion

Another possible mechanism for fatigue in these horses is hyperthermia. Prolonged submaximal exercise, particularly when performed under hot and humid conditions, may achieve a critical maximal body temperature that precipitates central fatigue. During prolonged exercise in ponies, a narrow range of core body temperatures between 40.2°C and 41.7°C occurred at fatigue (McConaghy et al., 2002). Electrolyte derangements such as hypocalcemia, hypomagnesemia, hypokalemia, and dehydration may also induce muscle weakness and fatigue in endurance horses sustaining electrolyte losses in sweat.

Central Fatigue

Many horses exhibit unwillingness to continue exercise prior to developing near catastrophic failure of muscle function. For some horses, fatigue may be of central origin, where nociceptive input from muscles to the brain reduces motivation to continue exercise (Noakes et al., 2004). Such circumstances are impossible to assess in horses, although, anecdotally, motivation to compete is highly variable between individual horses. A central role for fatigue in horses was support from studies where intravenous infusion of tryptophan to exercising horses resulted in a decrease in run time to fatigue at 50% $\dot{V}O_{2max}$ (Farris et al., 1998).

Central fatigue may be the result of an exercise-induced increase in brain serotonin concentration which may reduce motor drive. The possible mechanism for this may be increasing plasma FFA with exercise that displace tryptophan from binding sites on plasma albumin resulting in an increase in unbound or free plasma tryptophan. During exercise, muscle uptake of branched chain amino acids (BCAA) lowers plasma BCAA concentration, and the increase in free tryptophan coupled with the decrease in BCAA leads to enhanced transport of tryptophan across the blood–brain barrier. Because free tryptophan and BCAA compete for the same brain transport mechanism, the increased availability of brain tryptophan

provides substrate for increased synthesis and possible release of serotonin (Acworth et al., 1986; Farris et al., 1998).

In summary, the precise mechanisms by which fatigue occurs with submaximal exercise situations are unknown and likely encompass a variety of the factors described above, depending on the duration of submaximal exercise and environmental conditions.

MUSCLE ADAPTATION: EFFECTS OF AGE AND TRAINING

It is likely that muscle is the most adaptable of all tissues, showing a wide range of both immediate (exercise) and long-term (age, training, nutrition) responses. Changes can occur in the morphologic, metabolic, and contractile properties of muscle fibers. This has led to the term *plasticity* being used to describe the adaptability of skeletal muscle.

Changes in Early Life (Growth)

Growth occurs mainly via a marked increase in the length and cross-sectional area of existing muscle fibers after birth. Fibers lengthen through the insertion of new sarcomeres to existing myofibrils near tendinous attachments. Growth hormone, insulin, and thyroid hormone have an essential role in stimulating this development. Intermittent active stretching of sarcomeres, such as occurs during isometric contractions, and testosterone both prompt an increase in fiber diameter. Increased fiber diameters are mainly due to the enlargement and subsequent splitting of myofibrils at the Z-line.

The change in metabolic and contractile properties of the gluteus medius muscle with growth and development have been studied in Standardbreds, Quarterhorses, Andalusians, Arabians, and Thoroughbreds (Essen-Gustavsson et al., 1983, Galisteo et al., 1992; Kline and Bechtel, 1990; Rivero et al., 1993b; Roneus, 1993; Roneus et al., 1991; Thornton et al., 1987; Yamano et al., 2002; Yamano et al., 2005). In these studies CS or SDH have been used as markers for oxidative capacity, HAD as a marker for capacity for fat oxidation, and PFK and LDH as markers for glycolytic capacity. A confounding factor in some of these studies is the degree of standardization of the biopsy site. Ideally, the same relative position within the muscle would be sampled as the animal grows. In studies where standardization was not accomplished, variation in both metabolic and contractile properties with growth may in fact reflect the sample depth–age relationship. In particular, if biopsy sample depth was not increased as foals aged, samples from older foals could underestimate any real increase in oxidative fiber types or oxidative capacity.

Birth to 1 Year

Metabolic Changes

The metabolic changes that occur in gluteal muscle with growth are not consistent across the studies available. Standardbred weanlings followed from 6 months to 1 year of age showed no change in oxidative capacity and a decline in glycolytic capacity (Essen-Gustavsson et al., 1983). In contrast, a study of Arabian and Thoroughbred crossbred foals showed a 23% decline in oxidative enzyme activity and oxidative staining intensity of fast twitch fibers from 2 weeks to 8 months of age (Thornton et al., 1983). A 25% increase in glycolytic capacity was also recorded. It is unclear what the depth of sampling was in these foals. This decline in oxidative fast twitch fibers was also documented in Arabian and Andalusian horses from birth to 1 year of age in a study where depth was not adjusted (Galisteo et al., 1992). A semiquantitative study indicated an increase of oxidative capacity in all fiber types with growth in Thoroughbred foals (Eto et al., 2003). The activity of three enzymes involved in β-oxidation of fat in Thoroughbred foals was examined by the author using a biopsy technique that accounted for changes in muscle size with growth. The activity of the enzyme crotonase increased from 4.4 ± 0.4 μmol/g/min to 6.9 ± 0.9 μmol/g/min at 1 year of age, HAD increased from 4.1± 0.4 to 6.0 ± 1.2 μmol/g/min, while thiolase remained unchanged at 1.2 ± 0.1μmol/g/min. Thus it would appear that there might be a variable change in oxidative capacity during the first year of life and an increase in glycolytic capacity.

Contractile Properties

From 6 months to 1 year of age, Standardbred foals showed no change in the percentage of type I fibers, an increase in type IIA, fibers and a decrease in type IIB fibers (Essen-Gustavsson et al., 1983). In a study of Arabian and Andalusian foals, the percentage of type I and IIA fibers increased, and type IIB decreased from 10 days to 1 year of age (Galisteo et al., 1992; Rivero et al., 1993b).Thoroughbred foal gluteal muscles show no change in the percentage of type 1 fibers from birth to 1 year of age, an increase in the percentage of type 2a fibers and a decrease in 2x fibers (Eto et al., 2003). In Warmblood foals, the percentage of type 1 and type 2a fibers increased and the percentage of hybrid type 2a/2x and type 2x fibers decreased from birth to 1 year of age (Dingboom et al., 1999; 2002), Thus, there is a small increase in type 1 fibers and a consistent increase in fast-twitch type 2a (IIA) fibers and a decrease in type 2x (IIB) in foals of a variety of breeds as they reach 1 year of age. Between 1 year and 2 years of age these changes in fiber type proportions continue and then appear to level off at about 3½ years of age in those breeds that have been studied (Roneus et al., 1991).

In summary, fast contracting, glycolytic metabolism appears to be of prime importance in young foals, possibly reflecting their need to escape quickly in the wild. A gradual increase in the ratio of type IIA:IIB subsequently occurs over the first year of life.

Adaptations with Training

Whether skeletal muscle adaptations occur during a training regime or not depends on the frequency, intensity and duration of exercise, and the length of the conditioning program, as well as whether the amount of exercise reaches a plateau or is continuously increased during training. For example, training over 3 km for 6 weeks at an intensity of 40% $\dot{V}O_{2max}$ produced no change in oxidative capacity, whereas when speed was increased to 80% $\dot{V}O_{2max}$, a moderate increase in oxidative fibers occurred (Sinha et al., 1991). Depending on the discipline the horse is being trained for, there is an optimal desired level of fitness, and exceeding this may create a detrimental effect known as *overtraining*.

Results of scientific studies of training in horses are highly variable as a result of the variation in breed, age of horse, intensity, duration, and type of exercise utilized. A recent review by Rivero (2007) summarized very succinctly the results of the many training studies in horses with respect to the duration and intensity of conditioning programs in various disciplines. A broader overview of training is provided here.

Fiber Type Alterations

Short-term training studies often find no or only a small increase in type 1 fibers in the middle gluteal muscle. No change or a decrease in percentage of type 1 fibers was found in 70 Thoroughbreds examined at the beginning and at the end of a racing season 9 months later (Snow, 1983). In contrast, endurance training over 8 months produced an increase in the ratio of type 1 to type 2 fibers and an increase in MHC type 1 to type 2 isoforms (Serrano et al., 2000). Over the course of years of growth and training, small but significant changes have been found in the proportion of type I fibers of the middle gluteal muscle. In large groups of non–intensively trained Andalusian and Arabian horses ranging in age from 10 days to 24 years, an almost 100% increase in the proportion of type I fibers was recorded, with the largest change occurring progressively between 1 and 10 years (Rivero et al., 1993b). Similar changes have been found in large scale cross-sectional studies in both Thoroughbreds and Standardbreds (Roneus et al., 1991; 1992; Roneus and Essen-Gustavsson, 1997). The reasons for this are uncertain, since this increase could represent (1) splitting of type I fibers; (2) gradual transformation of type IIA to type I fibers, although the intermediate type IIC fibers are rarely recorded in adult muscle; or (3) a loss of fibers with age being greatest for type II fibers.

The most consistent change in fiber type proportions in equine training studies is a shift resulting in a higher type IIA to type IIB (2a:2x) fiber ratio. It is believed this is a result of transformation of myosin isoforms from type 2x to 2ax to 2a (Rivero et al., 2007). This transformation is characteristic for those training studies that incorporate longer durations of training and high enough exercise intensities to recruit type IIB (2x) fibers (Rivero et al., 2007). Thus, this shift in fiber type proportions has been shown in Standardbred training, endurance training, and intensive training of young Thoroughbreds. Studies of conventionally trained Thoroughbreds (Foreman et al., 1990; Hodgson et al., 1986; Snow, 1983) have not shown a decrease in type IIB fibers but rather an increase in the oxidative staining of type IIB fibers. In racehorses, a significant decline in IIB (2x) fibers seems undesirable, since this would be associated with a decrease in strength and speed of contraction. Even studies of elite endurance horses have shown a high proportion of type IIB fibers, suggesting that this fiber type is essential to equine athletes (Essen-Gustavsson et al., 1984; Hodgson and Rose, 1987; Rivero and Henckel 1996).

Fiber Sizes

It is obvious that the maximal increase in muscle mass occurs during the growth stage, and it is believed that this occurs as a result of hypertrophy rather than hyperplasia. In horses, approximately 80% of growth has occurred by 18 months, and therefore, it would be expected that the greatest change in fiber size would take place during this phase. A 30% to 70% increase in fiber size was found between 7 and 18 months of age (Essen-Gustavsson et al., 1983). However, in a cross-sectional study examining a large group of Standardbreds between the ages of 1 and 6 years, there was only a tendency for about a 10% increase in fiber areas with age (Roneus et al., 1991). An increase in fiber area was found in Thoroughbreds from about 1 year until they commenced training (Snow, 1983). This was essentially age related, since no change was seen in a group of Thoroughbreds during training.

With respect to fiber areas, reports have indicated increases, no change, or decreases in the size of type IIB fibers (Rivero, 2007). It is not surprising that little, if any, increase in fiber area occurs with aerobic training of mature horses because with training there is a gradual transformation of low-oxidative fast-twitch fibers to high-oxidative fast-twitch fibers, which usually have smaller cross-sectional areas. A decrease in fiber size may be favorable for aerobic exercise because it permits faster diffusion of oxygen through the muscle fiber and more rapid removal of waste products (e.g., CO_2). Although trained Standardbreds appear to have well-developed musculature, trained Standardbreds of a similar age have smaller gluteal fiber areas than inactive horses (Essen-Gustavsson and Lindholm, 1985).

In a 3-month study of endurance-trained horses of Andalusian and Arab breeds, overall, there were minimal effects of training state on myofiber size (Rivero et al., 1995). More recent studies of Thoroughbreds, Standardbreds, Andalusians, and Arabian horses have shown an increase in the area of type IIA (2a) fibers and a variable increase in the size of type I fibers (Rivero et al., 2007). Type 2a and 2x fiber hypertrophy was reported in a group of intensively trained Thoroughbreds (Rivero et al., 2007). Training over jumps has been shown to increase the area of type 2a muscle fibers (Rivero and Letelier, 2000). In addition, draught work or weight carrying in horses or ponies has been shown to increase the cross-sectional size of type IIA and IIB fibers (Gottlieb et al., 1989; Heck et al., 1996). What is certain is that regardless of the individual fiber area, with sufficient aerobic training, the proportion of muscle occupied by type IIA (2a) fibers is increased due to an increase in the number of type IIA relative to other fiber types.

Capillarization

Studies of Standardbred trotters and endurance trained horses have shown that an increase in the number of capillaries around muscle fibers occurs after 5 to 7 weeks of training (Essen-Gustavsson et al., 1989; Henckel, 1983; Rivero et al., 1993a). This facilitates direct delivery of oxygen and bloodborne substrates to myofibers and the rapid removal of lactic acid.

Metabolic Changes

Oxidative Enzymes

Virtually all cross-sectional and longitudinal studies in a variety of breeds are in agreement that training from about 2 years of age causes an increase, up to twofold, in skeletal muscle oxidative enzyme activity such as CS and an increase in high oxidative fibers (Rivero, 2007). This is reflected in an increased fast-twitch high-oxidative to fast-twitch glycolytic fiber ratio. The major oxidative increases occur quite rapidly, probably within the first couple of months of training. Increased activity is most pronounced in the first 2 years of training, with later increases being relatively small. In parallel with this increase in aerobic enzyme activity is an increase in volume density of mitochondria. Increased oxidative capacity influences performance by enhancing the efficiency of ATP generation, delaying muscle and blood lactate accumulation with anaerobic exercise, and decreasing glycogen utilization in favor of FFA metabolism during aerobic exercise.

These oxidative changes have been observed routinely in experimental training studies and in investigations of Standardbreds in training for racing, with two studies examining

conventional training of Thoroughbreds in Australia and the United States, one being experimental and the other in a racing yard finding no or little change in oxidative capacity and no change in fiber type composition (Foreman et al., 1990; Hodgson et al., 1986). In the latter study, even at the end of 12 weeks of training and the commencement of racing, 90% of the type IIB fibers were still low-oxidative. An extensive study of racehorses in Hong Kong prior to commencement of training and 9 months later found no change in the proportion of fast-twitch high-oxidative or type IIA fibers, although there was an increase in CS activity (Cutmore et al., 1985). Both longitudinal and cross-sectional studies in Sweden found that training caused an increase in CS activity (Essen-Gustavsson et al., 1983; Roneus et al., 1992). Other studies suggest that greater training adaptations (higher oxidative capacity, fast-twitch high-oxidative fibers, and type 2a:2x ratio) can be achieved in Thoroughbreds less than 2 years of age compared with those 2 to 3 years of age (Eto et al., 2003; Yamano et al., 2002). The differences among these studies may reflect different training programs used in different countries, ages of horses, or a considerably shorter time frame for training.

It has been well documented in other species that a beneficial effect of training is an increased utilization of FFA at submaximal workloads. Greater FFA utilization requires a high activity of enzymes responsible for their oxidation such as HAD. Studies in horses have not always reported an increase in HAD in parallel with a training induced increase in citric acid cycle enzymes. Both increases in the order of 400% (Rivero et al., 2000) and no change in HAD activity have been reported. The reason why both CS and HAD do not always show similar increases with training is not completely clear.

Glycolytic Enzymes

Indicators of glycolytic metabolism such as PFK, aldolase, triose phosphate dehydrogenase, and LDH generally have been found to be unaltered or, for LDH, decreased with training. This is in accord with findings in other species. In contrast, other studies showed a marked increase in PFK activity or LDH in training programs with an intensive anaerobic training component (Eto et al., 2004; Guy and Snow, 1977a; Yamano et al., 2002).

Other Enzymes

Only limited studies have been carried out investigating alterations in other enzymes with training. A marked increase in alanine aminotransferase (ALT) was considered to reflect an improved ability to metabolize pyruvate to alanine, reducing formation of lactate and increasing production of α-ketoglutarate, an intermediate of the citric acid cycle (Guy and Snow, 1977b).

Nucleotides

The activities of the key enzymes creatine kinase (CK) and AMP deaminase even in the resting state are found to be higher in the Thoroughbred horse than in other species (Cutmore et al., 1986). Muscle CK activity increased slightly with anaerobic training in an experimental study (Guy and Snow, 1977b), but no change was found in a longitudinal study of Thoroughbreds in race training (Cutmore et al., 1985, 1986). In other species, no change in muscle CK activity occurs with aerobic training, but an increase occurs with anaerobic training. Training results in an increase in AMP deaminase and other enzymes associated with purine nucleotide degradation. The increase in AMP deaminase with training may be beneficial in that it could ensure a rapid and near-maximal stimulation of glycolysis. Although training may increase enzyme activity, the concentration of the phosphogen pool remains unaltered.

Glycogen

Glycogen is present in higher quantities in equine muscle than in other species. The glycogen content of muscle in well-trained horses is generally on the order of 500 to 650 mmol glycosyl units per kilogram dry weight. Training can increase muscle glycogen concentrations. A detailed study of glycogen changes in a training program involving 2 days per week of intense exercise indicated that with adequate nutrition, training does not cause a progressive reduction in glycogen content that may affect performance (Snow et al., 1991) (Figure 12-12). However, training programs involving frequent bouts of prolonged submaximal or high-intensity exercise may lead to a gradual lowering of glycogen content as repletion occurs relatively slowly (Lacombe et al., 2003; 2004). Such an effect has been reported during an intensive endurance training program (Essen-Gustavsson et al., 1989).

Lipid and Carnitine

In contrast to what might have been expected, an intensive training program resulting in increased CS activity did not result in increased intramuscular triglyceride content. Carnitine increases with age and training, which is not surprising, since it is highly correlated to oxidative capacity of muscle fibers. To try to enhance muscle carnitine content, commercial carnitine preparations have been made available. However, this substance is only very poorly absorbed from the equine gastrointestinal tract, and muscle content does not increase even following intravenous administration.

Buffering Capacity

During intense exercise, the detrimental effects of proton formation on intracellular pH are partially offset by the buffering capacity of the cells and export via the bicarbonate system. The buffering capacity of equine muscles has been reported to be in the order of 210 µmol/ kg of dry muscle in a pH range of 7.0 to 6.0 (Marlin et al., 1991). A few studies have reported a marked increase or a slight increase in buffer capacity with training.

Within muscle, buffering occurs through phosphocreatine hydrolysis (dynamic) and physicochemical (static) buffering by proteins, dipeptides, and inorganic phosphate. The ability of muscle to buffer proteins may be a key determinant of sprint performance. Thoroughbreds have a very high buffering capacity when compared with humans (Harris et al., 1990). This may be related to equine myofibers containing considerably greater content of the histidine dipeptide, carnosine, than humans, contributing approximately 30% of the nonbicarbonate buffering. Carnosine content is related to fiber type, being highest in type IIB fibers where it contributes up to 50% of fiber buffering capacity. A decrease in muscle carnosine content with age and training was found in Thoroughbreds, which may be related to fiber transformation from type IIB to IIA fibers with training, where type IIB fibers have higher carnosine content (Sewell et al., 1992).

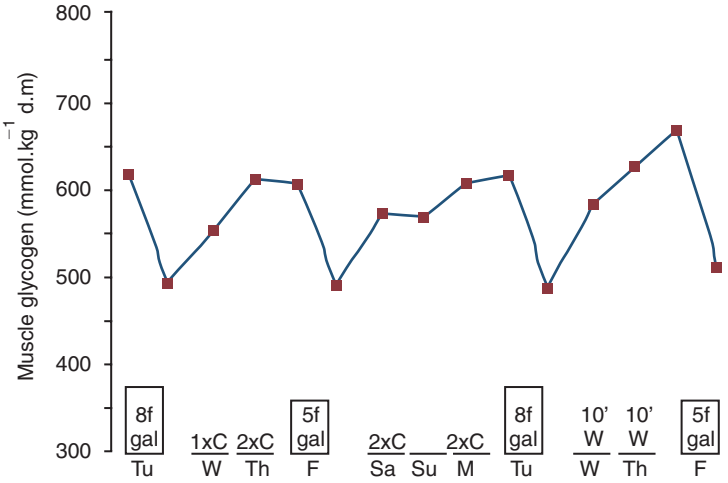

FIGURE 12-12 Mean changes in the glycogen content of the middle gluteal muscle during 2 weeks of training (*gal*, gallop; *8f*, 1600 m; *5f*, 1000 m; *c*, canter; *w*, walk). *(Adapted, with permission, from Snow DH, Harris RC: Effects of daily exercise on muscle glycogen in the thoroughbred racehorse. In Persson SGB, Lindholm A, Jeffcott LB, editors:* Equine exercise physiology 3*, Davis, CA, 1991, ICEEP Publications, p 299.)

Sarcolemma and Sarcoplasmic Reticulum

Training appears to increase the sodium ATPase activity (McCutcheon et al., 1999b) in skeletal muscle as well as calcium ATPase activity (Suwannachot et al., 2005; Wilson et al., 1998). This change should assist in quickly restoring resting sarcoplasmic calcium concentrations and resting membrane potentials between contractions.

Muscle Fiber Recruitment

Although very difficult to document, there is a learned component of training in which patterns of specific muscle contraction and relaxation are acquired and fixed for various types of activities (muscle memory). For example, in Standardbred trotters, maximal trotting speed is acquired over the first few months of training (Roneus et al., 1994; Roneus and Essen-Gustavsson, 1997) and in dressage horses the ability to perform collection is acquired as strength, coordination, and flexibility are built. An alteration in recruitment pattern and increase in strength has been demonstrated in an 8-month study of Andalusian horses. These horses showed an increase in stride frequency and a decrease in stride duration and stance time at a trot with training which occurred in conjunction with an increase in the ratio of type IIA (2a) to IIB(2x) fibers (Rivero et al., 2001).

Detraining (Relative Inactivity)

Horses have enforced periods of rest due to layoffs, injury, and illness during training. Early studies of the effect of forced inactivity of Thoroughbreds on muscle found a decline in oxidative enzyme activities after 5 weeks of inactivity, but after 10 weeks, there was an inexplicable increase (Guy and Snow, 1977a; Snow and Guy, 1979). In intensively trained Thoroughbreds, a 5-week period of inactivity resulted in no change in enzyme activities or fiber composition from values assessed immediately after training (Essen-Gustavsson et al., 1989). A 34-week training study in Standardbred horses showed a progressive decline in aerobic enzymes and increase in glycolytic enzymes during a 12-week detraining period (McGowan et al., 2002). Similarly, in a study of endurance-trained horses, a change in fiber type composition from type 1 to 2a to 2x occurred with a concomitant decline in muscle fiber sizes, oxidative capacity, and glycogen content 3 months after detraining (Serrano et al., 2000).

Summary of Training Effects

Changes occur within muscle during training mainly relate to improved oxidative capacity. Some adaptations occur rapidly, however, for major changes to occur, especially conversion of low-oxidative to high-oxidative fibers, a threshold of training intensity is required over a minimal training duration. Training effects will be more pronounced in previously untrained horses than in those re-entering a training program. For horses performing maximal intensity exercise, increased muscle oxidative capacity and increased proportion of fast-twitch high-oxidative fibers allows horses to achieve higher speeds before the onset of lactate accumulation and nucleotide depletion, which is reflected in improved performance. Horses performing at submaximal aerobic exercise intensities benefit from enhanced delivery of oxidative substrates to muscle fibers as well as improved oxidative metabolism of glycogen and FFA. Especially in endurance rides, greater FFA utilization leads to a glycogen-sparing effect and prolonged endurance capacity.

Although the preceding changes are considered to be desirable, there is controversy about the necessity of oxidative adaptations for horses competing in short-duration galloping races. An increased type 2a:2x ratio and increased oxidative capacity in type 2x fibers may occur in conjunction with a potential decrease in the cross-sectional area of type 2x fibers. This may, in turn, reduce maximal force output, acceleration, and possibly also stride length. Therefore, for horses competing over distances less than 1000 m, it may be more beneficial to maintain a high proportion of large type 2x (IIB) muscle fibers. For horses racing over longer distances, it is likely that a balance between stamina provided by high oxidative capacity, the ratio of 2a:2x fibers, and muscle fiber area has to be obtained.

Once optimal fitness is attained, training programs may plateau to sustain the desired degree of aerobic capacity and strength. From detraining studies it would appear that

metabolic changes can be maintained for several weeks with decreased activity. Therefore, in certain situations, for both physical and mental reasons, it would be quite advantageous to reduce training intensity.

RELATIONSHIP OF FIBER TYPE COMPOSITION TO PERFORMANCE

When the same muscle is sampled at a uniform site, differences are found between individuals. In humans, this difference has been related to athletic performance ability. Elite sprinters have almost all type II fibers in the vastus lateralis muscle biopsies, a muscle that is very active during running. In contrast, successful long-distance runners have almost all type I fibers in the same muscle. Middle-distance runners and the general population have approximately equal proportions of both fiber types (Saltin et al., 1977). From studies of monozygotic twins, the proportions of type I and type II fibers appear to be genetically influenced; however, environmental factors have a stronger impact (Bouchard et al., 1986; Lortie et al., 1982; Simoneau et al., 1986). Genetic factors have a stronger influence on activities of regulatory enzymes of the glycolytic (PFK) and citric acid cycle pathways and in their adaptation to training. These genetic effects reach only about 25% to 50% of the total phenotypic variation when data are adjusted for age and sex differences (Bouchard et al., 1986; Simoneau et al., 1986).

In horses, some relationship between performance and muscle fiber composition is indicated by differences between breeds, although within breeds, this fiber type composition can show considerable overlap. Thoroughbreds and Quarterhorses that were originally bred for short bursts of speed (1 mile and ¼ mile, respectively) have a considerably greater proportion of type II fibers compared with the Arabian, the Andalusian, or a heavy Hunter type of animal (see Table 12-4 and Table 12-5). Standardbreds have more type I and fewer type IIB fibers than do Thoroughbreds. In French Anglo-Arabian horses, the heritability of fast MHC (representing fast-twitch fibers) was estimated at 13%, and the best performing galloping and jumping horses had a higher proportion of fast versus slow MHC (Barrey et al., 1999). In a Spanish study, a significant influence of maternal lines on the percentage of type I and IIB fibers was found, and an influence of paternal lines was found on the percentage of type IIB fibers (Rivero et al., 1996).

These genetically based differences have raised the possibility of a muscle biopsy allowing selection of horses suited for a particular type of competition at an early age. In a study of Thoroughbred racehorses with proven performance, sprinters and medium-distance horses had a significantly higher proportion of type II fibers compared with stayers (2400 to 3000 m) (Snow and Guy, 1981). Biopsy findings from the semitendinosus of 14 yearling Thoroughbreds were compared with their 2- and 3-year-old racing performance (Barlow et al., 1984). Horses were divided into two groups: (1) those having less than 90% type II fibers and (2) those having more than 90% type II fibers. Results indicated a higher percentage of desirable performance characteristics in the horses with greater than 90% type II fibers. Interestingly, there was a marked difference in the distance the two groups raced over, being 1440 and 2720 inches, for the high and low type II groups, respectively. Quarterhorses that raced successfully had a significantly slightly higher proportion of type II fibers than those which were unsuccessful (Wood et al., 1988). In a small-scale study in Standardbreds, there was an indication that performance could be related to the percentage of type IIB fibers, but there was no relationship to type I (Roneus, 1993). Studies have indicated that endurance horses with the highest proportion of type I fibers usually performed best (Essen-Gustavsson et al., 1984; Hodgson et al., 1983; Snow et al., 1981). In an extensive study in Arabians and Andalusians, in which biopsies were collected from sites at three different depths, the most successful horses had a higher percentage and relative areas of type I and IIA fibers and with a greater homogeneity in size across the muscle (Rivero and Henckel, 1996).

However, in spite of these promising early studies, no readily applied method to use muscle fiber type composition is currently in practice to predict suitability for performance. This is likely because there are so many factors that influence performance that selection of horses based on fiber type composition alone is not practical. It may have more usefulness in eliminating horses with an undesirable proportion of a particular fiber type rather than selection of an ideal fiber type composition in extreme sports disciplines.

LIMITATIONS TO PERFORMANCE CREATED BY METABOLIC MYOPATHIES

To date, disturbances in mitochondrial oxidation, lipid oxidation, and glycogen synthesis have been described in horses that provide insight into the importance of cohesive metabolic pathways in equine athletes.

MITOCHONDRIAL MYOPATHY

The respiratory chain in the inner mitochondrial membrane has five multi-subunit enzyme complexes including complex I (NADH:ubiquinone reductase). These enzyme complexes serve to translocate protons from the matrix to the intermembrane space during electron transport providing the final step for synthesis of ATP. Complex I was found to be deficient in a young Arabian filly that was unable to trot for more than 10 minutes without developing marked fatigue (Valberg et al., 1994). There was no evidence of muscle cell damage following exercise (normal serum creatine kinase activity). Maximal exercise speed without a functional complex I was only 7 m/s. Lack of muscle oxygen extraction resulted in a rise in venous partial pressure of oxygen at a slow trot (4 m/s) to a value that was similar to that found in arterial blood. The horse also showed a drastically reduced maximum oxygen consumption ($\dot{V}O_{2max}$ 0.5 milliliter per kilogram per second [mL/kg/s]; Figure 12-13) and a marked plasma lactic acidosis (20 millimolar [mM]) at 7 m/s. Thus, although the cardiopulmonary system could deliver oxygen to muscles, muscles were unable to utilize the oxygen, which resulted in low $\dot{V}O_{2max}$ and near total reliance on anaerobic glycolysis even at low speeds. In spite of a normal glycolytic capacity, without a functional oxidative capacity, this horse could not produce bouts of speed. Histopathologic evaluation of muscle biopsies showed an increase in mitochondrial density in NADH stains equivalent to what would be expected in an extensively trained horse such as a Standardbred. Electron microscopy of muscle fibers, however, showed that the mitochondria were large and abnormally shaped with bizarre cristae formations (Figure 12-14). Biochemical analysis of oxidative enzymes revealed high activity of all enzymes except rotenone

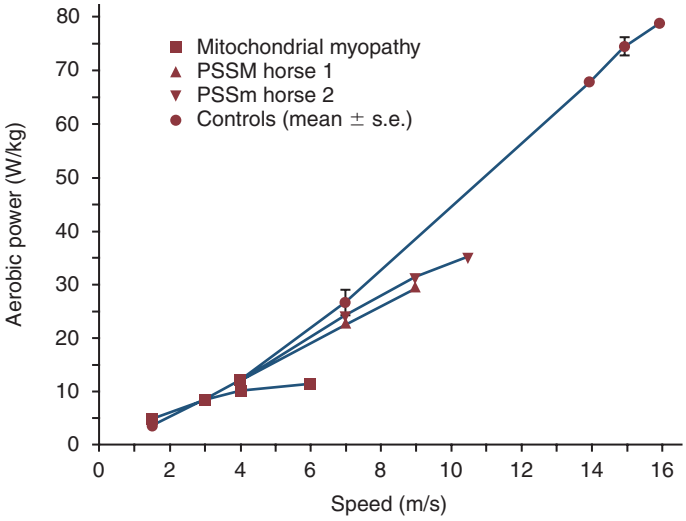

FIGURE 12-13 The aerobic power ($\dot{V}O_2 \times 20$ j/mL O_2) in control Thoroughbreds, an Arabian horse with a mitochondrial myopathy, and two horses with polysaccharide storage myopathy (PSSM) due to a mutation in *GYS1*. Note the marked limitation in aerobic power in the horse with a mitochondrial myopathy as well as the limited aerobic power in PSSM horses that may have limited glycolytic flux. Both myopathies result in early fatigue and low maximal exercise speeds. *(Modified from Valberg, et al: Skeletal muscle limitations to performance,* Equine Veterinary J *18(Suppl):736, 1995.)*

sensitive NADH-cytochrome c reductase indicating a complex I deficiency. Through the years, the horse remained healthy at rest on pasture with a gradual onset of generalized muscle atrophy.

DISORDERS OF LIPID OXIDATION

In horses, an acquired defect in fatty acid oxidation causes a highly fatal muscle disorder known as *atypical myoglobinuria*, or *pasture myopathy*. This disorder causes clinical signs at rest in the postural and respiratory muscles that are highly reliant on fat metabolism. An acquired defect in several mitochondrial dehydrogenases that use flavin adenine dinucleotide (FAD) as a cofactor has been suggested by characteristic profiles of organic acids and acylcarnitines in the urine and plasma of a few affected horses (Westermann et al., 2008). Long-chain fatty acids are transported as fatty acyl-CoA thioesters across the mitochondrial membranes by carnitine palmitoyl transferase enzymes. Short-chain and medium-chain fats do not require a transport system and form acyl-CoA thioesters within the mitochondrial matrix. The four steps in β-oxidation of lipid, dehydrogenation by FAD via the enzyme acyl-CoA-dehydrogenase, hydration by enoyl-CoA-hydratase, oxidation by NAD+ via β-HAD, and cleavage of β-ketoacyl-CoA by β-ketothiolase. Many of these enzymes have specificity for a particular chain length of fatty acid. This process continues until the entire chain is cleaved into acetyl CoA units.

A toxin called hypoglycin A is believed to be consumed by horses with atypical myoglobinuria from pastures during wet, windy spring or fall conditions (Valberg et al., 2013). Impairment of lipid oxidation appears to be most dramatic in postural, respiratory, and cardiac muscles, which show histopathologic evidence of acute myodegeneration and marked accumulation of lipid, particularly in oxidative myofibers. Horses develop acute signs of depression, which progresses to stiffness, sweating, trembling, inability to stand for prolonged periods, dyspnea, myoglobinuria, and death within 24 to 48 hours. Affected muscles are usually not firm on palpation. Many of the clinical signs are believed to be the consequence of lack of energy generated distal to the metabolic block and accumulation of toxic intermediates of fat metabolism that have a detergent-like effect in oxidative muscle fibers.

GLYCOGEN STORAGE DISORDERS

Two inherited glycogen storage disorders that cause exercise intolerance and muscle degeneration with exercise have been described in horses. One is an autosomal recessive mutation in the ubiquitous glycogen branching enzyme (*GBE1*) gene, which results in a loss of glycogen branching enzyme activity. The other is an autosomal dominant mutation in the skeletal muscle glycogen synthase 1 (*GYS1*) gene which results in an increase in glycogen synthase activity.

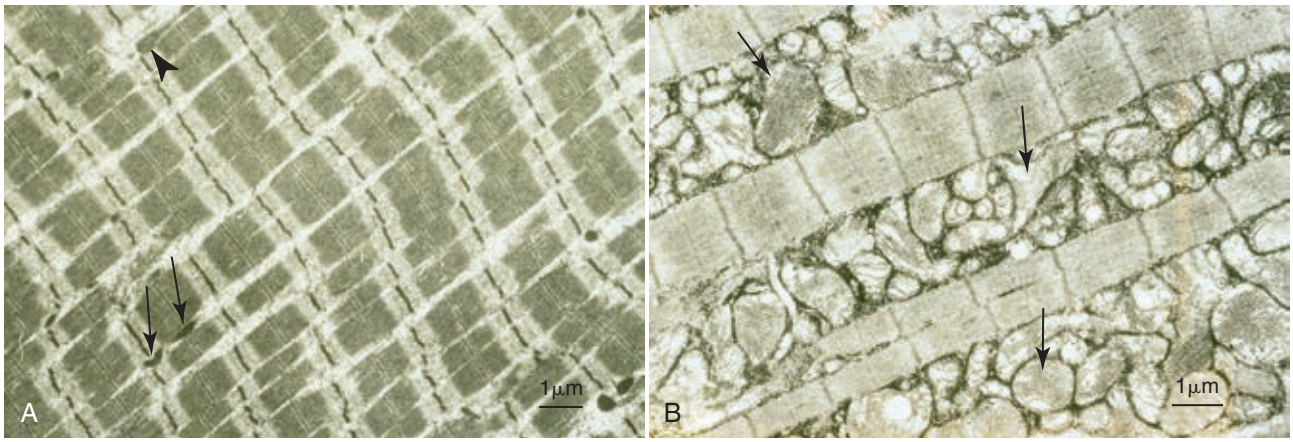

FIGURE 12-14 **A,** Electron micrograph of a normal horse showing a few mitochondria (*arrows*) between the uniform array of myofibrils. **B,** Electron micrograph from a horse with a mitochondrial myopathy showing numerous large bizarre shaped mitochondria (*arrow*) between myofibrils.

Glycogen Branching Enzyme Deficiency (GBED)

GBED is caused by a nonsense mutation in exon 1 of the *GBE1* gene at codon 102, which introduces a premature stop codon in Quarterhorse-related breeds (Valberg et al., 2001; Wagner et al., 2006; Ward et al., 2004). Homozygotes have no GBE activity in liver, cardiac, or skeletal muscle, whereas heterozygotes have half of the normal tissue GBE activity. Homozygous foals show fatal signs related to a lack of stored liver, skeletal, or cardiac glycogen. Foals with GBED are aborted, are stillborn, show signs of muscle weakness in the form of inability to stand or hypoventilation, or have hypoglycemia and collapse. The total fatality with this disorder highlights the complete reliance many tissues have on the storage of glucose as glycogen. The inability to store highly branched glycogen decreases the end points in the glycogen molecule for myophosphorylase to use as a substrate to liberate glucose. As a result, brain, heart, and skeletal muscle lack sufficient glucose for normal cellular metabolism, and foals are weak, exercise intolerant, and incapable of survival.

Nine percent of the Quarterhorse breed carries this autosomal recessive mutation, and 3% of abortions are attributed to this disease in Quarterhorses (Wagner et al., 2006). Persistent eucopenia, intermittent hypoglycemia, and high serum CK (1000 to 15,000 Units/L), AST, and γ-glutamyltransferase (GGT) activities are features of affected foals. PAS staining of skeletal muscle, heart, and liver show a notable lack of normal staining for glycogen as well as abnormal PAS-positive globular or crystalline (Figure 12-15, *A*) intracellular inclusions (see Figure 12-15, *B*). A diagnosis is best obtained by confirming the presence of the genetic mutation in tissue samples or by identifying typical PAS positive inclusions in muscle or cardiac samples.

Polysaccharide Storage Myopathy

In contrast to GBED, polysaccharide storage myopathy (PSSM) is characterized by 1.8 times higher muscle glycogen concentrations and accumulation of nonglobular abnormal polysaccharide in muscle fibers (see Figure 12-15, *C*) (Annandale et al., 2004). Clinical signs occur usually in adult horses within 10 to 30 minutes of submaximal exercise and include muscle pain and stiffness, accompanied by high serum CK activity, indicative of muscle damage (Annandale et al., 2005; Firshman et al., 2003; Valberg et al., 1997). Clinical signs are

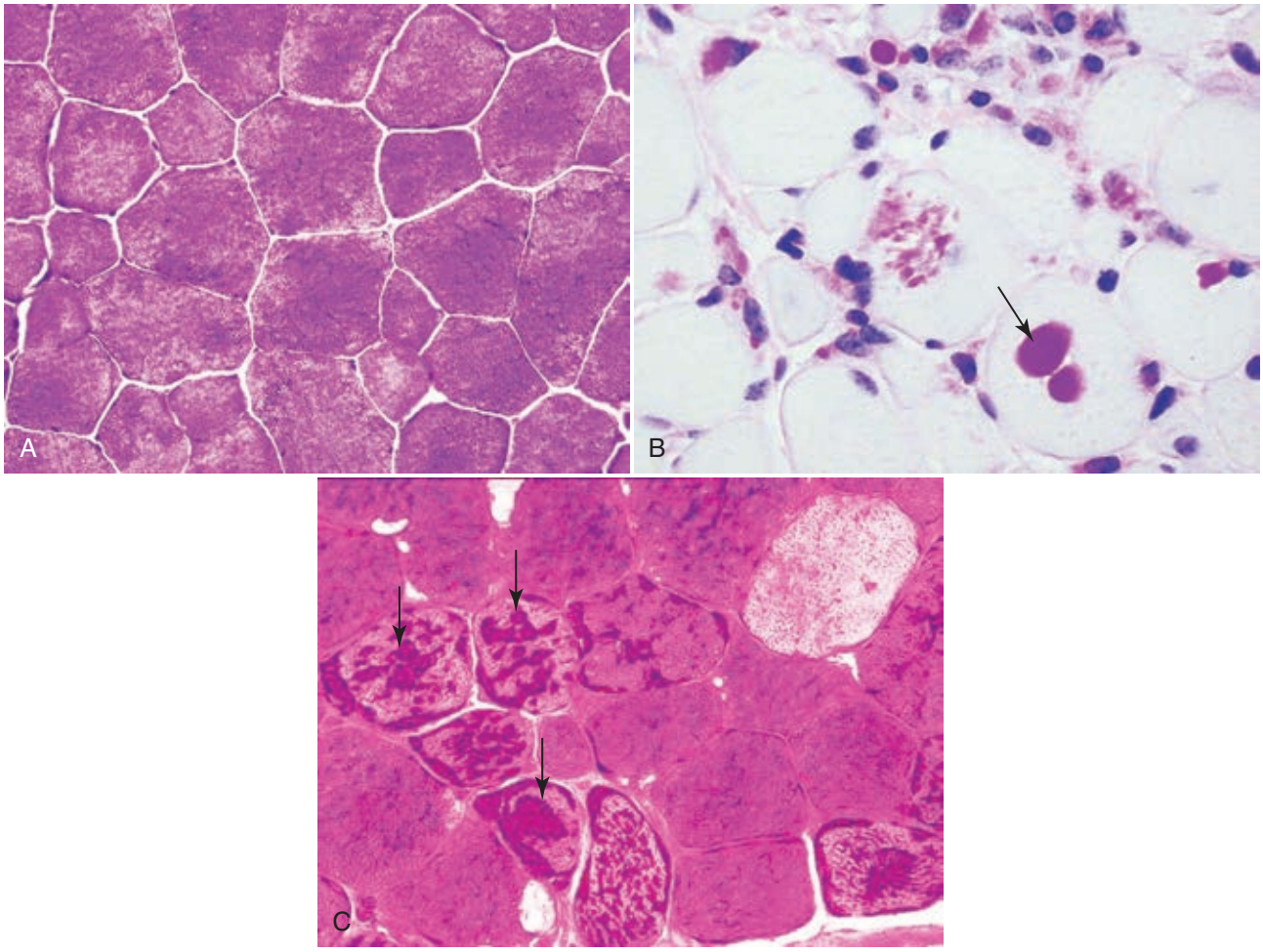

FIGURE 12-15 Periodic acid Schiff's stains of a normal horse (*A*), a foal with glycogen branching enzyme deficiency (GBED) (*B*), and a horse with polysaccharide storage myopathy (PSSM) (*C*). Arrows show abnormal polysaccharide which is globular in fibers with GBED (complete lack of branching) and granular in horses with PSSM (partial decrease in branching). Note the lack of any normal background Periodic acid Schiff's (PAS) staining for glycogen with GBED and dark staining indicating high glycogen concentrations with PSSM.

exacerbated when horses are fed high-starch and high-sugar diets. PSSM horses have normal activities of glycogenolytic and glycolytic enzymes and can metabolize glycogen during near-maximal exercise (Valberg et al., 1998, 1999). In addition, PSSM Quarterhorses may also have heightened sensitivity to insulin and enhanced skeletal muscle glucose uptake (Annandale et al., 2004; De La Corte et al., 1999; Firshman et al., 2005). The metabolic defect in PSSM involves a novel gain of function mutation in the gene glycogen synthase 1 (*GYS1*), which produces increased unregulated glycogen synthase activity and, thus, increased muscle glycogen (McCue et al., 2008). This form of PSSM has been found in a variety of breeds of horses.

The link between abnormal regulation of glycogen synthase and the development of rhabdomyolysis is perplexing. Despite the apparent availability of glycogen for energy metabolism, PSSM horses have an energy deficit during submaximal exercise, as shown by increased IMP in single muscle fibers. Concentration of IMP in single muscle fibers of horses with PSSM after exercise ranged from 0 to 9.8 mmol/kg, compared with 0 to 3.1 mmol/kg in control horses (Annandale et al., 2004). The IMP concentration in a few single muscle fibers of horses with PSSM were equivalent to concentration reported in single muscle fibers after maximal exercise. Thus, considerable metabolic stress is evident in skeletal muscle fibers of PSSM horses with as little as 11 minutes of light aerobic exercise.

The deficit in energy generation in PSSM horses appears to link regulation of glycogen synthesis with the availability of glycogen for muscle metabolism. Substrate limited oxidative metabolism may occur in a small number of muscle fibers recruited after 15 to 20 minutes of light exercise. This is suggested by studies of PSSM horses that show lower oxygen consumption during exercise (see Figure 12-13) and a modest increase in muscle lactate in PSSM horses on grain diets performing light exercise compared with healthy horses (Figure 12-16) (Valberg et al., 1995). Substrate limited oxidative flux in PSSM horses appears to lie downstream of the metabolism of glycogen to pyruvate because a major decline in muscle pyruvate concentrations does not occur in PSSM horses on grain diets with exercise (see Figure 12-16).

One potential link between glycogen synthase activity and impaired oxidative metabolism of pyruvate or fatty acids may be metabolic switches such as AMP kinase that profoundly regulate substrate flux within the cell. When energy supply is deemed to be insufficient (↓ [ATP]/[ADP]), activation of AMP kinase leads to the increased transcription of genes involved in oxidative metabolism, stimulation of fatty acid oxidation, and activation of pyruvate dehydrogenase, which increases carbohydrate oxidation (Hardie, 2004). It is possible that excessive stimulation of glycogen synthesis resulting from a gain of function mutation in glycogen synthase (stimulated further by insulin released when consuming grain) might be

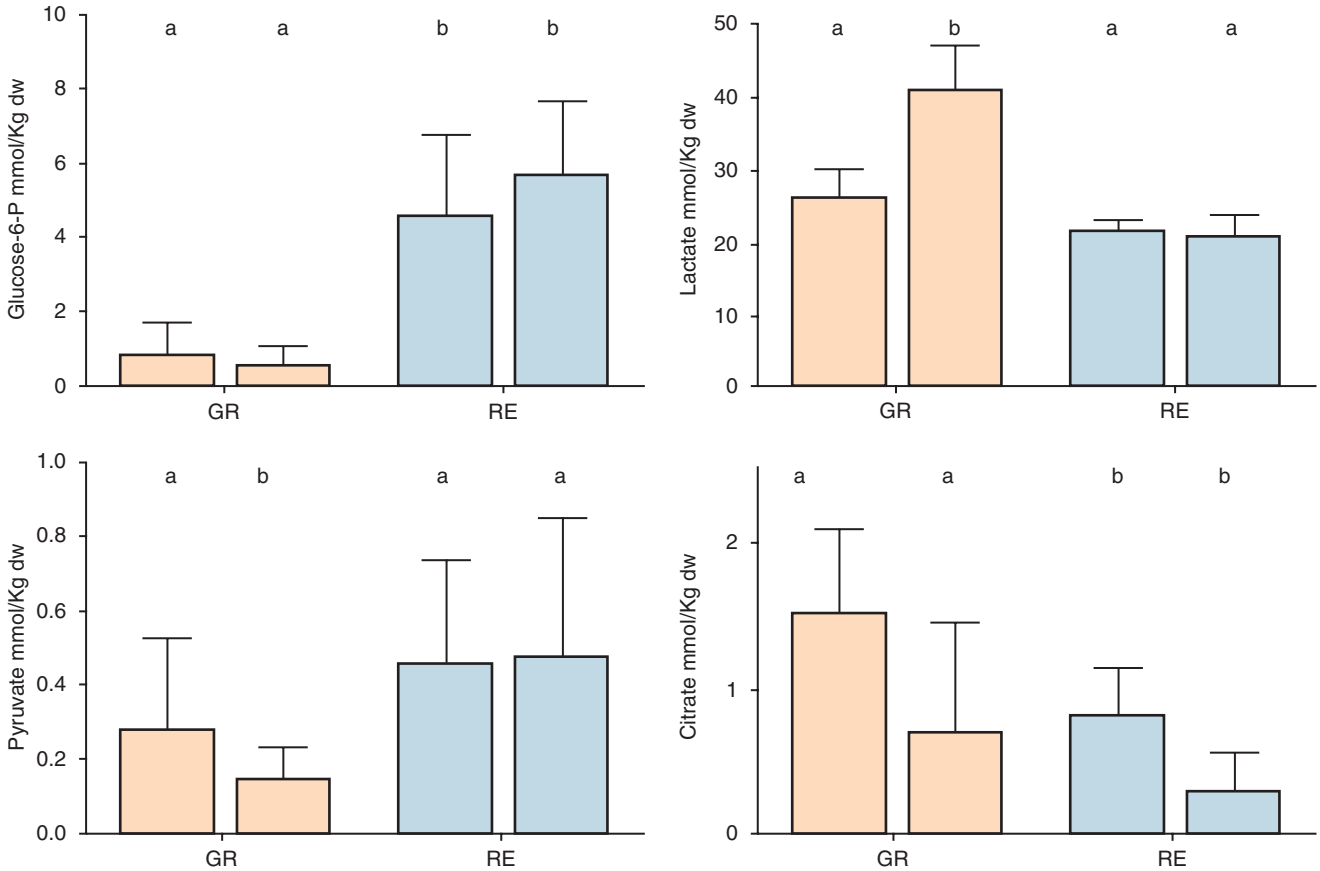

FIGURE 12-16 Gluteal muscle glucose-6-phosphate, lactate, pyruvate, and citrate concentrations taken at rest and after 15 minutes of walking and trotting from polysaccharide storage myopathy (PSSM) horses which were fed either a high-starch grain or isocaloric equivalent of a low-starch high-fat concentrate (Re-Leve). Note that the low-starch high-fat concentrate resulted in higher glucose-6-phosphate and pyruvate concentrations and lower lactate and citrate concentrations after exercise.

interpreted by nutrient sensors in the cell as an indication that glycogenolysis and lipolysis need not be activated. A potential scenario in PSSM horses on grain diets could be that nutrient sensors such as AMP kinase do not fully activate enzymes such as pyruvate dehydrogenase during exercise to produce adequate acetyl CoA for oxidative metabolism. Although acetyl CoA could also be supplied by fatty acid oxidation, PSSM horses on grain have low plasma FFA concentrations, possibly due to suppression of lipolysis by high insulin. A further factor reducing fatty acid oxidation in PSSM horses could be the high muscle citrate concentrations found in PSSM muscle on grain diets (see Figure 12-16). Citrate activates acetyl CoA carboxylase which converts acetyl CoA to malonyl CoA, the committed step for fatty acid synthesis, thereby directing acetyl CoA away from the citric acid cycle (Saha et al., 1999). In addition, accumulation of malonyl CoA causes inhibition of carnitine palmytoyl transferase (CPT1), the key enzyme necessary to transport long-chain fatty acids into the mitochondria for β-oxidation (McGarry and Brown, 1997; Ruderman et al., 1999). Thus, PSSM horses fed grain may be unable to generate sufficient acetyl CoA from either carbohydrate or fat metabolism to fuel muscle contraction during submaximal exercise.

The provision of long-chain fatty acids in the diet of PSSM horses significantly decreases exercise intolerance and rhabdomyolysis with exercise (Firshman et al., 2003; Ribeiro et al., 2004; Valentine et al., 2001). Fat supplementation increases plasma FFA concentrations and, thus, the availability of fats for oxidation in skeletal muscle. Increased β-oxidation of long-chain fats in PSSM horses on fat supplemented diets has been shown by measuring elevated postexercise C2 and C18:2 plasma acylcarnitine concentrations. Long-chain fat diets also appear to increase glycogenolytic or glycolytic and oxidative flux in PSSM muscle, as shown by higher glucose-6-phosphate concentrations, lower lactate, and higher pyruvate concentrations in the muscle of PSSM horses fed low-starch fat-supplemented diets with an isocaloric amount of grain (see Figure 12-16).

Daily exercise is another important means by which to decrease exercise intolerance and rhabdomyolysis in PSSM horses (Firshman et al., 2003; Ribeiro et al., 2004). Exercise may lower plasma insulin and increase plasma FFA concentrations, and training over time may enhance uptake of fatty acids into skeletal muscle and improve muscle oxidative capacity. Plasma FFA concentrations obtained 4 hours after exercise are at least double those of pre-exercise samples. In addition, it is possible that daily exercise may improve nutrient sensing and thereby affect the balance of energy supplied as carbohydrate and fat for oxidative metabolism (Baldwin et al., 1973; Greenberg, 2006). Regular exercise and diet do not appear to decrease muscle glycogen concentrations in PSSM horses (De La Corte et al., 1999; Valberg et al., 1999).

CONCLUSION

As described in this chapter, equine skeletal muscle is essentially similar to all mammalian muscles. However, through evolution and, more recently, selective breeding, the horse has developed muscle characteristics that allow breeds to have great strength (draft breeds, Shetland ponies), speed and stamina (the Arabian), or sheer speed (Quarterhorse, Thoroughbred).

Great advances have been made in the understanding of skeletal muscle fiber types, adaptations with training, and the cause of fatigue through the ease of taking sequential biopsies. Our knowledge in this area is almost equal to that about humans and some laboratory animals. These studies have shown that adaptations with training superimposed on genetic traits have allowed muscles to be extremely efficient in generating and maintaining high-energy turnover. Activities of key metabolic enzymes as well as glycogen are extremely high. Experimental studies have enabled a better understanding of the causes of fatigue as well as nutritional requirements, allowing veterinarians to give scientifically based advice to trainers and owners. An understanding of normal muscle metabolism in horses has led to the discovery of several enzymatic defects that profoundly affect life and exercise tolerance. The extensive studies in this area also have helped to dispel many myths and left many new areas open to further investigation. Although much has been learned, there is still a great deal more information required.

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CHAPTER

13 Tendon, Ligament, Bone, and Cartilage: Anatomy, Physiology, and Adaptations to Exercise and Training

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The musculoskeletal system of the horse is highly developed and specialized, allowing this large animal to roam extensively in search of food and to attain high speeds (in excess of 15 meters per second [m/s]) from a very early age to evade predators. The effective length of the limbs has been extended by elongation of distal segments, reduction in number and size of the distal limb bones, and adoption of a digitigrade stance. High stride rates are possible because of the proximal placement of the heavy limb musculature, and the long digital tendons that span the elongated distal bones form an energy-storing mechanism for efficient locomotion. Intensive selective breeding has increased the size and speed of domestic horses, but the cost has been a reduction in the mechanical safety margins of musculoskeletal connective tissues and a consequent high frequency of distal limb injury. Exercise-induced injury to bone, cartilage, tendons, and ligaments is the main cause of failure of equine athletes to train, compete, and perform; such injuries can be life threatening or life ending. Musculoskeletal injury is the major cause of loss of income and value in the equine industries, in addition to being a significant animal welfare concern that raises negative emotional responses from the public with regard to the use of horses for athletic and recreational activities.

Tendons, ligaments, bone, and cartilage are connective tissues in which the activities of various cellular populations are responsible for synthesis and maintenance of large amounts of extracellular matrix that should, theoretically, be dynamically optimized to respond to mechanical demands. One of the major reasons postulated for training horses is to increase musculoskeletal strength that would result in increased resistance to injury. However, many injuries are preceded by failure of the tissue to adapt to exercise, an imbalance between subclinical damage (microdamage) and cellular reparative activity, or both. Our knowledge of how various physical stimuli influence adaptation or damage of equine connective tissues prior to clinical injury is surprisingly limited, even in the case of bone that is more easily assessed than cartilage, tendon, or ligament tissue. It has become increasingly evident that a significantly greater understanding of the responses of cellular populations to various exercise regimens is required, including how various types, frequencies, magnitudes, rates, and durations of mechanical loading may be transduced into coordinated cellular control of matrix synthesis, degradation, and turnover. In tendon, in particular, the cell types and their connection into functional networks are very poorly defined. Ultimately, all of the macroscopic, microscopic, ultrastructural, or biochemical changes occurring in response to exercise are determined by cellular activity.

Training methods vary significantly and are currently not fully informed by scientific research. Knowledge specific to horses is limited, yet significant progress has been made with recent research on (1) epidemiologic analysis of injury incidence and risk factors, (2) defining the physiology and cell biology of these tissues, and (3) measuring the effects, mainly in young horses, of controlled training programs on adaptation and injury of bone, cartilage, and tendon. Further, although direct extrapolations of findings from other species (including humans) to the horse may be questioned because of differences in anatomy, environment, function, evolution, and endocrinology in this highly adapted athletic species, many current, relevant, and exciting key developments in the definition and understanding of the mechanisms and pathways of adaptive and reparative cellular responses to exercise have comparative relevance. Of equal importance is the determination as to whether, theoretically, adaptive or degenerative responses to exercise in the musculoskeletal system actually reduce or increase the likelihood of injury occurrence. This has not been clearly defined for any species, but internationally collaborative studies have begun to explore injury reduction as an end point, with some initial evidence of success.

Finally, in reviewing the various equine training studies that have been performed, it is important to recognize the difficulties involved in their direct comparisons. Quantification of the exercise performed has not been standardized, including calculation of loading cycles and magnitudes of forces applied, and in many, the intensity of training has been altered to allow for individual horses' "fitness." A cumulative work index, including distances worked at various speeds in various periods, has been proposed, but that requires refinement in terms of application to different locations; stages of training; and individual horse factors, including age and different environments (Firth and Rogers, 2005a). In some studies, researchers have effectively immobilized horses in control or "exercise" groups; in those studies where control animals

were not confined, quantifying the amount of exercise during periods of natural activity has usually not been attempted. There is very limited information on "normal" frequencies, durations, and magnitudes of various activities for horses of various ages, breeds, and functions, in the enormous diversity of situations in which they either are kept or live in the wild. Studies using the global positioning system (GPS) technology are few. The "optimal" mechanical environment, in terms of appropriate growth, development, and adaptation of the musculoskeletal system during training and competitive athletic activity, remains unknown. This, together with the background of enormous individual variation, means that resistance to injury has not yet been maximized.

TENDONS AND LIGAMENTS

Horses can attain high speeds largely because specific tendons and ligaments store and return elastic strain energy as the foot is set down and then lifted. This, effectively, halves muscular work during galloping. The tendons act as "biologic springs," and horses are one of the species where tendons have reached their most extreme development. As a result, these are also clinically the most important tendons because of their susceptibility to injury; most current research information relates to superficial digital flexor tendon (SDFT) injury, one of the most common causes of lameness in Thoroughbreds in both racing and training and in sport horses. The mid-metacarpal region of the forelimb SDFT is most frequently injured, with rupture often initially located in the tendon core. In the suspensory ligament (SL), lesions may occur in the proximal one third (proximal suspensory desmitis), middle third (body lesions), or branches (branch lesions) (Dyson et al., 1995). It has been estimated that 10% to 30% of racehorses and horses in training experience SDFT or SL injury, with long periods out of work (up to 18 months) (Avella et al., 2009; Goodship et al., 1994; Pinchbeck et al., 2004; Williams et al., 2001). In the long term, in many horses, the SDFT or other components of the suspensory apparatus is reinjured. In one Japanese study, 70% of horses that had sustained tendon injuries failed to return to their previous level of performance (Jorgensen and Genovese, 2003; Jorgensen and Genovese, 2003; Oikawa and Kasashima, 2002). However, in a more recent study of Thoroughbred racehorses in the United Kingdom, SDFT injury did not cause a significant reduction in Racing Post Rating (published by the *Racing Post* newspaper) when these horses returned to racing (O'Meara et al., 2010). In the same study, it was concluded that the assessment of the outcome of an SDFT injury requires a minimum completion of five races on return to competition. Tendon injuries seem to occur more often in horses used for steeplechasing, and other types of high-level competition, including elite eventing and showjumping (Murray et al., 2006; Singer et al., 2008; Takahashi et al., 2004; Williams et al., 2001). Increased age was a significant risk factor in several epidemiologic studies (Avella et al., 2009; Perkins et al., 2005; Williams et al., 2001), which can be related to the pathophysiology of exercise-induced tendon injury. In trotters, there appears to be a high prevalence of SL injury, which is thought to reflect differences in load distribution between the flexor structures at the gallop and at the extended trot (Goodship et al., 1994).

TENDON AND LIGAMENT ANATOMY AND FUNCTION

Anatomy of Tendons and Ligaments of the Distal Limb

The suspensory apparatus of the fetlock (metacarpophalangeal and metatarsophalangeal joints) comprise the SDFT, the deep digital flexor tendon (DDFT), accessory ligaments of the flexor tendons, the SL, proximal sesamoid bones, intersesamoidean ligaments, and distal sesamoidean ligaments (Figure 13-1, *A* and *B*). The digital flexor muscles are thought to act largely to dampen the high-frequency and potentially damaging oscillations occurring in the limb during high-speed locomotion (Wilson et al., 2001). However, results of a recent study of muscle force and fascicle length in horses walking and cantering on a treadmill indicate that the muscle of the DDFT does play a role in supplementing mechanical work, in contrast to that of the SDFT (Butcher et al., 2009). Cross-sectional areas (CSAs) vary markedly along the lengths of digital tendons, with significant differences existing between individual horses; these differences are not based on height, weight or mid-metacarpal circumference (Smith et al., 1994). There are also breed differences, with ponies and purebred Spanish horses having smaller CSAs in the metacarpal region compared with Thoroughbreds (Agut et al., 2008; Smith et al., 1994).

In the forelimb, the accessory ligament of the SDFT (radial or superior check ligament) originates on the caudomedial aspect of the distal radius and joins the tendon immediately proximal to the carpus. The SDFT measures approximately 45 cm in length, extending through the carpal canal, where it has a round shape, and down the palmar surface of the third metacarpal bone (Mc3), where it is crescent-shaped (Denoix, 1994). The SDFT widens and flattens considerably in the fetlock region, forming a fibrous ring (manica flexoria) proximal to the proximal sesamoid bones, through which the DDFT passes. The SDFT branches at the distal end of the proximal phalangeal bone (P_1), with each branch inserting on the scutum medium, a fibrocartilaginous structure attached to the proximopalmar aspect of the middle phalangeal bone (P_2) (Denoix, 1994). The DDFT also has an accessory ligament (carpal or tarsal check ligament, or inferior check ligament), which, in the forelimb, originates from the palmar carpal ligament, joining the tendon in the mid-metacarpal region (Shively, 1983); the accessory ligament of the DDFT in the hindlimb originates from the joint capsule and plantar ligaments of the tarsus but is poorly developed or absent in horses. The accessory ligaments are thought to prevent overstrain of the digital flexor muscles, functioning as part of the passive "stay" apparatus. The DDFT is round in cross-section in the metacarpal–metatarsal region, becoming oval as it passes through the manica flexoria and then widening at the palmar aspect of the fetlock joint. It passes between the SDFT insertions and over the palmar aspect of the navicular (distal sesamoid) bone to insert on the distal phalangeal bone (P_3).

The SL, underlying the DDFT, is a highly modified interosseous muscle that still contains large numbers of skeletal myofibers. It ranges from 20 to 25 cm long in the forelimb and 25 to 30 cm in the hindlimb (Denoix, 1994). Muscle tissue located within the structure of the SL is also thought to have a "damping" effect (Wilson et al., 1991). In the forelimb, the SL originates from the palmar carpal ligament and proximal palmar aspect of the third metacarpal bone and descends between the second and fourth metacarpal bones; in the hindlimb, the SL originates largely from the plantaroproximal aspect of the

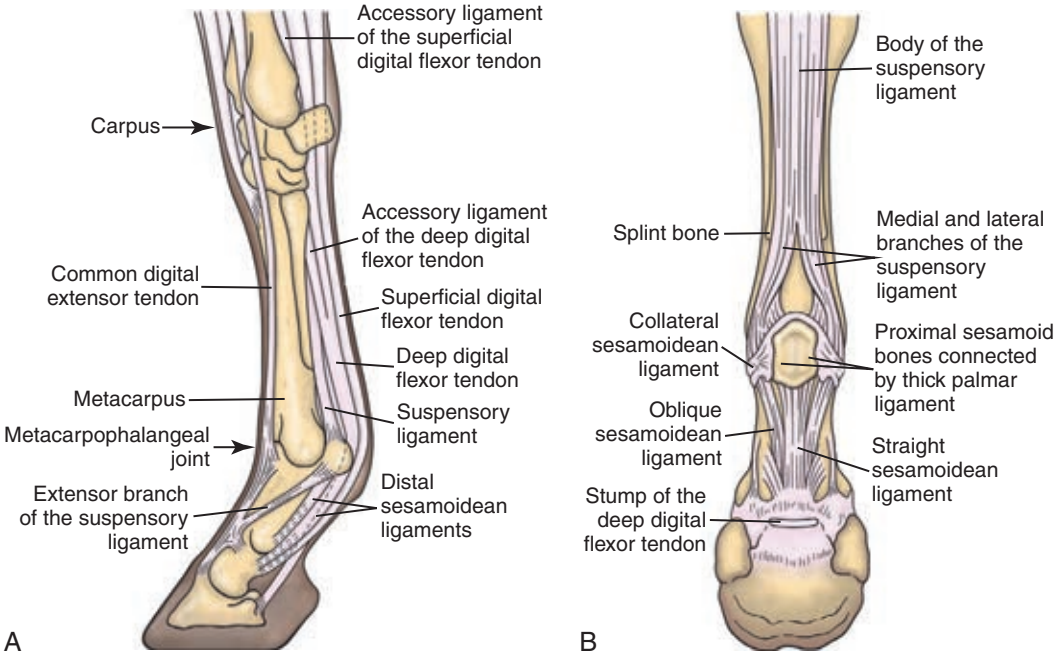

FIGURE 13-1a **A,** Anatomy of tendons and ligaments of the distal limb of the horse. **B,** Anatomy of the suspensory ligament and other structures supporting the metacarpophalangeal (fetlock) joint. (***A,*** *Adapted from Stashak TS, editor:* Adams' lameness in horses, *ed 5, Philadelphia, PA, 2002, Lippincott Williams & Wilkins, p 71, with permission;* ***B,*** *Adapted from Dyce KM, Sack WO, Wensing CJG:* Textbook of veterinary anatomy, *ed 4, Philadelphia, PA, 2010, Saunders, p 604, with permission).*

third metatarsal bone. The SL divides in the distal metacarpal–metatarsal region (the exact site varies) into two branches, each of which inserts on the abaxial surface of the corresponding proximal sesamoid bone, with thin extensor branches passing forward to join the common digital extensor tendon (CDET). The extensor branches may limit P_1–P_2 joint flexion and have been suggested to assist in orienting the foot just prior to impact (Jansen et al., 1993; Wilson et al., 1991). Distally, the suspensory apparatus continues as the sesamoidean ligaments (straight, oblique, cruciate, short), which are highly modified in horses in terms of number, arrangement, strength, and area of insertion, this being correlated in an evolutionary sense with development of the single-toed foot and the passive spring apparatus (Camp and Smith, 1942).

In some sites, tendons are surrounded by a synovial sheath; a carpal synovial sheath surrounds the SDFT and DDFT in the carpal canal, with a mesotenon between them. The carpal synovial sheath extends 7 to 10 cm proximal to the antebrachiocarpal joint to the distal extent of the proximal third of the metacarpus (Denoix, 1994). An incomplete digital synovial sheath begins 4 to 7 cm proximal to the proximal sesamoid bones and extends to the middle of P_2. When not within a synovial sheath, the tendons are surrounded by the *paratenon*, a layer of connective tissue that overlies loose connective and vascular tissue comprising the *epitenon*. The blood supply of tendons arises from the musculotendonous junction and the osseous insertion, between which are longitudinal intratendonous vessels and extratendonous vessels. The extratendonous vessels are located in (1) the paratenon, in extrasynovial locations or (2) the mesotenon that is variably present as remnants, termed *vinculae*, and pass from the wall of the tendon sheath to the tendon proper (Smith and Webbon, 1996). Two major blood vessels extend longitudinally in the lateral and medial borders of the mid-metacarpal SDFT, with extensively anastomosing vessels between them. Where tendons are surrounded by synovial sheaths, diffusion of nutrients is also important; injuries are less frequent in such regions in the horse (Smith and Webbon, 1996). Tendon tissue where it inserts into the bone may be fibrous or fibrocartilaginous, the latter with a transitional zone of hyaline cartilage.

Hierarchical Structure, Microanatomy, and Matrix Composition

The microanatomy of tendons and ligaments is poorly understood in relation to other connective tissues. Despite their bland gross appearance, they have a complex uniaxial hierarchical structure (Figure 13-2), which has not yet been completely defined in terms of mechanical or biologic properties. There is disagreement in the literature on the nomenclature and nature of various hierarchical levels and subunits with certain terms, including collagen "fiber," having many different definitions. Some of this confusion may relate to the initial studies of Kastelic et al. (1977), which were based on rat tail tendons, the structure of which cannot necessarily be directly extrapolated to locomotor tendons or to the specialized structures found in the equine distal limb. This model is the most commonly cited, but there is variation in its interpretation. The discussion below refers to data obtained from examination of equine tendons, wherever possible, and avoids the use of "fiber" as a term.

Fascicles

Fascicles are the largest tendon subunits, measuring approximately 1 mm in diameter in the equine SDFT, with a variable polygonal shape in cross-section (see Figure 13-2) and an age-related reduction in CSAs (Gillis et al., 1997). They are separated by small amounts of loose connective tissue,

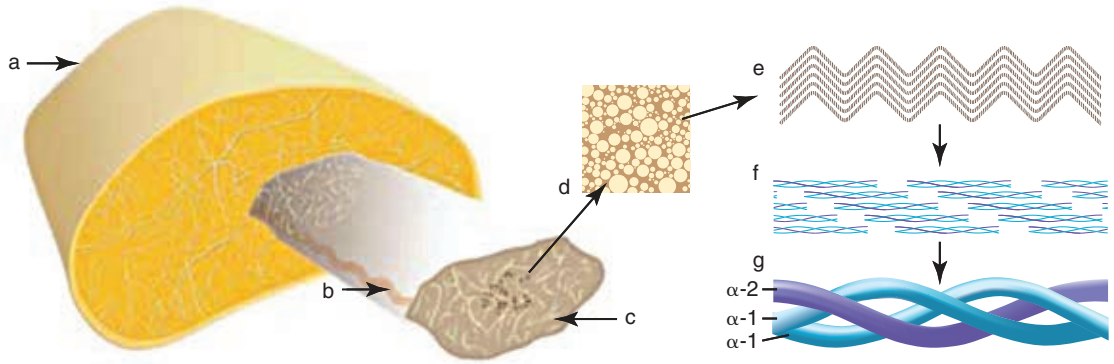

FIGURE 13-2 The hierarchical structure of a superficial digital flexor tendon (*a*). The "crimp" (*b*) is visible on surfaces of the tendon proper and on fascicles (*c*), the latter being the largest subunits of tendon and measuring approximately 1 mm in diameter. Fascicles comprise collagen fibrils with intervening noncollagenous matrix and cells. Collagen fibrils are the units of tensile strength of tendon (10–300 nm diameter), seen here (*d*) as circular in cross-section and including large- and small-diameter structures. The collagen fibrils follow an in-register planar zig-zag waveform (*e*; crimp), and each fibril has a banding pattern when visualized in the longitudinal axis under the electron microscope. The banded appearance is due to the arrangement of collagen molecules in a quarter-stagger pattern (*f*) that creates empty (hole) zones and overlap zones. Each collagen molecule (*g*) comprises three α-chains arranged as a triple helix; type I collagen molecules, which predominate in adult tendon, are each composed of 2 α_1 chains and 1 α_2 chain.

termed the *endotenon*, which contains the vasculature. The inner surface of the epitenon, a fine connective tissue sheath underlying the paratenon that surrounds the whole tendon, is continuous with the endotenon. Fascicles appear to move independently, on the basis of in vitro cyclical loading studies of both equine and human tendon specimens, with small or negligible lateral force transmission (Goodship et al., 1994; Haraldsson et al., 2008). In addition to low interaction between fascicles, results from loading studies using human Achilles tendon specimens suggest that the overall mechanical responses of tendons may be determined by a subset of the "strongest" fascicles (Komolafe and Doehring, 2010). In contrast to rat tail tendon, it is not an easy matter to dissect out individual fascicles because of branching and spiraling along the longitudinal axis. Fascicles comprise collagen fibrils, with the extracellular matrix between them, and tenocytes. Blood vessels do not penetrate into the fascicle under normal circumstances.

In the SL, skeletal muscle tissue comprises 2% to 11% of the total area (Dyson et al., 1995), arranged in bundles that vary in size and arrangement, often in the form of two longitudinally oriented bundles within the proximal part and body of the ligament that are less organized distally and differ in shape between the forelimb and the hindlimb (Wilson et al., 1991). Muscle tissue is associated with longitudinal sheets of adipose tissue. Standardbreds have 40% more muscle in the SL compared with Thoroughbreds, and higher muscle content in the hindlimb SL than in the forelimb SL (Wilson et al., 1991).

Collagenous Matrix

Approximately 70% of the tendon matrix consists of water. Collagen comprises approximately 75% to 80% of the dry weight of tendon tissue. Elastic fibers are also present but are a minor component in most tendons and ligaments. Collagen fibrils are the units of tensile strength in tendons and ligaments, in which they are submicroscopic cylindrical structures arranged in parallel in the longitudinal axis of the fascicle (see Figure 13-2); in ligaments, there tends to be greater variation in orientation. Although equine digital tendons show significant variation in CSAs along their lengths, the CSA is inversely proportional to total collagen content, dry weight of collagen, and percentage occupied by collagen fibrils (Birch et al., 2002; Riemersma and de Bruyn, 1986). This implies that (1) the injury-prone mid-metacarpal SDFT site with the smallest CSA would not be expected to be the weakest point, and (2) variation in CSAs in these tendons is largely due to the noncollagenous components of the matrix. In adult tissue, collagen fibrils consist of predominantly type I collagen (95%). Type III collagen occurs in small amounts within fascicles and is also found in the endotenon; small amounts of other collagen types, including III, IV, V, VI, XII and II, have also been identified. Type II collagen, normally associated with cartilage, is present in the fibrocartilaginous tendon regions that are subjected to compressive forces as they "wrap around" bony prominences.

Collagen fibrils, like other matrix components, are synthesized by tenocytes (tendon fibroblasts) that are arranged in longitudinal rows between them. Tenocytes extend cytoplasmic processes to each other between and within their rows. It has been proposed that these cytoplasmic processes are sheetlike, enveloping bundles of collagen fibrils (McNeilly et al., 1996; Ralphs et al., 2002). Collagen fibrils and fibril bundles are thought to be assembled within complex and overlapping extracellular spaces formed by the tenocyte cytoplasmic membrane, which begin with the fusion of intracellular collagen secretory vacuoles with the surface (Birk and Trelstad, 1986). More recently, plasma membrane protrusions with lumens, termed "fibropositors," have been identified in embryonic tendon projecting into parallel channels between cells, and the tips of these structures are the sites of fibril deposition in the extracellular matrix (Canty et al., 2004). The collagen fibrils follow a planar zigzag waveform, termed the "crimp," which is visible histologically under polarized light as black-and-white banding and may be generated during embryonic development by tenocyte contraction (Herchenhan et al., 2012) (see Figure 13-2 and Figure 13-3). The crimp is thought to act as a mechanical safety buffer; straightening occurs under load (in the toe region of the

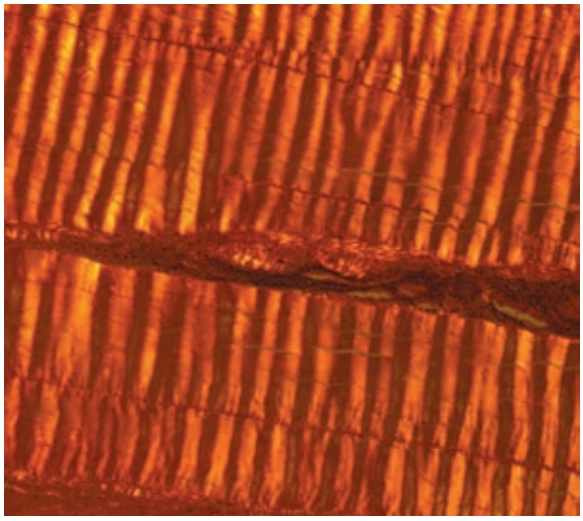

FIGURE 13-3 The "crimp" pattern of the superficial digital flexor tendon, as seen using the polarizing filter of a light microscope. Magnification ×40.

stress–strain curve) before the fibril is itself stretched. A fibril with a low crimp angle will straighten and fail before a fibril with a relatively high crimp angle (Patterson-Kane et al., 1997a; Wilmink et al., 1991) (Figure 13-4). Collagen fibrils are thought to comprise subfibrils (as defined during in vitro collagen fibrillogenesis studies), which are, in turn, proposed to be formed by microfibrillar units. Each microfibril is a grouping of five tropocollagen molecules, which are arranged in a parallel, quarter-stagger array responsible for the repeating bands of fibrils visible in longitudinal sections under the electron microscope (see Figure 13-2). Each molecule comprises three polypeptide α-chains (in type I collagen two α-1 and one α-2), each of which is coiled in a left-handed helix (see Figure 13-2) with

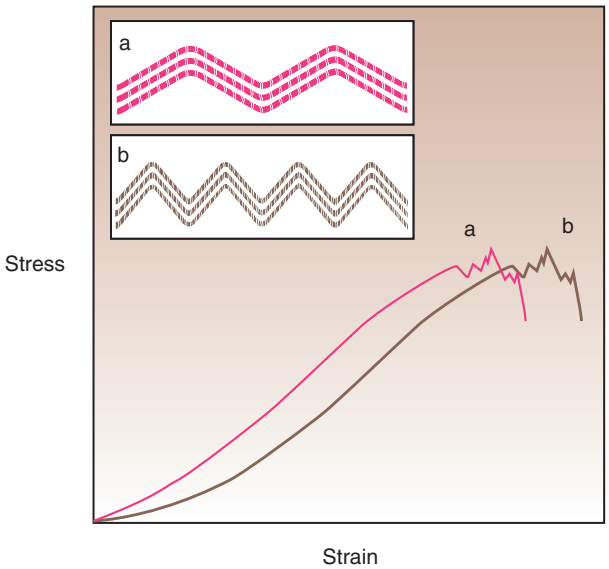

FIGURE 13-4 Diagram comparing the (in vitro) stress–strain behaviour of collagen fibrils with differing crimp angles. The arrow indicates the beginning of the failure zone for the fibril with the smaller crimp angle (*A*). At that level of strain the fibril with the larger crimp angle (*B*) would not be damaged. *(Adapted from Patterson-Kane JC, et al.: Agerelated differences in collagen crimp patterns in the superficial digital flexor tendon core region of untrained horses,* Aust Vet J *75:39, 1997.).*

approximately 100 amino acids. Almost two thirds of the collagen molecule is composed of three amino acids: (1) glycine (33%), (2) proline (15%), and (3) hydroxyproline (15%). Tenocytes synthesize larger precursor (procollagen) molecules that are secreted extracellularly, followed by enzymatic cleavage of aminopropeptides and carboxyl propeptides. It is also possible for collagen synthesis and fibril formation to occur intracellularly (Canty et al., 2004). Cross-links are formed within and between the three helices of a molecule but also form between molecules, this being essential for the strength of the collagen fibril. Lysyl oxidase converts lysine and hydroxylysine residues to aldehydes, which can form reducible or nonreducible cross-links (e.g., lysylpyridinoline and hydroxylysylpyridinoline); levels of reducible cross-links decrease with age.

In neonatal foals, the fibril diameter distribution in tendons and ligaments is unimodal (i.e., the histogram has a single peak), becoming bimodal during maturation (Parry et al., 1978a) (Figure 13-5). In theory, large fibrils are stronger because of their lower surface area per unit volume and consequent higher intrafibrillar density of collagen cross-links. Smaller fibrils, although weaker, may have greater interaction with the surrounding noncollagenous matrix, reducing interfibrillar slippage and contributing to elasticity (Parry et al., 1978a). Type III collagen has been associated with smaller fibril diameters (Birk and Mayne, 1997) and is found in greater amounts in (weaker) immature and healing tendons and in ligaments; as the fibril distribution becomes bimodal in tendons during maturation, the collagen type changes to a clear predominance of type I. As small numbers of large-diameter fibrils can make a large contribution to tendon strength, the diameter distribution is often expressed as the mass-average diameter (MAD). The MAD is effectively the mean of the fibril diameter-area distribution as derived from the diameter-frequency distribution of a given collagen fibril population and correlates with tendon tensile strength (Parry and Craig, 1988). There are significant differences in collagen fibril diameter distributions between tendons with different functions (see Figure 13-5). In the SDFT, with conflicting requirements for strength and elasticity, both large and small diameter fibrils are prominent. Similarly, the SL contains large numbers of small diameter fibrils, but in the DDFT, most are of large diameter (Patterson-Kane et al., 1998a). Mean mass-average diameters of approximately 132 nm, 202 nm, 114 nm, and 271 nm have been reported for the SDFT core, DDFT, SL, and CDET, respectively, in young adult Thoroughbreds (Edwards et al., 2005; Patterson-Kane et al., 1997d; 1998a). MADs in the SDFT and DDFT peak at 12 to 18 months and 2 years, respectively. Crimp angle and period length in the digital flexor tendons reduce from birth until approximately age 2 years, and "mature" (hydroxylysylpyridinoline) collagen cross-links increase to almost maximal levels by age 12 to 18 months (Patterson-Kane et al., 1997b). The digital flexor tendons are, therefore, considered mature by the time horses begin to train and race.

Noncollagenous Matrix

Proteoglycans (PG) comprise up to 20% of solids in the matrix and are molecules that play significant roles in structural integrity and regulation of the matrix. Each PG molecule has a protein core, to which one or more sulfated polysaccharide side chains, termed *glycosaminoglycans (GAGs)*, are attached. Small PGs have one to two GAGs, with decorin and fibromodulin being the most abundant in equine tendon (Smith and Webbon, 1996). Small PGs are thought to play an important role in the regulation of

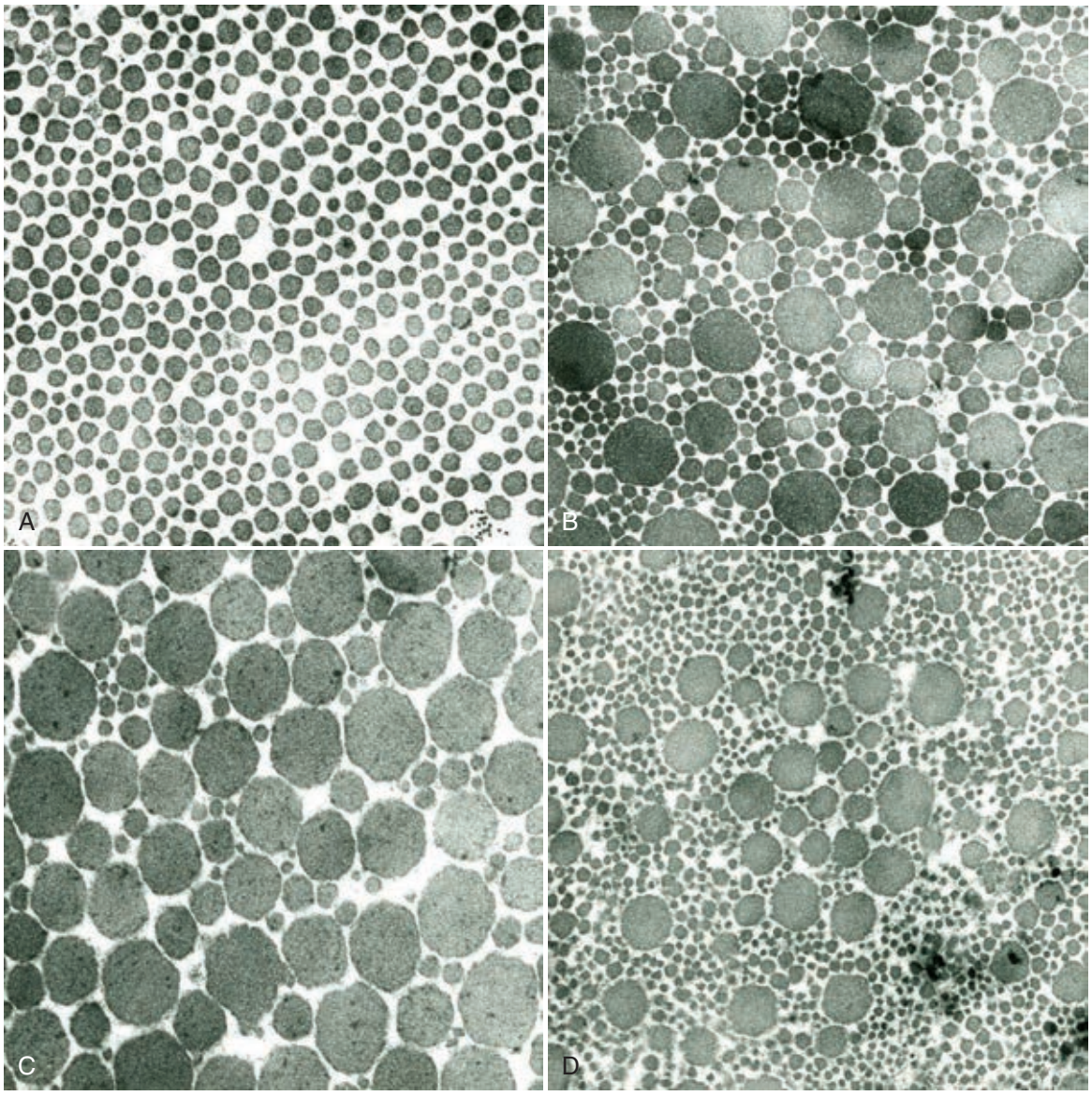

FIGURE 13-5 Collagen fibril morphology in transverse sections of (*A*) the superficial digital flexor tendon of a neonatal foal, (*B*) the superficial digital flexor tendon of an adult horse, (*C*) the deep digital flexor tendon of an adult horse, and (*D*) the suspensory ligament of an adult horse. The fibril diameter distribution in the neonate is unimodal, whereas in the adult, there are large- and small-diameter fibrils in all three structures that provide strength and elasticity respectively. Most fibrils in the deep digital flexor tendon (DDFT) are large-diameter as this is a supportive rather than an energy-storing tendon. (***C,*** *Patterson-Kane JC, et al.: Effects of training on collagen fibril populations in the suspensory and deep digital flexor tendon of young Thoroughbreds,* Am J Vet Res *59:64, 1998, with permission).*

collagen fibril diameter, and interfibrillar PG bridges have been visualized in tendons and other connective tissue (Scott and Tomlinson, 1998; Watanabe et al., 2005). It has been proposed that these PGs also contribute to matrix strength and elasticity through their collagen fibril surface interactions and that they may bind various growth factors; however, the results of one recent study of human patellar tendon, some fascicles in which were treated with chondroitinase ABC to digest GAGs, suggested that proteoglycan-associated GAGs have no effect on tensile force transmission (Svensson et al., 2011). Small PGs, in general, are more abundant in regions of tendons subject largely to tensile loading (e.g., the metacarpal region of the SDFT), with greater prominence of large PGs, including aggrecan and versican, in fibrocartilaginous wrap-around regions, including the dorsal aspects of tendons traversing the palmar surface of the fetlock and plantar surface of the hock (tarsus) region.

Another abundant noncollagenous molecule that has received significant research attention is cartilage oligomeric matrix protein (COMP), a five-armed disulfide-bonded calcium-binding glycoprotein with globular C-terminal domains, which is found in load-resistant tissues. C-terminal domains bind to fibrillar collagen (I, II) and collagen type IX in a zinc-dependent matter. COMP, which was first identified in cartilage, has been proposed to assist in collagen molecule alignment and fibrillogenesis in addition to providing structural integrity; in the SDFT, a positive correlation was seen between high COMP immunolabeling and the percentage of small fibrils (<60 nm) (Södersten et al., 2005). Levels of COMP in the SDFT are

influenced by weightbearing; levels in the tensile region are low in neonates, reach a peak at approximately 2 years (skeletal maturity), and then rapidly decline (Smith et al., 2002b). This decline does not occur in the fetlock region of the SDFT. Highest levels of COMP are found in tendons experiencing the highest tensile loads; levels are lower in the DDFT and the CDET (Smith et al., 1997; 2002b).

There is significant regional variation in the biochemical composition of tendons, in particular, between tensional and wrap-around regions. Glycosaminoglycan (GAG) concentrations are higher in the sesamoid region than the metacarpal region of the SDFT. In the adult bovine DDFT, there is higher expression of messenger ribonucleic acid (mRNA) for collagen types I and II, decorin, biglycan, and aggregan in the wrap-around region (Perez-Castro and Vogel, 1999). The equine SDFT is not homogeneous (i.e., not "biochemically blank") at birth, which has been suggested to be caused by fetal movement in utero; however, there are significant and differential changes in the metacarpal and sesamoid regions during postnatal growth and development associated with changes in the mechanical environment (Lin et al., 2005). This essentially fibrocartilaginous differentiation in wrap-around regions has been reduced in rabbit flexor tendons by surgical translocation to eliminate compression; that is, even in adult tendons, load-modulated remodeling can occur within a short time (Gillard et al., 1979).

Biomechanical Properties of Digital Tendons and Ligaments

Tendons transmit muscular forces to bones, often crossing one or more joints and enabling muscles to (1) move skeletal parts distant from their own positions and (2) coordinate contractile forces of agonist and antagonist (groups of) muscles for precision of movement. Ligaments by definition act passively to link bones and resist their distraction, limiting the motion of some joints and contributing to antigravity mechanisms (Hildebrand and Goslow, 2001). The equine digital flexor tendons and the SL deviate from these traditional definitions, as part of a unique passive apparatus.

Structural and Material Properties

It is important to understand the meaning of terms used to describe the mechanical properties of tendons, ligaments, and other connective tissues. Tendons and ligaments stretch and narrow when subjected to tensile loads. In vitro, a tendon loaded at a constant rate elongates until rupture occurs; the resulting load-elongation curve has a characteristic sigmoidal shape, and properties of the tendon or ligament as a whole structure can be calculated from it. In the initial "toe" region, there is easy stretching in response to low loads, which has been proposed to be caused by straightening of the collagen fibril crimp, the interfibrillar sliding, or both (Nordin et al., 2001). As loading continues, the straightened fibrils are stretched, and there is a "linear" region, with progressively greater force required to produce equivalent amounts of elongation. The slope of this region is the structural stiffness of the tendon. At increasing loads, the curve becomes nonlinear, with the point at which this occurs being termed the "yield point" (although in practice this point is not always easily determined), at which time stretching and slipping of collagen fibrils and molecules begin to occur. Deformations below the yield point are fully recoverable, with the tendon returning to its original shape (elastic deformation); if loading continues, there is increasing collagen deformation with defibrillation and rupture. The ultimate load is the force at complete failure.

Because tendons differ in size between individuals and anatomic sites, the load can be normalized as stress, that is, the internal transmission of the load. This is the force per unit CSA, usually expressed in kilograms per centimeters squared (kg/cm^2) or megapascals (1 MPa = 10.2 kg/cm^2). The resultant deformation is normalized as *strain*, which is a dimensionless term, defined as the percentage change in the length relative to the original "resting" length. The stress–strain curve is also sigmoidal (Figure 13-6, *A*), but properties calculated from it refer to the material that the tendon or ligament is composed of and not the structure as a whole. The slope of the curve in the linear region is a measure of the intrinsic stiffness of the material and is termed the "modulus of elasticity" (Young's modulus, E). It is important not to confuse E with (1) elasticity, that is, the ability of the tendon or ligament

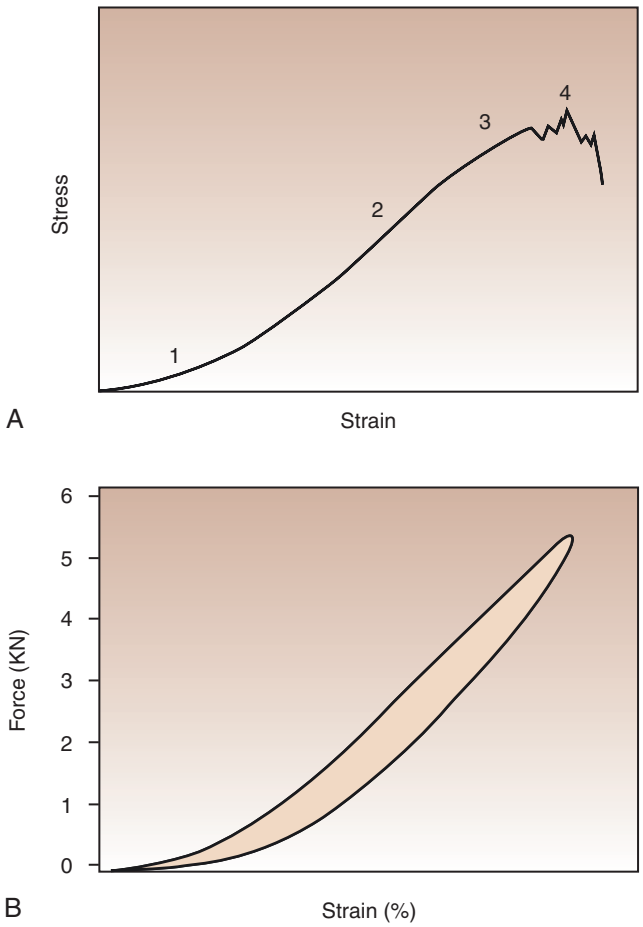

FIGURE 13-6 **A,** A representative stress-strain curve generated during in vitro testing of a tendon. *Zone 1*, the "toe" region, represents straightening of the crimp. *Zone 2*, the linear region in which the now straightened fibrils are themselves stretched. The slope of the linear region is the modulus of elasticity (a measure of stiffness of the tendon material). *Zone 3*, the yield point, irreversible damage occurs. Zone 4 fibrils rupture, leading to complete failure of the structure. **B,** Typical loading and unloading curves of a tendon tested in vitro. The area between these two curves represents the loss of energy (hysteresis). *(Adapted from Goodship AE, et al.: The pathobiology and repair of tendon and ligament injury,* Vet Clin N Am Equine Pract *10:323, 1994, with permission).*

to return completely to its original shape after the load is removed, and (2) structural stiffness (as described above). The ultimate stress and strain values, at which the tendon breaks, are independent of the size and shape of the tendon. Measurements made in vitro do not completely predict the behavior of tendons and ligaments in vivo, as these structures are not linearly elastic, that is, stress and strain do not alter in phase. They are defined as "viscoelastic," which means that they exhibit time-dependent behavior under loading, reflecting the complex interactions between collagen fibrils, the noncollagenous matrix, and water. This can be modeled as a combination of the response of a viscous fluid and an elastic solid (spring). When strain rates are increased, the linear region of the stress–strain curve becomes steeper, that is, the tissue becomes stiffer, and the curve shifts slightly to the left. During repeated cyclical loading, however, the stress–strain curve moves to the right along the strain axis (i.e., the tendon stiffness reduces) until it reaches a steady state. This has been suggested to be caused by packing of collagen fibrils and redistribution or elimination of water from the matrix. In the creep test, under a static load below the linear region of the stress–strain curve, there is an initial rapid deformation followed by slower deformation until equilibrium is reached. Whether dynamic or static loading is applied, this is termed "preconditioning" and is relatively short-lived and eventually recoverable, with no effect on the eventual maximal load.

Some energy input is lost, mostly as heat, due to internal resistance processes, and this is termed "hysteresis," or energy dissipation. Under cyclical loading conditions, the unloading curve will be below the loading curve, with the area between the two representing the energy lost by viscous damping (see Figure 13-6, *B*); energy dissipation in equine tendons and ligaments is approximately 5%. Mathematical modeling predicted that the temperature of the core of the SDFT would plateau at 11°C above that of the surface at a sustained gallop due to insufficiency of the blood supply in dissipating energy lost as heat. Subsequently, peak intratendonous temperatures of up to 45°C were recorded (Wilson and Goodship, 1994); it is possible that core temperatures might be even greater under some circumstances, including extended overground exercise.

Under static loading conditions in vitro, the toe region for the SDFT extends to approximately 3% to 4% strain, with the linear region from 4% to 11%, yield strains of 5% to 13%, yield stresses of 48 to 52 MPa, ultimate tensile strains between 10% and 27%, and ultimate tensile stresses of 65 to 120 MPa (Birch, 2007; Crevier et al., 1996; Dowling et al., 2002; Wilson, 1991). The ultimate tensile strain varies along the length of the SDFT, with highest values in the metacarpal region (Crevier et al., 1996). Values for the elastic modulus for the forelimb SDFT have varied from 1097 MPa to almost 2500 MPa (Batson et al., 2003; Birch, 2007; Crevier et al., 1996; Denoix, 1994). For the DDFT, an ultimate tensile stress of 89 MPa has been reported, with elastic modulus values of 613 to 1585 MPa (Denoix, 1994; Smith, 2006). For the SL, ultimate tensile strains of 10% to 12% and an ultimate tensile stress of 78 MPa have been measured, with an elastic modulus of 510 to 1100 MPa (SL body) (Birch 2007; Denoix, 1994; Smith 2006). The significant individual variation in structural and material properties of each structure suggests that certain horses are more prone to injury. For example, large differences are noted between individuals in both SDFT CSA and ultimate tensile force, with up to twofold differences in the latter (9.5–20 kN [kilonewton]) (Birch 2007; Goodship et al., 1994; Smith et al., 1994; Wilson, 1991). There are also significant differences between horses in the ultimate tensile stress (i.e., the material properties vary between animals); horses with weak tendon tissue tend to have weak bone tissue, raising the possibility that type I collagen quality may vary as in humans (Draper et al., 2004). Alternatively, however, a weaker tendon might exert less force on the attached bone either directly or by signaling earlier "switch-off" of muscular contractile force, resulting in a lesser cortical thickness, lower bone size, or even lower bone density parameters.

Strains occasionally have been measured in vivo, with weightbearing values in the SDFT of ponies and horses of 2% to 4% at a walk and 4% to 10% at a trot (the latter value with a rider), and peak loads of 0.8 kN to one third of bodyweight (Lochner et al., 1980; Meershoek and Lanovaz, 2001; Platt et al., 1994; Riemersma et al., 1996; Silver et al., 1983; Stephens et al., 1989,). Strain measurements of 11.5% and 16.6% were obtained from the SDFTs of two galloping horses (Stephens et al., 1989). Strain rates of up to 200% (i.e., the rate at which deformation occurs) have been predicted and recorded in the SDFT during the early weightbearing phase (Lochner et al., 1980; Stephens et al., 1989; Wilson, 1991). Maximum stresses of 40 to 50 MPa have been calculated to act in the SDFT, the DDFT, and other forelimb and hindlimb tendons at speeds up to 7.4 meters per second (m/s) (Biewener, 1998); Thoroughbreds gallop at speeds up to 19 m/s. Although inherent experimental errors preclude direct comparison, the in vitro and in vivo measurements of SDFT strains support the concept of a narrow mechanical safety margin, thought to be typical of energy-storing tendons (Riemersma and Schamhardt, 1985; Wilson, 1991). Additionally, it is important to consider differences in the relative loading of the flexor structures during weightbearing and interacting external factors, including the weight of the rider, surface conditions, and hoof angle and conformation. The SDFT and the SL are loaded with greater strain rates and are maximally loaded earlier in the stride compared with the DDFT at the walk and the trot (Platt et al., 1994). Strains of 3.4% to 5.9% at the walk, 5.6% at the trot, and 6.3% at the slow canter have been calculated or measured for the SL with smaller maximal stresses of 18 to 25 MPa for speeds up to 7.4 m/s than those for the flexor tendons (Biewener, 1998; Jansen et al., 1993; Keegan et al., 1992). However, it has been suggested that at the extended trot, significant loads are redistributed to the SL. Lowering of toes with respect to heels or raising of heels with respect to toes reduces loading of the DDFT and increases loading of the SDFT and the SL (Denoix, 1994; Stephens et al., 1989). Some computerized analysis of conformation has been performed, showing an increased risk of SDFT injury in racehorses that have increased metacarpophalangeal joint angle or carpal valgus conformation (Weller et al., 2006).

Energy-Storing versus Positional Tendons

Tendons such as the CDET, which position the digit prior to weightbearing (see Figure 13-1, *A*) and function only to transmit muscular force, are defined as "positional tendons." Certain specialized tendons, including the SDFT of horses, also have a significant function in storing elastic energy, resulting in significant savings in muscular work during high-speed gaits, with important biomechanical and injury-risk

implications central to equine evolution and survival. This concept was first proven in kangaroos hopping at high speeds, their gastrocnemius and plantaris tendons being obvious examples of such "biologic springs"; it was calculated that almost 40% of the positive and negative work performed during hopping was accounted for by tendon stretching and subsequent elastic recoil (Alexander and Vernon, 1975). As the horse bears weight, extension of the metacarpophalangeal joint stretches the SDFT, the DDFT, and the SL, including the accessory ligaments of the SDFT and the DDFT, storing kinetic and potential energy as elastic energy to be released during the subsequent propulsion phase. Proximal limb joints, including the elbow and the shoulder, may contribute to this elastic energy storage and release (Clayton et al., 1998). The percentage recovery of mechanical work by tendon elastic energy savings at a gallop has been estimated at 36% (7.4 m/s) (Biewener, 1998).

Because of their dissimilar functions, the SDFT and CDET experience different mechanical environments; the CDET is not a load-bearing tendon and is thought to experience maximum strains of approximately 2.5% (Birch et al., 2008b). The SDFT must be strong enough to deal with the high locomotor and gravitational forces it experiences, but must be able to stretch significantly to store sufficient amounts of energy. Although some researchers have claimed that tendons with different functions do not differ in their mechanical properties, there is clear evidence from in vitro studies of significant differences between the energy-storing SDFT and the positional CDET of horses. Although as a structure the SDFT has a larger CSA and, therefore, fails at higher loads, as a material, it is able to stretch more to store elastic energy (Batson et al., 2003). In two studies of tendons from adult horses, the SDFT had a significantly higher CSA, structural stiffness, and failure load compared with the CDET which had no difference in ultimate stress (Batson et al., 2003; Birch, 2007). However, the failure strains were higher and the mean elastic moduli (1086 ± 261 MPa, per Batson et al., 2003; 970.8 ± 60.4 MPa, per Birch, 2007) lower in the SDFT compared with the CDET (1586 ± 279 MPa, per Batson et al., 2003; 1236.3 ± 209.6 MPa, per Birch, 2007) in both studies. Greater material stiffness is an advantage for the CDET as it functions only to transfer muscle force for accurate limb movement and positioning. These results were supported by measurements of the SL, which is also thought to store elastic strain energy during locomotion. The SL from the same horses in the more recent study had an elastic modulus (643 ± 130 MPa) significantly lower than that of either the SDFT or CDET (Birch, 2007; Smith 2006).

CELLULAR BASIS OF TENDON AND LIGAMENT MATRIX MAINTENANCE AND RESPONSE TO EXERCISE

Central Role of the Tenocyte

Tenocytes are fibroblastic cells within fascicles responsible for the synthesis, maintenance, and degradation of the matrix. Surprisingly, most research on tenocytes has been conducted only in the last 20 years; prior to that most tendon research focused on the matrix. As a result, tenocytes are still a poorly defined population with no generally agreed specific markers. A very small number of genes, including tenomodulin, are expressed in tendons and ligaments but not in other musculoskeletal tissues of rats (Archambault et al., 2006), and scleraxis is a specific marker for tendon and ligament during embryogenesis. Tenocytes were traditionally thought to be relatively inert, but recent studies in human athletes have demonstrated by using tendon biopsies rapid upregulation of collagen synthesis following single bouts of exercise (Magnusson et al., 2007). They are arranged in interlinked networks with connection of their cytoplasmic processes by both gap junctions (GJ) and adherens junctions (AJ) (McNeilly et al., 1996; Ralphs et al., 2002). Tenocytes are immediately surrounded by small amounts of specialized (pericellular) matrix containing type VI collagen, versican, and fibrillin-2 (Ritty et al., 2003).

Tenocytes comprise a very small proportion of the tissue, with cellularity in the young adult SDFT (2–7 years of age) of only 400 cells/mm^2, dropping to less than 300 cells/mm^2 in older horses (Stanley et al., 2007). They are capable of oxidative energy metabolism, suggesting that they may be susceptible to hypoxia (Birch et al., 1997a). In addition to synthesizing matrix components, tenocytes also produce enzymes involved in matrix degradation, much of which is thought to occur extracellularly. These include MMPs, ADAM (A distegrin and metalloproteinases) and ADAMTS (A distegrin and metalloproteinases with thrombospondin motifs). Actions of the proteinases are inhibited by tissue inhibitors of metalloproteinases (TIMPs).

There are also fibroblastic cells in the paratenon, epitenon, and endotenon, the roles of which are not certain. It is known that transforming growth factor-β (TGF-β1) is found in only the endotenon in the adult SDFT (Cauvin et al., 1998). The existence of stem cells in tendon tissue was first suggested by isolation of cell lines with pluripotential behavior, with subsequent direct demonstration within mouse and human tendon tissue of a minor population of cells exhibiting universal stem cell characteristics that are now termed *tendon stem or progenitor cells (TSPCs)* or *tendon-derived stem cells (TDSCs)* (Bi et al., 2007; Lui and Chan, 2011; Salingcarnboriboon et al., 2003). It has been suggested, but not demonstrated, that TDSCs have a perivascular location within the endotenon (Lui and Chan, 2011). However, other authors, on the basis of studies of mouse tendon tissue, claim that the TDSCs are within the extracellular matrix of fascicles and that the stem cell "niche" is determined by the nature of that matrix (Bi et al., 2007). Adult equine tendon-derived cells have some degree of multipotential differentiation capability, but it is less than that of bone marrow–derived mesenchymal stem cells (Strassburg et al., 2006). Identification of TDSCs in situ in equine tendon has not yet been achieved.

Cell Types and Networks

Tenocyte Types

Histologically, three tenocyte "types" have been defined in equine tendon on the basis of their nuclear morphology. It is not known if these are true functional cell types and if they are the equivalents of osteocytes, osteoblasts, and osteoclasts in bone. Type 1 tenocytes have thin spindle-shaped nuclei, whereas those of type 2 cells are more rounded and cigar-shaped nuclei (Figure 13-7, *A*). In the SL, there are larger numbers of cells than in the SDFT, with predominance of type 2 nuclei (Smith and Webbon, 1996) (see Figure 13-7, *A* and *B*). Three dimensionally, type 1 and 2 tenocyte nuclei are slightly flattened ovoids; the range of nuclear widths is greater for the type 2 cells (Doube, 2001, Stanley, 2005). Type 3 tenocytes are found in wrap-around regions and have a more chondrogenic phenotype (see Figure 13-7, *B*). Type 3 tenocytes express significantly less gap junction proteins or none at all (Ralphs et al., 1998). In other species, tendon cells equivalent to type 2 and type 1 cells in

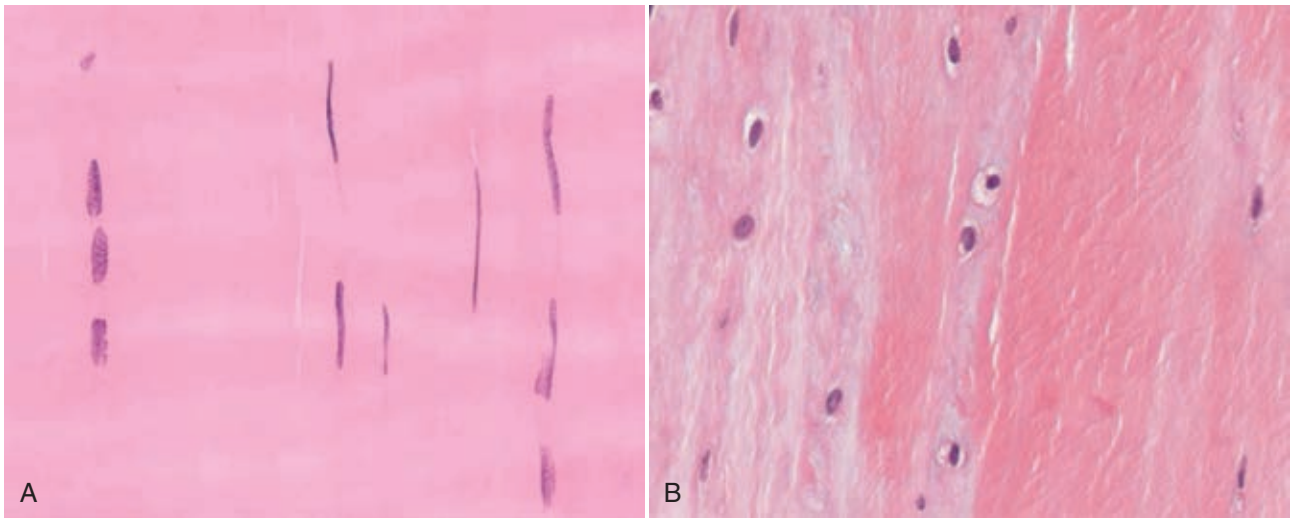

FIGURE 13-7 Photomicrographs of tenocytes. **A,** Rows of type 1 tenocytes with thin, spindle-shaped nuclei (*arrows*), and of type 2 tenocytes with rounded, cigar-shaped nuclei in the mid-metacarpal region of the superficial digital flexor tendon of a 5-year-old horse. **B,** Type 3 tenocytes in the metacarpophalangeal region of the deep digital flexor tendon of a 6-year-old horse with chondrocyte-like appearance, surrounded by matrix with increased basophilia. Hematoxylin and eosin. Magnification ×400. (Courtesy of R. L. Stanley.)

horses have been referred to as "tenoblasts" and "tenocytes," respectively (Chuen et al., 2004; Ippolito et al., 1980; Kannus, 2000). It has been proposed that as they age, the tenoblasts become elongated and transformed into tenocytes, although there is only indirect evidence for this (Ippolito et al., 1980; Nakagawa et al., 1994). Age-related increases in the proportions of type 1 tenocytes and in nuclear lengths have been measured in the equine SDFT and CDET, along with reductions in type 2 cells and overall cellularity (Stanley, 2005). Type 2 tenocytes (tenoblasts) do appear to be more synthetically active compared with type 1 cells; in nonequine tendons the type 2 tenocytes have been shown to contain more extensive synthetic organelles, express higher levels of certain proteins including type I procollagen and matrix metalloproteinase 1 (MMP1), and have higher proliferative and apoptotic indices (Chuen et al., 2004; Ippolito et al., 1980).

Tenocyte Network

The ability of tenocytes to sense and respond to load is central to maintenance of the matrix. Detection of load signals is thought to occur through deformation of the cytoplasmic membrane, the cytoskeleton, or both, and growth factors may be required for this process (Banes et al., 1995; Wang, 2006). Tenocytes are attached to extracellular matrix proteins, including collagen and fibronectin by integrins, these being transmembrane mechanoreceptors with α- and β-subunits (Wang et al., 1993). Adhesion to collagen via α1β1 integrins, which preferentially bind to nonfibrillar collagen types, promote cell growth, and inhibit collagen synthesis. Adhesion via α2β1 integrins, which preferentially bind to fibrillar collagens (including type I), inhibit the cell cycle, and promote collagen synthesis and degradation. Tenocytes attach more strongly together in response to mechanical loading, with upregulation of n-cadherin (an AJ component) and vinculin, an actin-binding protein that links both integrin molecules and AJ components to the cytoskeleton, and assemblage of more of their actin fibers into stress fibers with tropomyosin (Ralphs et al., 2002). Tenocytes have been suggested to establish an internal cytoskeletal tension through their extracellular interactions, resulting in a mechanostat set point that determines whether cells will respond in an anabolic manner or a catabolic manner to mechanical loading (Lavagnino and Arnoczky, 2005). The nucleus itself may be deformed, as demonstrated in tendons in vitro by using confocal laser scanning microscopy, and may also play a significant role in the signal transduction pathway (Arnoczky et al., 2002). Additionally, tenocytes are now known to express a single primary cilium, this being a microtubule-based sensory organelle that projects into the extracellular matrix parallel to the longitudinal axis of the tendon; the deflection of cilia in response to tensile loading that has been demonstrated experimentally supports the concept that these structures are involved in mechanotransduction (Donnelly et al., 2010, Lavagnino et al., 2011).

Tenocytes are also linked into coordinated networks by GJ, that allow metabolites, ions, and small molecules <1 kilodalton (kDa) to pass directly between the cytoplasm of neighboring cells. Each GJ comprises two annular hemichannels that are embedded in the cytoplasmic membranes of apposing cells and dock to form a tightly sealed channel. Each hemichannel comprises connexins, these being transmembrane proteins of which 21 genes are known in the human genome. Connexins 43 and 32 (Cx43, Cx32) have been identified in equine and nonequine tendons (McNeilly et al., 1996; Stanley et al., 2007; Figure 13-8). Both isoforms may be found in the GJ linking tenocytes in the same row, but only Cx43 forms the GJ channels responsible for lateral links (McNeilly et al., 1996). Calcium wave propagation is blocked in avian tendon cells if they are cultured in the presence of GJ chemical blockers or connexin antibodies (Boitano et al., 1992). GJ blockade also prevents upregulation of collagen synthesis by tendon cells in response to cyclic loading (Banes et al., 1995, 1999). There is some evidence from a study of avian tenocytes treated with antisense oligodeoxynucleotides that Cx43 and Cx32 may differentially modulate collagen synthesis, with Cx43 being

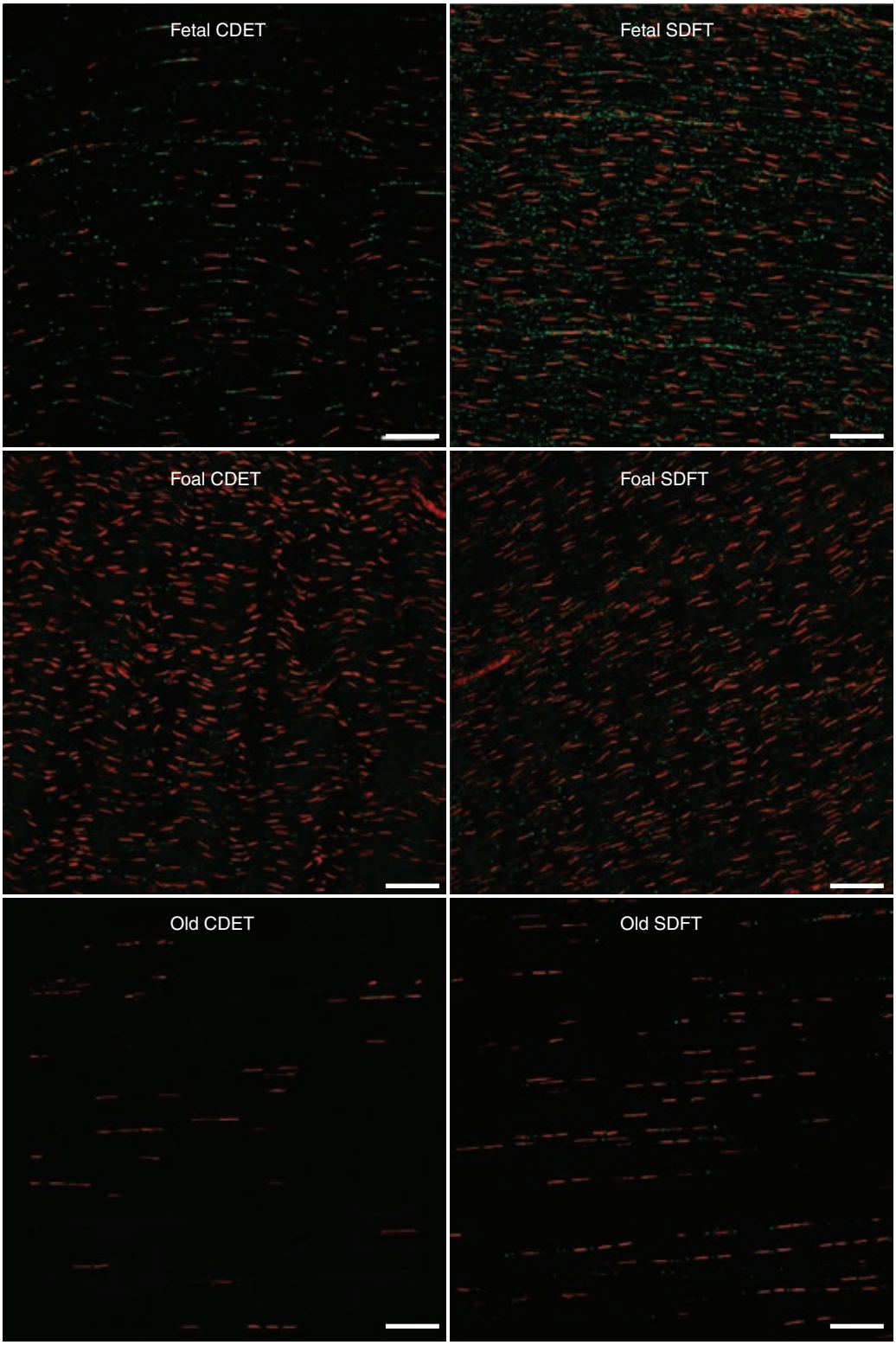

FIGURE 13-8 Longitudinal cryosections from the superficial digital flexor tendon and common digital extensor tendon of a fetus, a foal, and an old horse, immunolabeled for connexin 43 (*green plaques*). Tenocyte nuclei are counterstained with propidium iodide (*red*). Immature tendons were significantly more cellular, and greater expression of the gap junction protein per cell was measured in the fetal structures. Bar = 80 µm. *(From Stanley RL, et al.: Gap junction protein expression and cellularity: comparison of immature and adult equine digital tendons,* J Anat *211:325, 2007, with permission).*

inhibitory and Cx32 stimulatory (Waggett et al., 2006). In wrap-around regions of tendons, where type 3 tenocytes predominate and the expression of GJ is low to nonexistent, coordination of tenocyte behavior must occur by other means (e.g., via indirect cytokine and growth factor signaling) (Ralphs et al., 1998).

Tendon-Specific and Age-Specific Differences in Tenocyte Networks

Significant changes in cellularity, tenocyte morphology, and tenocyte activity with both maturation and aging have been documented in equine digital tendons, with marked differences between energy-storing tendons and positional tendons. In the adult horse, the SDFT has a significantly higher cellularity than the CDET (Stanley, 2005; Stanley et al., 2007; Young et al., 2009). However, there is increasing evidence that these more numerous SDFT tenocytes are less active. In the SDFT of adult Thoroughbreds, age-related increases in collagen-linked fluorescence were documented, but these did not occur in the CDET; this suggested, but did not directly prove, that the turnover of collagen reduces with age in the SDFT (Batson et al., 2003). Subsequent analysis of aspartic acid racemization of collagen in horses 4 to 30 years of age indicated mean collagen half-life values of 198 years and 34 years for the SDFT and the CDET, respectively (Thorpe et al., 2010). Analysis of tissue showed lower relative levels of mRNA for type I and III collagen and for MMPs per cell in the SDFT compared with the CDET (Birch et al., 2008b). There were high levels of the cross-linked carboxyterminal telopeptide of type I collagen (ICTP) (a marker of collagen synthesis) and lower levels of tissue fluorescence in the CDET; that is, this was further support for the hypothesis that tenocytes are less active in the SDFT than in the CDET of the adult horse, with reduction or inhibition of collagen turnover.

In immature horses, however, the SDFT tenocytes are significantly more numerous and appear to be more active. This is not surprising, as increases in total bodyweight are very rapid in foals, particularly within the first 90 days of birth (Hintz et al., 1979). The SDFT cross-sectional area increases more than twofold between 50 and 365 days, necessitating significant synthetic cellular activity (Kasashima et al., 2002). In histomorphometric and immunofluorescent labeling studies, the density of tenocytes was greatest in tendons of late-term equine fetuses and foals, with higher expression of GJ proteins, and type I and III collagens in immature horses than in adults for the SDFT (Stanley et al., 2007; Young et al., 2009) (see Figure 13-8). In the CDET, there was actually an increase in Cx43 and collagen expression with maturation, exceeding levels in the more cellular SDFT (Young et al., 2009). Cultured immature SDFT-derived cells produced more collagen and COMP compared with adult cells in another study, with no such maturational changes in CDET cells (Goodman et al., 2004). When gap junction permeability was studied in cell culture, there was a reduction with maturation in the SDFT but not in the CDET (Young et al, 2009). Additionally, significantly higher levels of TGF-β (a potent stimulator of type I collagen synthesis) were measured in young equine tendons compared with adult structures (Cauvin et al., 1998). It has been suggested that despite the theoretically higher demand for matrix renewal compared with positional structures, energy-storing tendons, including the SDFT, specifically "switch off" synthetic cellular machinery once maturity is reached to maintain the matrix within narrow optimal limits of elasticity and stiffness (Smith et al., 2002a). This has also been measured in the bovine DDFT, with no expression of mRNA for collagen or PG in the tensional region of the adult structure (Perez-Castro and Vogel, 1999). The mechanisms by which this cellular switchoff occurs are not understood; however, it is a key consideration in the response of the SDFT to imposition of increased exercise through training, racing, or other athletic activity.

FUNCTIONAL ADAPTATION OF TENDON

There is very little information on the effects of exercise on tendons in any species, and much of it is inconsistent, as opposed to the well-documented changes that occur in skeletal muscle tissue and bone. To some extent, this can be attributed to the lack of a suitable animal model in which tendons can be isolated and differentially loaded, e.g., as for the functionally isolated avian ulna model used for bone research (Rubin and Lanyon, 1984b). Additionally, it is important to consider whether the changes that have been documented have any adaptive significance. For example, changes in tendon stiffness will not necessarily maximize elastic energy storage if forces applied to the tendon are not also substantially altered (Buchanan and Marsh, 2002). Moreover, increases in strength or stiffness may not necessarily increase resistance to fatigue damage. A number of controlled exercise studies using foals or young adult Thoroughbreds have been performed in an attempt to determine effects of specific training regimens on tendon structure and composition (Table 13-1 and Table 13-2); these studies will be discussed below.

Evidence of Exercise-Induced Microdamage

Tenocytes constantly repair small amounts of damage to the matrix under normal circumstances; otherwise, all tendons would weaken and rupture (Ker, 2002). It has been suggested that damage is actually the trigger for tenocytes to produce "better" material to resist fatigue (Ker et al., 2000). However, excessive and repetitive loading may cause levels and types of subclinical matrix damage (microdamage) that cannot be repaired by the tenocytes for various reasons, including insufficient rest periods between episodes and simple overwhelming of cellular capacity due to a high frequency or nonuniformity of overstrain. Tenocytes could potentially be damaged themselves, either dying or responding with inappropriate synthetic or degradative activity. Prolonged cyclical loading of equine SDFT explants at 5% strain (1hertz [Hz] for 24 hours) resulted in significantly reduced matrix strength, a process that required viable cells and MMP activity (Dudhia et al., 2007). Experimental work with cultured human tenocytes has suggested that upregulation of MMP expression is associated with high levels of strain (Yang et al., 2005). Some researchers have hypothesized the opposite, that is, the occurrence of isolated fibril rupture near the end of the linear region of the stress–strain curve, which results in mechanobiologic understimulation of tenocytes in the region of microdamage with resultant upregulation of expression of various MMPs (Arnoczky et al., 2007). Degradation of the pericellular matrix would further compromise cell–matrix interactions and signaling. Mechanical stress–deprivation has also been shown to upregulate tenocyte apoptosis in tendon explants (Egerbacher et al., 2008).

Temperatures of up to 45°C as documented in the SDFT core of galloping horses could be an important factor. Although

TABLE 13–1

Controlled Exercise Studies Using Young Adult Thoroughbred Horses

Name of study	Breed, gender	Exercise type	High-speed training regimen	Controls	Number of horses	Duration	Age at start (± SD)
18-month Bristol study.	TB fillies	High-speed equine treadmill.	Per wk: Mon 3 km @ 12 m/s; Weds 2x 1.5 km @ 12 and 14 m/s (5 min recovery); Fri 3x 1 km @ 12, 13 and 15 m/s (5 min recovery). (Low intensity 10 min trotting Tues, Thurs, Sat; 40 min in mechanical horse walker 6 d).	Per wk: 40 min in mechanical horse walker 6d.	Ex: 5 Control: 6	18m	Ex: 21.3 ± 1.1mo Control: 20.7 ± 1.1mo
18-week Bristol study.	TB fillies	High-speed equine treadmill.	Per wk: Mon 4.8 km @ 12 m/s; Weds 3x 0.8 km @ 13 m/s (1 min recovery); Fri 2x 1.3 km @ 11 m/s (2 min recovery). (Low intensity 20 min trotting Tues, Thurs, Sat; 40 min in mechanical horse walker 6 d).	Per wk: 40 min in mechanical horse walker 6d.	Ex: 6 Control: 6	18 wks	Ex: 19.4 ± 0.6mo Control: 19.2 ± 1.2 mo
Massey University Grass Exercise Study (MUGES).	TB fillies	Grass or sand racetracks.	Exercised 6d per wk. Stage 1 (wks 1-4): canter @ 7.5 m/s. Stage 2 (wks 5-8): canter @ 9.0 m/s. Stage 3 (wks 9-13): canter @ 8.4 m/s, galloping 2x/wk @ 14.6 m/s. 3 horses did not complete training due to lameness. Mean distance galloped 1066m and 4450 m for medium and high exercise intensity subgroups.	Grass enclosures, exercise at will.	Ex: 7 Control: 7	13 wks	Ex: 662 ± 26d. Control: 652 ± 34d
Colorado State University Study	Quarter horses and Quarter horse crossbred, gender not indicated.	High-speed equine treadmill.	Exercised 5d per wk. Initially: 2 min trot (4-5 m/s), 3 min gallop (9 m/s), 2 min trot. After 36 d, gallop speed increased to 12 m/s.	Hand-walked for 7 min 5 d/wk	Ex: 6 Control: 6	6 m	2.5 yo

TB = Thoroughbred, Ex = exercised group.

high survival of tendon-derived cells after 10 minutes at that temperature was documented in one study (91% ± 4%) (Birch et al., 1997b), those cells were in suspension, a situation in which cells are known to have greater heat resistance and are not linked by the GJ. Subsequent work using SDFT-derived cellular monolayers indicated significant cell loss following a 10-minute period, during which the temperature was increased to 45°C (Burrows et al., 2008). Loss of even small numbers of tenocytes in this lowly cellular tissue could have significant consequences in terms of collagen synthesis and repair potential; however, it has not been directly demonstrated that apoptotic cells occur in equine tendon that is degenerate but not clinically injured.

Given that the SDFT has narrow mechanical safety margins and that the number of tenocytes and their level of synthetic activity and matrix turnover in the adult tendon are low under normal circumstances, (1) this energy-storing tendon may occasionally or even frequently be loaded into the "yield" phase of the stress–strain curve during exercise, resulting in microdamage; and (2) the risk of an imbalance between cellular repair and matrix microdamage is likely to be high. A "tendonosis cycle" has been proposed, with accumulation of damage that weakens the matrix and predisposes to further insult. The ultimate result in some individuals will be a partial or complete rupture during normal athletic activity. It is generally agreed that a large proportion of tendon injuries in humans occur in this way, as opposed to rupture of a healthy tendon under extreme physical circumstances, and there is also significant evidence for exercise-induced degenerative change (i.e., "overuse") in the SDFT of significant numbers of horses (Goodship et al., 1994; Józsa and Kannus, 1997; Smith et al., 1999). This concept is supported by results of epidemiologic studies, showing an increased risk of SDFT and suspensory apparatus injury in older horses that have theoretically experienced greater numbers of

TABLE 13–2

Controlled Exercise Studies Using Foals

Name of study	Breed, gender	Exercise type	Training regimen	Controls	Number of horses	Duration	Age at start (± SD)
Dutch EXOC (Examination OsteoChondrosis) study.	Dutch Warmblood. Colts and fillies (blocked for sex and sire).	Mares and foals chased in a paddock with sand surface over a concrete foundation.	Pasture group: moved freely in the paddock (24h), no stabling. Observed mean gallop time 3.5min/d divided over 40 sprints. Boxtraining group: kept in 3 x 3.5 m box stall up to 5 m with 12 gallop sprints/d (40 m each) for 6d/wk a from 7d (of age), increased gradually up to 32 and 16 sprints on alternate days from 39d to 5m (total 3 min galloping/d). At 5m 8 foals per group were euthanatized; the reminder in this group underwent normal paddock activity until 11m.	Boxrest group: permanently stabled up to 5m, normal paddock activity 5-11m (n=6).	Pasture: 15 Boxtraining: 14 Boxrest: 14	5 m, 11m	7d
Japanese Racing Association (JRA) study.	TB Colts and fillies (blocked for sex).	Horse walker followed by high-speed equine treadmill.	Pasture exercise 4h/d. At 55d (of age) 960m walking @ 1.6m/s for 10min. From 60d an ascending program of treadmill exercise: trotting for 4min (2.5m/s initially, 3.3m/s from 150d) interspersed with 5x 15s cantering periods (5 m/s at 60d to 11m/s at 440d).	Pasture exercise 4h/d, remainder of time spent in a stall.	Ex: 7 Control: 7	440d	birth
Global Equine Research Alliance (GERA) study.	TB Colts and fillies (blocked for sex and sire).	515m purpose-built turf track with a thin superficial layer of sand. Mares and foals trained to trot and canter adjacent to the inner rail.	CONDEX group: kept on pasture with track exercise 5d/wk over 1030m. Phase 1A (start 21±19d) base average velocity 5.4m/s. Phase 1B (weaning (138±10d) until completion of first sprint) base average velocity 7.5m/s. Phase 1C (first sprint (236±31d) to 18m) base average velocity 9.6m/s, sprint average velocity 12.5m/s after first 500m @ base speed.	PASTEX group: Pasture exercise 24h/d.	CONDEX: 18 PASTEX: 15	16-18 m (n=6 per group); remainder monitored until end of the 3-year-old racing season.	3w

TB = Thoroughbred, Ex = exercised group.

loading cycles at high speed (Avella et al., 2009; Perkins et al., 2005; Williams et al., 2001). Results from several studies have suggested that exercise and age have synergistic effects that can be difficult to separate in uncontrolled circumstances. In one study of feral horses, that is, those experiencing "natural" levels of activity, mean crimp angles in the core of the mid-metacarpal SDFT reduced with age such that they were lower than those in the periphery of the tendon cross-section in horses 10 years of age and older (Patterson-Kane et al., 1997a). This normally age-related change was found to be accelerated in racehorses, and in the horses in the Bristol study that were trained on a high-speed treadmill for 18 months, that is, in animals experiencing a greater number of loading cycles earlier in life (Patterson-Kane et al., 1997c, 1998b) (Table 13-3). As fibrils with lower crimp

TABLE 13–3

Collagen Fibril Mass-Average Diameters (nm ± SD) for Core and Peripheral Regions of the Mid-Metacarpal Superficial Digital Flexor Tendon Cross-Section of Control and Exercised Horses After 18 Months Treadmill Exercise (the Reference is Below).

	Horse	Central	Peripheral
Controls	2	119.1	74.8
	4	136.8	107.7
	6	126.8	150.8
	8	127.8	140.8
	10	153.5	104.3
	12	126.0	128.1
	Mean	131.7 (4.9)*	117.8 (11.3)
Exercised	1	110.5	140.0
	3	117.2	86.0
	5	106.3	123.9
	7	100.8	133.2
	11	91.7	152.3
	Mean	105.3 (4.3)	127.1 (11.3)

*significantly greater than the corresponding value for the exercised group ($p<0.05$) (Patterson-Kane et al. 1997d).

angles will fail at lower levels of strain, the exercise-induced changes in crimp morphology were interpreted as microdamage.

Similarly, collagen fibril diameters have been previously shown to reduce with aging in the equine SDFT and SL (Parry et al., 1978b). In the 18-month Bristol study, there was a reduction in the MAD in the SDFT core of the exercised horses, which did not occur in equivalent segments of the DDFT, the SL, or the CDET (Edwards et al., 2005; Patterson-Kane et al., 1997d; 1998a) (see Table 13-3). As there was no evidence of synthesis of new collagen in the SDFT core in the form of detectable levels of immature collagen cross-links or alterations in levels of tissue fluorescence, it was suggested that there had been breakdown of larger fibrils (Birch et al., 2008a; Patterson-Kane et al., 1997d; Smith et al., 2002); fibrils from rat tendons were noted to split longitudinally in response to high mechanical loads in in vitro experiments (Torp et al., 1975). Additionally, in the same site, there was exercise-related acceleration of normal maturational loss of COMP and a reduction in GAG content (Birch et al., 2008a; Smith et al., 1999). As SDFT COMP concentrations for 2-year-old horses have been correlated with ultimate tensile stress, modulus of elasticity, and tendon stiffness, this change in the 18-month Bristol study was interpreted as being one that would predispose to further injury (Smith et al., 1999; 2002a).

Increased amounts of (weaker) type III collagen have been measured in the SDFT core in older horses relative to the periphery of the cross-section (Birch et al., 1999a). Levels of type I collagen degradation markers, that is, collagenase-generated neoepitope and cross-linked telopeptide of type I collagen have also been determined to increase with age in this tendon (Thorpe et al. 2010). In areas of red discoloration of the SDFT core in other horses (Figure 13-9), there were also increases in the proportion of type III collagen in addition to increased total sulfated GAG content and a decrease in collagen-linked fluorescence relative to the periphery, that is, an increase in collagen turnover and deposition of an abnormal collagen type (Birch et al., 1998). Such changes were previously interpreted as subclinical damage. However, in vitro analysis has revealed that tendons with red core lesions are enlarged and contain central hypoechoic areas; that is, this is more likely to be a reparative response than preclinical microdamage (Smith, 2003).

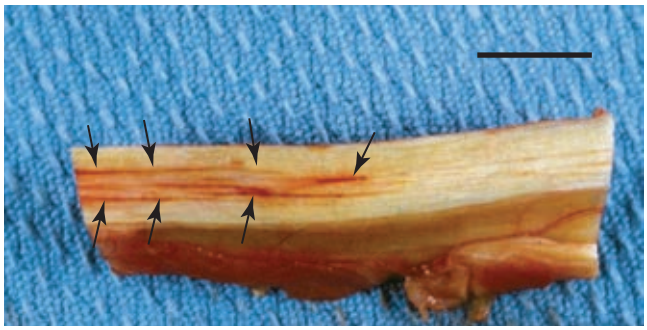

FIGURE 13-9 Sagittally sectioned mid-metacarpal segment of the superficial digital flexor tendon of a Thoroughbred racehorse. The arrows indicate red discoloration of the tendon core, a change that has been associated with increased collagen turnover and deposition of weaker type III collagen, that is, a reparative response. Bar = 1.5 cm. (*From Patterson-Kane JC, Firth EC: The pathobiology of exercise-induced superficial digital flexor tendon injury in Thoroughbred racehorses,* Vet J *181:79, 2009, with permission*).

Effects of Training on Equine Digital Tendons

Most studies of the response of equine tendons to training have focused on the SDFT, sometimes with comparison with other digital tendons. Information relating to the SL is very limited. In one in vitro study of the limbs of a small number of Thoroughbreds and Standardbreds, the point of failure of the suspensory apparatus from horses that had been in training or racing ($n = 6$) was the proximal sesamoid bones, rather than the SL itself, as was observed in control horses ($n = 6$) (Bukowiecki et al., 1987). This was attributed to exercise-induced strengthening of the SL. In another study, Thoroughbreds (but not Standardbreds) in race training had a lower muscle content in the SL compared with those not in training; however, only two horses not in training were analyzed (Wilson et al., 1991).

Controlled Treadmill Exercise Studies

Minimal evidence has been obtained from these studies (see Table 13-1) to indicate that digital tendons can adapt to exercise. In the 18-week and 18-month Bristol studies, exercised horses were galloped on a high-speed equine treadmill three times weekly with additional trotting and walking exercises. Control horses underwent walking exercise only for 40 minutes daily (6 days per week). There were no changes in the CSA of the SDFT; however, there was an acceleration of normal maturation-related hypertrophy of the CDET (Birch et al., 1999b). As stated previously, as the CSA of the digital flexor tendons of horses is inversely proportional to collagen content, an increase does not necessarily imply increased resistance of the material to tensile loading (Birch et al., 2002; Firth, 2006; Riemersma and de Bruyn, 1986). In the periphery of the CSA of the SDFT in the 18-month Bristol study, there was a significant exercise-related increase in collagen fibril crimp angle that was interpreted as adaptive, in contrast to changes in the injury-prone SDFT core (Patterson-Kane et al., 1998b) (see Table 13-3).

Controlled Racetrack Exercise Studies

In the "Massey University Grass Exercise" (MUGES) study (see Table 13-1), 2-year-olds galloped on grass and sand tracks for 13 weeks had significantly larger SDFT CSA as measured ultrasonographically at five pooled levels in the metacarpal region, compared with control horses (Perkins et al., 2004). These findings were substantiated by tissue examination of the tendons; the CSAs, weight, and volume of the SDFT and the CDET were greater in the exercised group, and there was no clinical, ultrasonographic, or histologic evidence of injury (Firth et al., 2004a). Larger tendons will be stronger under tensile loading; however, this does not imply that the material comprising the tendons changed or adapted in any way. The SDFT echogenicity of the trained horses was lower at most sites, and it was proposed that increases in the CSAs were largely due to changes in the noncollagenous matrix, including water content (Firth et al., 2004a). It is important to differentiate apparent adaptive increases in SDFT CSAs from injury. In an uncontrolled Californian study of the SDFTs of six Thoroughbreds during the first 4 months of race training, there were significant ultrasonographically measured increases in CSA that could not be interpreted as adaptive, as two of the animals developed clinical signs of SDFT injury (Gillis et al., 1993).

Should Foals Be Trained from Soon after Birth?

The more abundant and apparently more active tenocyte population in the SDFT of foals may provide a "window of opportunity" in terms of stimulating adaptive change or at least conditioning the horse before skeletal maturity. Several controlled studies of the effects of exercise on the musculoskeletal system of foals are summarized in Table 13-2. It is important to keep in mind that there are few studies on the workloads of foals kept completely at pasture and of feral horses in the wild (Hampson and Pollit, 2008; Kurvers et al., 2006) and that there are wide variations within both environments; the result is that valid comparison of the two to determine what is "natural" or normal locomotor activity in the young horse is extremely difficult or impossible. Foals stabled for some proportion of the day show some compensatory activity that is probably insufficient to reach the levels of those kept outside. This implies that foals not given continuous access to pasture exercise are effectively partially immobilized. The type and timing of any exercise superimposed on normal pasture activity is also likely to be important.

In the Dutch EXOC study, three groups of foals experienced box stall rest (boxrest group), sprint exercise superimposed on box stall rest (boxtraining group) and pasture exercise, respectively, until they were 5 months of age, with some foals from all groups subsequently having 24-hour pasture access up to 11 months of age (Cherdchutham et al., 1999; 2001). By 5 months, there were greater numbers of small-diameter fibrils in foals that had been exercised, particularly in the pasture group; this did not occur in boxrest foals until they had access to pasture. At 11 months, the development of collagen structure in the boxrest and boxtraining groups still lagged behind that of pasture group, and it was proposed that the immobilization in box stalls may have had long-term negative effects on tenocyte metabolism.

In the Japanese Racing Association (JRA) foal study, control and exercise groups received 4 hours of pasture exercise per day from 2 to 15 months of age, with the exercise group receiving an additional short daily period of treadmill exercise (Kasashima et al., 2002). Again, neither group received "normal" levels of pasture exercise. In the treadmill-exercised foals, the CSAs of the SDFT were larger at several time points, with a greater rate of increase as a function of time. This was interpreted as modulation of tendon development by the exercise regimen; however, the long-term effects were not known; there was no significant difference between the groups at the end of the study because of high variance.

In a study conducted by the Global Equine Research Alliance (GERA), foals in the control group were allowed free pasture exercise (PASTEX), whereas foals in the exercise group (CONDEX) were also kept at pasture but began conditioning exercise from 21 days of age over 1030 m on a grass track until 18 months of age. Workload for the CONDEX group was approximately 30% higher than that for the PASTEX group (Rogers et al., 2008). There were no significant increases in SDFT CSAs in the CONDEX group (Moffat et al., 2008). The GERA and JRA studies are difficult to compare. In the JRA study, the distance travelled was 11% less initially, the training was conducted on a treadmill rather than a turf track, sprint velocity was less but for approximately three times the distance, and the pattern of exercise including acceleration and deceleration was more complex. The growth in the CSAs of the SDFT differed at all of the time points between the two studies. Despite these differences during the studies, neither exercise regimen resulted in injury to the SDFT or caused a lasting change in SDFT CSAs. As it is debatable as to whether a small increase in the CSAs of the SDFT would be considered an adaptive change that could reduce susceptibility to injury, further information is required on possible changes in the tendon material. There was no effect of exercise on tenocyte number per unit area or tenocyte nuclear morphology in the GERA study; however, in this histomorphometric study there was no analysis of cellular activity (Stanley et al., 2008).

BONE

The equine skeleton is highly adapted for speed, requiring high resistance to deformation but low mass to minimize energy expenditure. The skeletal elements are a series of rigid, supportive levers on which forces are exerted by muscles via tendons and by ligaments to produce movement and maintain posture. Bones, of course, play additional roles in the protection of internal organs, mineral and energy metabolism, and provision of a framework for the production of hematopoietic tissue.

EQUINE SKELETAL ANATOMY AND FUNCTION

Fractures are a major cause of loss of horses from the racing industry and other athletic activities worldwide. They were estimated to be involved in 60% to 80% of racecourse fatal injury events (Johnson et al., 1994; Riggs 2002). In a 2-year study of British Thoroughbred racehorses, the nontraumatic fracture incidence was 1.15/100 horse months and 78% of these occurred during training (Verheyen and Wood, 2004). There is increasing evidence that a significant number of fractures should be classified as "stress fractures"; that is, they occur following weakening of the bone matrix by accumulation of fatigue damage (microdamage) (Riggs, 2002). At least 57% of fractures documented in the 2-year study of racehorses in training in the United Kingdom were considered to be in this category (Verheyen and Wood, 2004). This indicates that a significant understanding of responses of bone cells to exercise and exercise-induced damage is required, particularly those at well-documented sites of frequent injury. It remains to be determined how different forms of mechanical loading influence both cortical and cancellous bone,

including modulation of remodeling, whether such regimens can increase bone strength and, more specifically, resistance to fracture without compromising high-level athletic function, or whether some (combinations of) exercise regimens actually decrease bone strength.

Macroscopic Anatomy

The ability of a bone to function and resist deformation that is sufficient to cause fracture is determined by geometry, mass, and tissue material properties. The basic forms of most bones are controlled genetically, as demonstrated in studies where fetal bone rudiments transplanted to other sites or grown in vitro acquired similar shapes and sizes to those in adults. However, architectural refinements, including mass, distribution of mass, three-dimensional structure, and microstructure are dynamic and largely a consequence of loading related to the mechanical demands (strain) imposed on the skeleton.

Long bones (femur, humerus, radius, tibia, and the third metacarpal and metatarsal bones) have a hollow cylindrical shaft, termed the *diaphysis*, consisting mostly of compact or cortical bone, on the endosteal surface of which is a zone (of variable width) of cancellous or trabecular bone. The marrow of the intertrabecular spaces is directly contiguous with that in the diaphyseal cavity (Figure 13-10). The diameter of long bones is greatest at the metaphysis, which generally is maximal at the level of the physis or the epiphysis, both of which accommodate cartilage tissue. The outer layer of the metaphysis and epiphysis and cuboidal bones, including carpal and tarsal bones, is a thin shell of cortical bone, beneath which is cancellous bone. In the immature animal the epiphysis is separated from the metaphysis by the metaphyseal growth plate (physis), which, after longitudinal growth ceases, is replaced by cancellous bone (see Figure 13-10).

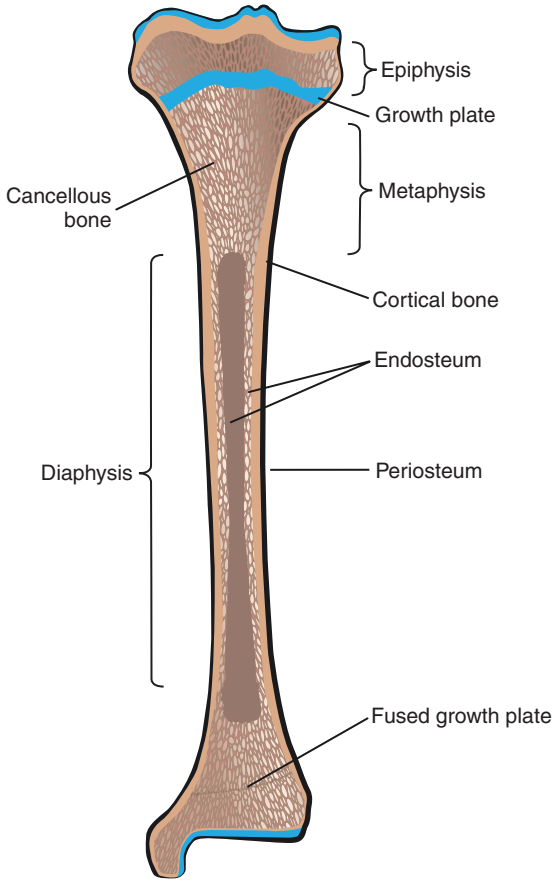

FIGURE 13-10 The sectioned surface of a long bone showing a growing proximal end with a growth plate and a distal end with the epiphysis fused to the metaphysis. *(Adapted from Weiss L, ed.:* Cell and tissue biology, a textbook of histology, *Baltimore, MD, 1988, Urban & Schwarzenberg, Inc., p 219, with permission).*

Most outer surfaces of bones are covered by the periosteum, or a periosteal–perichondrial complex at the level of the physes, which contains both arteries and veins and includes a thin layer of undifferentiated cells (the osteogenic layer) beneath a thick, dense fibrous layer familiar to surgeons. The endosteum is the internal equivalent, being a "membrane" of bone surface cells (osteoblasts, osteoclasts, and bone-lining cells; see below) that lines the diaphyseal marrow cavity, the trabeculae of cancellous bone, and the canals of compact bone. In long bones, the blood supply is via one or more principal nutrient arteries, which branch within the cavity and then arborize as they traverse to the peripheral reaches of the cortex. The main direction of flow is from central arteries to peripheral arteries, although it may be centripetal from periosteal arteries at the site of muscle attachments (Brookes, 1971). Metaphyseal arteries enter the bone just diaphysad to the physis, and their branches anastomose within the bone and with nutrient artery branches, as well as with epiphyseal arteries following closure of the physis. Larger and smaller epiphyseal arteries enter the epiphysis, and their branches supply epiphyseal bone, with arterial loops nourishing the thick unossified epiphyseal cartilage in the young; some branches cross the physis into the metaphysis and apparently act as a temporary reserve vascular supply of the chondro-osseous junction on the M-side, after which the branches traverse up to, but not into, the physeal and articular cartilage (Firth and Poulos, 1982; 1993).

To achieve an appropriate geometry, bones are modeled and remodeled throughout life. Modeling not only allows the development of normal architecture during growth, when it is most prominent, but also allows alteration in the adult. During modeling, resorption and formation of bone tissue take place at different sites, with relatively large changes resulting in changes in three-dimensional shape and position (Figure 13-11). Modeling may result in bone apposition on bone surfaces without prior resorption. For example, the diameter of a bone may increase by periosteal deposition with concurrent endosteal resorption, that is, increased outer cortical and medullary cavity diameters. These diameters are determined at each position and surface by

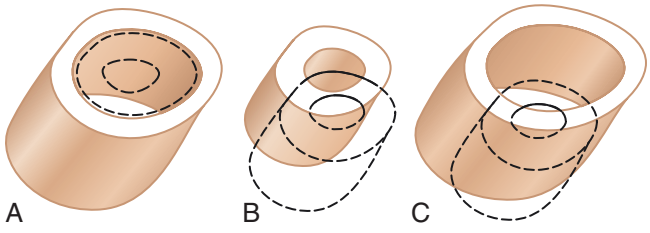

FIGURE 13-11 Diagram showing growth and modeling of the midshaft of a long bone. **A,** The growth process causes uniform enlargement. **B,** The modeling process shifts bone surfaces in space (also known as *drift*). **C,** Growth and modeling acting together enlarge the bone shaft and alter the shape. *(Adapted from Weiss L, editor:* Cell and tissue biology, a textbook of histology, *Baltimore, MD, 1988, Urban & Schwarzenberg, Inc., p 240, with permission).*

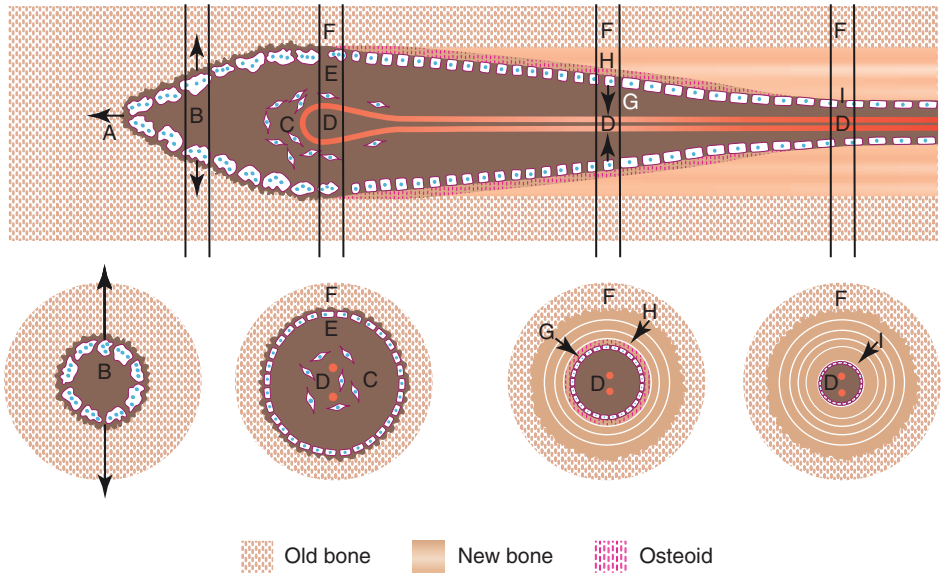

FIGURE 13-12 Diagram showing a longitudinal section through a remodeling unit in cortical bone, with corresponding transverse sections (*below*). **A,** Apex of osteoclastic front moving from right to left. **B,** Multinucleated osteoclasts advancing centrifugally to enlarge a small resorption space. **C,** Perivascular spindle-shaped precursor cells. **D,** Capillary loop. **E,** Mononuclear cells lining reversal zone. **F,** Cement line separating new bone from old. **G,** Osteoblasts advancing centripetally during radial closure. **H,** Osteoid seam separating osteoblasts from recently formed bone. **I,** Flattened cells lining canal of completed Haversian system. *(Adapted from Parfitt AM: The actions of parathyroid hormone on bone: Relation to bone remodeling and turnover, calcium homeostasis, and metabolic bone disease. I.* Metabolism *25:816, 1976, with permission).*

local loads, resulting in complex shapes of bone shafts, which are generally round, elliptical, or triangular. Flat bones also increase in size by coordinated accretion and resorption at the two active surfaces: endosteal and periosteal (Parfitt et al., 2000). Significant geometric information is available for certain equine bones, including the third metacarpal bone (Mc3), a focus of research because of the high incidence of stress fractures of the dorsal surface in Thoroughbred racehorses (Nunamaker et al., 1989; Piotrowski et al., 1983). Remodeling is a dynamic, lifelong process occurring at multiple sites, which involves bone resorption, followed by (i.e., coupled with and usually balanced by) formation at the same site (Figure 13-12). It results in continuous reshaping of bones (1) during growth, (2) in response to changing functional demands, and (3) following injury; this process also plays an important role in calcium homeostasis.

Microanatomy and Matrix Composition

Cortical or cancellous bone may be woven or lamellar. Woven bone is a less organized, provisional material found in the immature skeleton or following injury, in which collagen fibrils are not aligned in any particular direction (Figure 13-13). Lamellae are approximately 3 to 7 μm thick and are visible under polarized light in histologic sections; collagen fibers within each lamella are mutually parallel, but the direction changes from one lamella to the next in the so-called "twisted plywood structure" (Weiner et al., 1997). Within the mineralized matrix of both woven and lamellar bone, cells are located in small ellipsoidal cavities (lacunae) that are connected by thin channels, the canaliculi.

Cortical and Cancellous Bone

Cortical bone (called *compacta*) is essentially solid, with a porosity of approximately 5%. The osteon, often termed the *primary structural unit of cortical bone*, is a cylindrical structure in which a central canal containing blood vessels is surrounded by 20 to 30 concentric lamellae (Jee, 2001). The central canals are classically described as being longitudinally oriented and linked by transverse Volkmann's canals. The central canals link with the periosteum and bone marrow. It is not possible to clearly define the length of an osteon, as at the endosteal surface, a clear-cut interface is not seen between compact and cancellous tissue, with grading of properties and widening of Haversian canals into bone marrow spaces (Boyde, 2003). The equine immature diaphysis comprises primary osteons embedded between more highly mineralized concentric plates

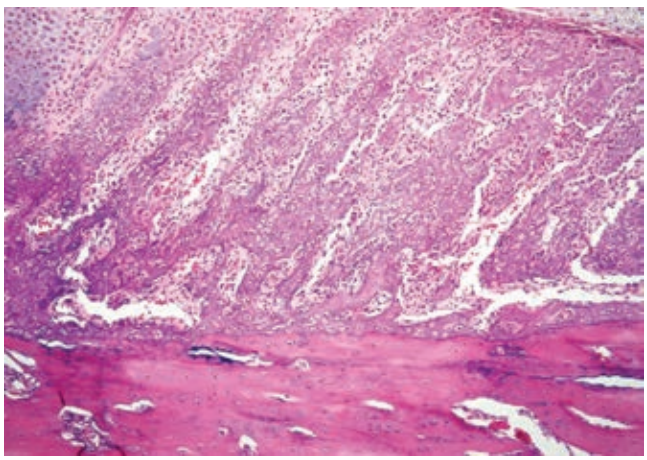

FIGURE 13-13 Photomicrograph of a fracture healing site. More highly cellular and less well-organized woven bone overlies preexisting lamellar bone. Hematoxylin and eosin. Magnification ×100. *(Courtesy of K. G. Thompson.)*

of woven bone. Primary osteons are the first to be laid down in early life, that is, where bone did not exist previously, and are relatively small structures. During postnatal growth, new woven bone forms in the periosteum of long bones and is transformed into new primary osteons (Figure 13-14). Subsequently, in all areas, there is resorption of primary osteons (Haversian reconstruction), with deposition of larger, secondary osteons that measure in the order of 150 to 250 μm in diameter in equine bone and are initially less mineralized (Gibson et al., 2006; Stover et al., 1992b) (see Figure 13-14). Secondary osteons are continually resorbed and renewed throughout life and are limited by a collagen-deficient "cement line," which is often scalloped, as it represents a previously resorbed bone surface. There are irregular pieces of lamellar bone (interstitial lamellae) between the secondary osteons that represent remnants of former osteons removed during remodeling. Appositional bone growth slows in horses at 2 years of age, with new subperiosteal bone at this stage being circumferential and lamellar (Stover et al., 1992b).

In cancellous bone (called *spongiosa*), the matrix and the associated cells are arranged as a three-dimensional mesh of thin interconnecting rods and plates oriented in relation to principal axes of mechanical strain experienced at each location. The lamellae are aligned with the orientation of the trabeculae. The main structural unit is the hemiosteon or trabecular packet, which is crescent-shaped, measures approximately 50μm × 1 mm, and is also delineated by a cement line. The packets are also thought to have some reality in cortical bone, but they are complex in both types and branch at the branching points of the capillary bed (Boyde, 2003). Osteons can also be found in transitional areas of cancellous bone, where trabeculae are >350 μm thick. A higher proportion of younger, less mineralized bone is found in trabeculae, versus cortical bone matrix, because they have a higher turnover rate. The matrix itself in cortical and trabecular bone is very similar, but cancellous bone has a lower bone volume fraction (e.g., 0.47–0.82 for third carpal bone compared with 0.92–0.98 for

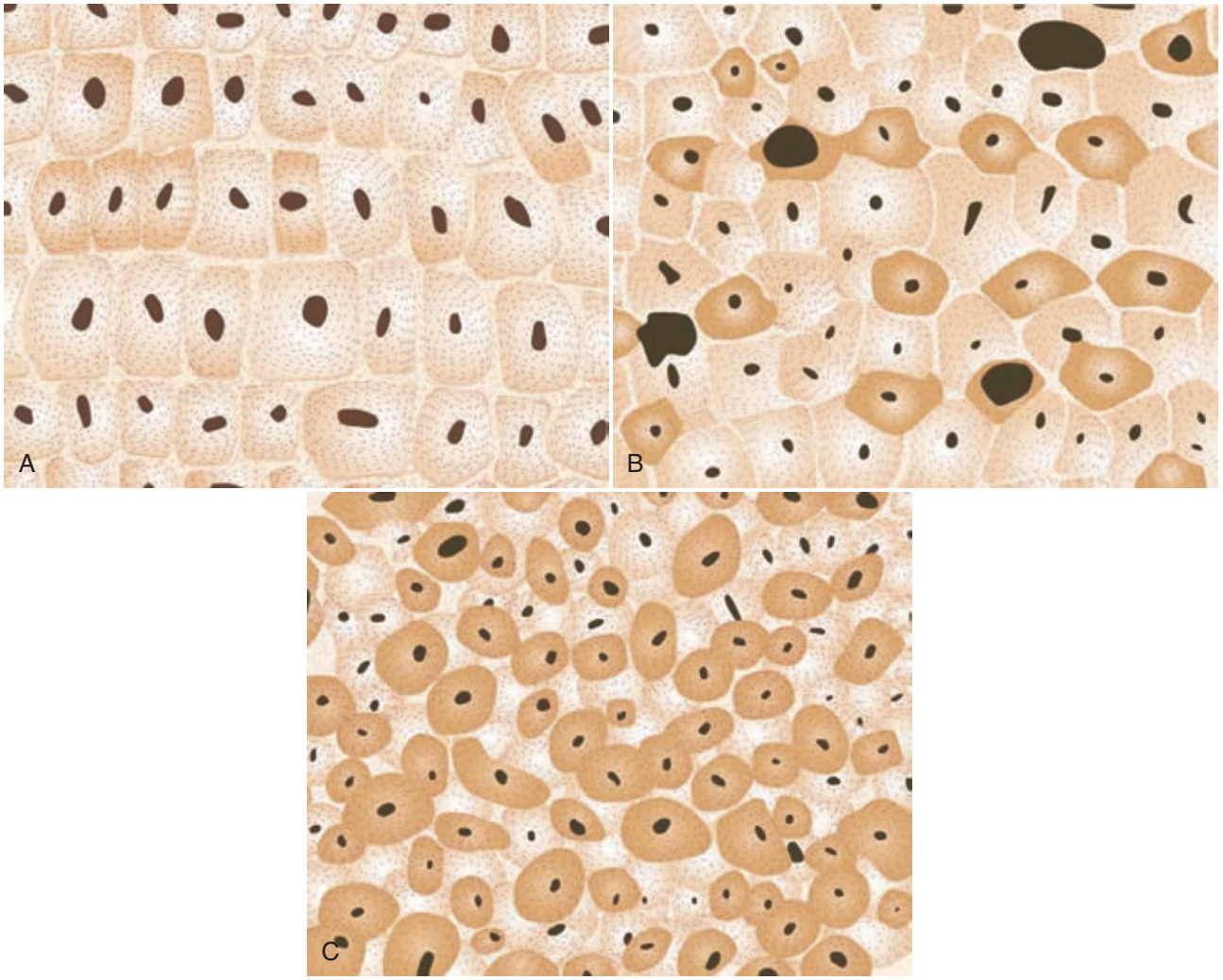

FIGURE 13-14 Diagrams of intracortical regions of the dorsal cortex of the third metacarpal bone during maturation. **A,** 54-day-old foal, showing rows of tightly packed primary osteons (*light brown*) embedded between thin plates of more highly mineralized, thin, concentric plates of woven bone. **B,** 1-year-old horse, showing progression of remodeling with resorption cavities (*black spaces*) and the presence of complete and incomplete secondary osteons (*dark brown*). **C,** 6-year-old horse (i.e., adult), with extensive remodeling by dark brown, secondary osteons. *(Adapted from Stover SM, et al.: Histological features of the dorsal cortex of the third metacarpal bone mid-diaphysis during postnatal growth in thoroughbred horses,* J Anat *181:455, 1992, with permission).*

mid-radial cortex) because of the spaces between trabeculae (Firth, 2004; Riggs et al., 1993b).

Bone Matrix

The inorganic fraction contributes approximately 60% to 70% of the dry mass, 75% of the weight, and 50% of the total volume of bone matrix and comprises an analog of the geologic mineral hydroxyapatite ($Ca_{10}[PO_{4]6}[OH]_2$). Bone apatites are deficient in calcium and hydroxide and contain numerous impurities, in particular, carbonate. Compact bone has the highest mineral content. Apatite crystals are relatively small, particularly in young bone, which facilitates (1) incorporation and adsorption of various ions, (2) dissolution by osteoclasts, and (3) provision of structural rigidity to associated collagen fibrils. The crystals fuse as bone undergoes maturation and undergo changes in composition, including carbonate substitution, which results in increasing density of the bone material in the months after matrix mineralization begins.

The unmineralized bone matrix component, or organic fraction, is termed *osteoid*. This fraction comprises 30% to 40% of the dry mass, and 90% of it is type I collagen. There are traces of collagen types II, V, and X, with most of the remaining 10% of the organic fraction consisting of noncollagenous proteins, including glycoproteins and PGs. The most abundant of such proteins in bone are osteocalcin, osteonectin, osteopontin, and bone sialoprotein. Collagen fibrils provide elasticity and flexibility and play a major role in guiding matrix organization. As in tendon, the collagen molecules making up fibrils have a quarter-staggered arrangement, and the cross-links between collagen molecules play a large role in the mechanical properties. However, in the bone matrix, calcification occurs in the gap regions of this arrangement, with the long axes of apatite crystals and collagen fibrils aligned in parallel. The orientation of these collagen fibrils at a given site in cortical or cancellous bone is related to the pattern of mechanical loading and the load paths along which forces are passing.

Biomechanical Properties

Various sites in a bone are subjected to various combinations of compressive, tensional, and torsional forces, being loaded in four possible ways: (1) axial compression, (2) bending, (3) twisting, and (4) shear. Within certain limits, bones will deform elastically in response to loading, with irreversible damage occurring should the degree of deformation or the number of repetition cycles exceed certain thresholds. Any consideration of the structure and function of bones in horses requires an understanding of how to define their complex mechanical environment and their equally complex responses to it. Unfortunately, there are few directly quantified data on cyclic deformation in horses.

Structural and Material Properties

The properties of bones as structures and the properties of the material of which they are composed are both important and interlinked but may be altered differentially. Adaptations to loading almost always involve modeling responses that change the three-dimensional geometry and, therefore, the properties of the structure, independent of the material of which the bone is composed. Any estimate of the effect of increased or reduced exercise on bone strength, thus, should include geometric information. The mineral mass in the bone and the distribution of the mass around the loading axis adjust to achieve the biologic optimum after imposition of a given mechanical environment. For example, most long bones are largely subjected to bending forces, with some variable compression and torsion. A wider bone with distribution of the cortex further from the neutral axis, as achieved by periosteal bone deposition and endosteal resorption, has greater resistance to bending; that is, the same bone mass (and weight of the limb) lends greater bone strength. This is because bending strength is proportional to the second moment of inertia, which is related to the fourth power of the bone radius (r^4). The length of a bone is also important; longer bones experience higher bending moments and, therefore, higher tensile and compressive loads. Bone mass is very important in athletic animals, as heavier bones increase energy costs during locomotion, especially in the distal limb, at a greater distance from the center of rotation. Due to the effect of radius, a larger bone can have greater strength compared with a smaller bone, even if the cortex is thinned to reduce mass; in fact, there is no correlation between bone mass and external volumes of long bones (Seeman, 2006). A corollary of this is a reduction in safety margins: In many land-dwelling mammals, bones can withstand loads that deform them three to four times the usually experienced maximum (Skerry, 2008). Equine distal limb bones are lighter to facilitate high-speed locomotion, but have been said to have reduced safety margins and an increased risk of fracture (Vaughan and Mason, 1975).

The degree of deformation of a bone in response to loading is a function of load magnitude and of bone stiffness, that is, resistance to deformation. In vitro, load-displacement curves may be generated in tensile or compressive tests to determine ultimate load, work to failure, and ultimate displacement (the latter being related to bone brittleness). Past the yield point, there is slippage at cement lines, with trabecular microfractures and propagation of pre-existing cracks. In long bones, stiffness is favored over flexibility, and in both cortical and cancellous bone, stiffness is strongly correlated to yield strength and ultimate strength (Forwood, 2001). At the mid-shaft of the Mc3, the dorsopalmar bending stiffness is approximately two thirds of the mediolateral stiffness, with torsional stiffness being highest proximally in the bone, reflecting design of this bone to resist the complex loading patterns during locomotion (Piotrowski et al., 1983). For example, the neutral axis of Mc3 rotates and translates through the gait cycle, as measured during trotting, such that the dorsal region is subjected to tensile force but part of the lateral cortex is alternately loaded in tension and compression (Gross et al., 1992).

The properties of bones can be discussed at macroscopic or microscopic levels. Strain can be tensile (positive elongation) or compressive (negative elongation) and is often expressed in microstrain ($\mu\epsilon$), which is unitless, being the ratio of deformation over the original length. Most bones experience very complex strain patterns with tensile, compressive, and torsional components; however, at least 70% of locomotion-induced strains measured in equine limb bones are thought to be tensional and compressive strains produced by bending (Mason et al., 1995). Strains in short bones and extremities of large bones are predominantly compressive. Bone is a linear viscoelastic material below certain thresholds of tensile and compressive strain, calculated to be at approximately 2000 and 4000 $\mu\epsilon$, respectively (Pattin et al., 1996). The viscoelasticity in bone is attributed to relative slip of lamellae within osteons along with the flow of interstitial fluid. Above critical strain thresholds during cyclic loading, accumulation of internal microdamage occurs with increasing nonlinearity of the

stress–strain relationship, increased area of the hysteresis loop, and reducing specimen stiffness (cyclic softening) (Fleck and Eifler, 2003).

There are difficulties in defining material properties of bone specimens in vitro. Bone material is not homogeneous because of the mineralized and fibrous organic components and is also anisotropic; that is, the mechanical properties are dependent on the orientation of the measurement, and the strain occurring will not necessarily be in the same direction as the applied stress. Cortical bone specimens have different values for modulus of elasticity, ultimate tensile stress, and ultimate compressive stress when tested in vitro in longitudinal and transverse directions (Brinckmann et al., 2002). Bone is also viscoelastic, so the deformation velocity (or strain rate), that is, whether it occurs over a short or long time interval, will determine the material property values; cortical bone has a higher modulus of elasticity at higher increased deformation velocities. As for structural stiffness, the elastic modulus (stiffness of the material) is higher if the degree of mineralization is greater: modulus is directly related to volumetric bone mineral density (vBMD) within the physiological range (Ferretti et al., 1996).

Bone Strains in Equine Athletes

Rosette strain gauges can be applied to bone surfaces both in vitro and in vivo, directly measuring deformations and allowing indirect calculation of stresses. This does involve extrapolation and some simplification of the loading environment, as the gauges respond to only the surface to which they are directly attached. Loads on bones of the appendicular skeleton have been found to increase linearly with speed, along with peak strain magnitudes and strain rates; at the extended trot abnormally high levels of strain for that speed were measured (Rubin and Lanyon, 1982). Large compressive strains occurred on the caudal surfaces of equine long bones, which exceeded the large tensile strains on the cranial surfaces and indicated craniocaudal bending with axial compression during the stance phase. Magnitudes of principal strains did not vary through the stance phase at each gait, and their orientations did not vary significantly between gaits, but there were differences in strain magnitude between walk–trot and trot–gallop transitions (Rubin and Lanyon, 1982). Another group did record a change in peak strain direction between a trot and a fast gallop, suggesting that training at low speeds will not necessarily stimulate appropriate bone modeling or remodeling for galloping activity (Nunamaker et al., 1990). The strain levels measured in various major long bones in horses have shown similar patterns at various locations to those in other species, at various gaits; however, the strain levels tend to be significantly higher (Turner et al., 1975). Functional bone strains generally fall between –2000 and –3200 με for the most vigorous activities in many species, with strain rates usually <100,000 με/s (Rubin and Lanyon, 1984a). Extremely high strains, some of the highest recorded in any animal (–4841 με), were measured on the Mc3 of a 2-year-old galloping Thoroughbred (16–17 m/s) (Nunamaker et al., 1990). At a trot, high compression (–1900 με) and shear strains (–1400 με) were reported in a 5-year-old Thoroughbred, which even at a walk had strains of up to –1400 με on the lateral surface of Mc3 of the inside leg during turning (Davies and Merritt, 2004). Combined beam theory and finite-element modeling were used to calculate normal strains in the Mc3 of a trotting horse (3.6 m/s) of –2400 με in compression, 810 με in tension, and a shear strain of 1500 με (Gross et al., 1992). Peak strains at maximal galloping of –3200 με and –2900 με, respectively, have been recorded on the equine tibia and radius respectively (Rubin and Lanyon, 1982).

There have been almost no studies in horses of age-related changes in stress–strain properties of bone tissue. In humans, there is significant loss of bone mass with aging, which varies in different skeletal sites and is associated with imbalance or uncoupling of resorption and formation in the remodeling process; in older women, loss of trabecular connectivity occurs because of an increased imbalance caused by estrogen deficiency (Seeman, 2006). Although –4841 με was measured on the Mc3 of a 2-year-old horse during galloping, a lesser level of –3317 με was measured in a 12-year-old under the same conditions and was related to significantly different mid-shaft geometry in older horses (Nunamaker et al., 1990). Given the current significant populations of older horses (>20 years of age) used for athletic activity, particularly outside the racing industries (McKeever, 2002), the effect of aging on structural and material properties of equine bone requires further research attention.

Bone Mechanics at a Microanatomic Level

There is significant regional variation in microanatomy and material properties in bones, thought by many researchers to be related to differential compressive–tensional strain distributions. Cortical bone of the equine radius has been the focus for researchers attempting to correlate prevalent strain environments with osteonal microstructure, as muscle attachments are not present along most of the diaphysis (simplifying the environment), and in vivo strain data over a range of gaits are available (Biewener et al., 1983; Mason et al., 1995). The equine radius is curved, with the neutral axis of bending passing through the medullary canal in a mediolateral direction in the proximal to mid-diaphysis; as a result of this bending during locomotion, the cranial cortex undergoes predominantly tensional strains ("tension" cortex, up to 900 με) and the caudal cortex predominantly compressive ("compression" cortex, up to –1400 με) (Schneider et al., 1982). These differing strain environments are reflected by longitudinal orientation of collagen and mineral in the cranial cortex and oblique to transverse orientation in the caudal cortex of this region (Mason et al., 1995). In vitro, bone from the caudal cortex was stronger than the cranial cortex when compressive loading was applied but weaker under tensional loading (Reilly and Currey, 1999; Riggs et al., 1993b). Tension cortex bone was stiffer under both tensional and compressive loading (Riggs et al., 1993b). In skeletally immature horses, the primary bone has predominantly longitudinal orientation in both sites, but as horses grow and mature, there is greater remodeling in the caudal cortex, producing greater numbers of secondary osteons (Mason et al., 1995; Riggs et al., 1993a; 1993b). There were similar structural findings in tension and compression regions of cortical tissue of the Mc3 (Skedros et al., 1996). Measurements of strains in the radius and Mc3 indicated that the regional distribution is similar at different gaits, this consistency facilitating economical adaptation of the bone tissue (Biewener et al., 1983).

In cancellous bone, the mechanical properties of trabeculae are determined by the density of the bone material of

which they are composed, the distances between them, their relative orientation and interconnection (Brinckmann et al., 2002), and possibly the presence of microinjury. It is recognized that trabecular plates and beams are strategically placed in relation to the summing of strains, as indicated by strain distributions measured on bone surfaces. For example, trabeculae in the distal condyles of the Mc3 form robust plates with sagittal orientation and less significant lateral–medial connections; this provides maximal strength when the bone rotates in the sagittal plane during locomotion but offers minimal resistance to fracture propagation in that plane (Boyde et al., 1999). If the material properties of cortical and cancellous bone are approximately equal, the properties of the two tissues were long thought to vary because trabecular bone has a lower bone volume fraction; the apparent density, sometimes referred to as *tissue density*, a measurement taking porosity into account, is above 1.5 grams per centimeters cubed (g/cm^3) for cortical bone and 0.1 to 1.0 g/cm^3 for cancellous bone (Brinckmann et al. 2002). However, in horses cortical bone density varies across the cortical thickness of the long bone and can be lower than this figure, and epiphyseal trabecular density can be as high as cortical density (Firth et al., 2005). It is technically difficult to test trabeculae because of their small size, but recent studies have suggested that the mechanical properties of cortical and cancellous bone may actually be very similar despite these microanatomic differences (Huiskes and van Rietbergen, 2005).

CELLULAR BASIS OF BONE MATRIX MAINTENANCE AND RESPONSES TO EXERCISE

Osteoblasts, Osteocytes, and Osteoclasts

The major cell types in bone are well-defined, with increasing research focus on the considerable interplay between them. Osteoblasts are the cells primarily responsible for synthesis and organization of the extracellular matrix, including secretion and mineralization of osteoid, bone resorption, and regulation of calcium and phosphate flux. They are highly responsive to factors that regulate bone turnover, including cytokines, parathyroid hormone (PTH), and sex steroids. Osteoblast precursors are located near bone surfaces and arise from mesenchymal stem cells (MSCs) under the influence of bone morphogenetic proteins (BMPs); the precursor cells proliferate and differentiate into preosteoblasts and then into mature osteoblasts. Preosteoblasts are less cuboidal compared with active osteoblasts, are not located at the bone surface, do not deposit bone matrix, and retain the ability to divide. Osteoblasts are arranged in single layers on bone surfaces, do not divide, and are cuboidal or polygonal when active (Figure 13-15) with expression of high levels of phenotypic bone markers, including bone sialoprotein, osteocalcin, alkaline phosphatase, and type I collagen. A resting osteoblast is a flat, elongated bone-lining cell that may have previously been active. Osteoblasts serve as a barrier between lacunocanalicular and interstitial fluid compartments, regulating the ionic milieu and controlling the growth of bone crystals. Bone-lining cells may also secrete growth factors to initiate proliferation of neighboring osteoblast progenitor cells.

Osteocytes comprise 95% of the bone cell population and cover 94% of all bone surfaces. In normal bone, osteocytes are approximately 10 times more numerous than are osteoblasts, and their lifespan is thought to be significantly greater, with an estimated half life of 25 years in humans (Parfitt, 1990).

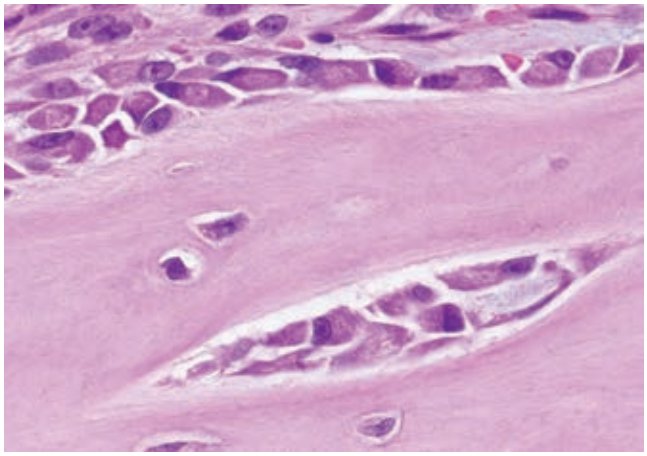

FIGURE 13-15 Active, cuboidal and polygonal osteoblasts on bone surfaces. Hematoxylin and eosin. Magnification ×1000. *(Courtesy of K. G. Thompson.)*

Cells at various stages of differentiation and maturation have been variably defined, with one model proposing eight stages based largely on morphologic characteristics: (1) preosteoblast, (2) preosteoblastic osteoblast, (3) osteoblast, (4) osteoblastic osteocyte (type I preosteocyte), (5) osteoid–osteocyte (type II preosteocyte), (6) type III preosteocyte, (7) young osteocyte, and (8) old osteocyte (Franz-Odendaal et al., 2006). Initial enclosure of the osteoblast by osteoid is not the end of the transformative process, and the exact mechanisms by which this "entrapment" occurs have not been defined. As transition and maturation occurs, organelles are reduced, particularly the endoplasmic reticulum and Golgi apparatus, with downregulation or "switching off" of many previously expressed bone markers when mineralization of the osteoid occurs. The time taken for this transformation varies between species, bones, and with age and gender but is not dependent on bone deposition rate (Franz-Odendaal et al., 2006). The shape of the embedded osteocytes may depend on bone type, but ultimately they are stellate with up to 80 long cytoplasmic "dendritic" processes within canaliculi that link via the GJ with other osteocytes (at a cell-to-cell distance of 20–30 μm), and with bone surface cells including osteoblasts, bone-lining cells, periosteal cells, and vascular cells (Figure 13-16). Cells at all stages of bone formation, from preosteoblast to mature osteocyte, remain connected.

Osteocytes, in collaboration with the surface cells, are thought to function in ionic regulation and to play a central role in mechanotransduction and microdamage repair. It has been suggested that osteocyte number determines bone mass; a relationship between bone volume fraction and osteocyte density has not been demonstrated in the equine Mc3, but a wide range of bone volume fractions has not been examined (Da Costa Gómez et al., 2005). Some osteocytes can deposit and resorb bone in the lacunae ("osteocytic osteolysis"), which may be limited to situations where mobilization of minerals from the skeleton is required. Osteocytes are highly dynamic cells, known to be able to extend and retract their dendritic processes and deform their bodies (Veno et al., 2006). Some osteocytes do eventually undergo apoptosis, the amount of which appears to increase with age, and osteocytic death has been proposed to be a major trigger of the remodeling process (Noble et al., 2003).

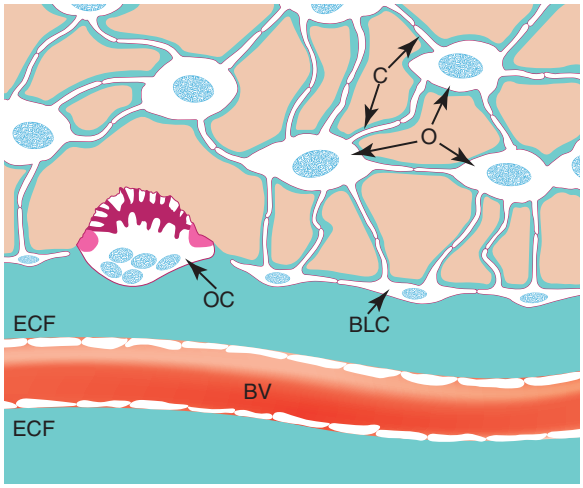

FIGURE 13-16 Diagram of the network of osteocytes (O) in lacunae in the bone matrix (tan), with long cytoplasmic processes extending within canaliculi (C). The osteocytic processes are linked to each other and to bone-lining cells (BLC) and the vasculature by gap junctions (*not shown*), forming a continuous network. An osteoclast (OC) is also shown on the bone surface. ECF, extracellular fluid; BV, blood vessel. *(Adapted from Weiss L, editor:* Cell and tissue biology, a textbook of histology, *Baltimore, MD, 1988, Urban & Schwarzenberg, Inc., p 229, with permission).*

Osteoclasts are multinucleate giant cells derived from mononuclear or phagocytic lineage cells in bone marrow. As both osteoblasts and osteoclasts are derived from precursors in bone marrow, their development is interlinked. A lysosomal enzyme, tartrate-resistant acid phosphatase (TRAP), is used as a phenotypic marker. Osteoclasts may be active or inactive, and it is not clearly understood how they are precisely targeted to sites where resorption is needed. It has been suggested that osteocytes send an inhibitory signal to osteoclasts to downregulate bone resorption and that this may be proportional to numbers of osteocytes linked to lining cells and inversely proportional to the distance of osteocytes from the surface (Marotti et al., 1992; Metz et al., 2003). The microvascular endothelium may also play a critical role, including direction of osteoclastic precursors to specific areas, sensitivity to bone regulators including PTH, and expression of factors that alter osteoblastic activity in response to varying levels of mechanical strain and oxygen tension (Kanczler and Oreffo, 2008).

Modeling and Remodeling at the Cellular Level

Responses to alterations in the mechanical environment can involve modeling, remodeling, or both. Increases in force occur most effectively and predominantly during growth and in response to novel forces imposed during athletic training, with modeling preventing bone strains from reaching levels at which damage will occur. Remodeling is a different and ongoing process occurring throughout life after the initial modeling activity in the growing animal; as in the soft connective tissues, the cellular population must constantly monitor local strain, renew matrix to maintain its quality or competency, and repair microdamage.

Cellular Responses to Mechanical Strain

The often-quoted Frost's mechanostat hypothesis is frequently presented as a modern version of the well-known Wolff's law of mechanically mediated bone adaptation, with remodeling "on" or "off" thresholds at the end of a wide range of physiologic strains and feedback loops to limit responses. In general, strains between 200 and 2000 με are thought to maintain remodeling at a steady state, although there is contention as to the value above which strain is osteogenic; for each surface, the balance between modeling and remodeling may vary, and the strain values are affected by other factors, including reproductive hormones. The magnitude, rate, site, frequency, type, and duration of dynamic strains are all important factors, in addition to interpolation of rest periods. Only a small number of strain cycles are required per day for stimulation of bone production, with a daily duration as short as one minute (Lanyon and Rubin, 1984; Rubin and Lanyon, 1984b).

Osteocytes are increasingly being proposed to be the major bone cell type responsible for sensing and transducing mechanical strain; however, the exact mechanical environment experienced in lacunae is not certain. There is some evidence that perilacunar tissue strains and canalicular deformations are significantly greater than bone surface strain levels (Bonewald and Johnson, 2008). Many theories of mechanotransduction have focused on perturbation of lacunocanalicular fluid leading to shear stress and, therefore, disturbance of the cell membranes of cell bodies and dendritic processes through tethering elements (integrins). In reality, it is likely that bone cells respond to more than one component of their mechanical environment, allowing for significant differences in response in different anatomic locations or types of bone (Ehrlich and Lanyon, 2002). Gap junctions facilitate transmission of signals (e.g., calcium waves) from osteocytes within the bone matrix to bone-lining cells on quiescent surfaces (Yellowley et al., 2000) (see Figure 13-16). More recently, primary cilia of osteocytes have been shown to be involved in mechanotransduction (Malone et al., 2007; Temiyasathit and Jacobs, 2010).

Bone Remodeling Unit

A bone remodeling unit (BRU) or basic multicellular unit (BMU) is defined as a group of bone cells that removes and replaces one bone structural unit (osteon or hemiosteon) (see Figure 13-12). The BMU is theorized to be the unit facilitating the adjustment of the microstructure of bone to maintain maximum strains at a "set point value" (Martin, 2007). This unit has six sequential phases: (1) resting (80%–95% of normal surfaces); (2) activation, including recruitment of osteoclasts and retraction of bone-lining cells; (3) resorption by osteoclasts in Howship's lacunae in cancellous bone, or "cutting cones" in cortical bone; (4) reversal, which refers to a 1- to 2-week interval between completion of resorption and initiation of bone matrix formation; (5) formation and mineralization of bone matrix, with complete mineralization taking approximately 3 to 6 months; and (6) return to resting.

Osteoblasts produce colony-stimulating factor to increase numbers of preosteoclasts from precursors in bone marrow. Osteoclasts are regulated by numerous factors, but the final two effectors are osteoprotegerin (OPG/RANK) and OPG ligand (RANKL). Both are secreted by osteoblastic lineage cells, with RANK-L stimulating osteoclast differentiation and mature osteoclast activity and inhibiting osteoclast apoptosis and RANK having opposite biologic effects (Jee, 2001). Therefore, the osteoclastic pool may be regulated by RANK–RANKL ratios in the microenvironment. For osteoclasts to attach to osteoid, the bone-lining cells must contract and secrete collagenase to

remove the surface layer of unmineralized matrix; these cells identify the site of resorption and also play a role in recruiting osteoclastic precursors from the circulation and enhancing their differentiation. The osteoclast adheres around the periphery of a selected resorption site using an area of their cytoplasm termed the *clear zone*, which is devoid of organelles and isolates the local environment. Inside this, the "ruffled border" of plasma membrane at the resorbing surface lowers the pH using an active proton pump that generates hydrogen ions (pH 2–3), and various proteolytic enzymes, including MMPs, are secreted to resorb matrix and osteocytes. Resorbing osteoclasts release TGF-β, which promotes osteoblastic maturation. Osteoclasts express calcitonin and vitronectin receptors, with resorption being inhibited by calcitonin. Their responses to PTH are mediated by osteoblasts, and 1,25(OH_2) vitamin D is another factor that stimulates differentiation of osteoclastic precursors. The lifespan of osteoclasts is thought to be up to 7 weeks, with apoptosis occurring when resorption is complete.

In cortical bone, "cutting cone" osteoclasts tunnel into the matrix (see Figure 13-12), whereas in cancellous bone, resorption occurs on the trabecular surface, with direct removal of old bone followed by filling in of the cavity with new matrix. Cutting cones can also be seen in trabeculae. In cortical bone, the resorptive cavity measures approximately 200 μm in diameter and can move through bone at a speed of 40 μm per day (Taylor et al., 2007); in young Thoroughbreds, such cavities have been observed to be the latter approximate diameter and advance at a speed of 11 μm per day (Boyde and Firth, 2005). It has been widely accepted that repair and replacement is site-coupled to prior osteoclastic absorption, as described above, but exercise-induced apposition of new bone can occur in the equine Mc3 and Mt3 epiphyseal trabeculae without an intervening resorptive event (Boyde, 2003).

Following the resorption and reversal phases, active osteoblasts synthesize bone matrix on the pre-existing osteoid, with mineralization occurring at the junction of pre-existing and newly formed osteoid, that is, the mineralization front. In adult bone, mineralization lags behind matrix formation by approximately 10 days, so an osteoid seam of unmineralized matrix can be seen. In woven bone, the mineralization lag time is very short, and the seam is either not noted or very thin. Primary mineralization increases mineral density to approximately 70% of maximum in a few days, while secondary mineralization increases it to 90% to 95% over several months. At the end of their bone-forming phase, osteoblasts can (1) transform into (inactive) bone-lining cells, (2) undergo apoptosis, (3) transform into cells that make chondroid matrix, or (4) become embedded in the newly synthesized osteoid in lacunae as osteocytes. The proportion of osteoblasts following each of these fates differs with age, between species, and between types of bone.

FUNCTIONAL ADAPTATION OF BONE

It is well known that bone has a remarkable ability to respond to increased levels, frequency of loading, or both and also to underuse or immobilization. Horses reach 85% to 90% of adult weight and height by 18 months but frequently begin to train at 16 months and may race as early as 22 months (Firth, 2004; Frape, 2004). At age 2 years, the digital tendons are mature (Patterson-Kane et al., 1997b); however, the skeleton is still growing slowly, with closure of growth plates occurring at ages from a few months up to approximately 42 months (Strand et al., 2007). Intuitively, it seems reasonable to aim at increasing bone strength in horses being trained while still completing their growth, without inducing accumulation of significant microdamage. In reality, there is little detailed information on rates of specific orthopedic injuries in racehorses, with even less for horses involved in jumping, dressage, and eventing; accurate workload has been difficult to quantify. Therefore, it would be very difficult to determine if there was a positive effect of any interventional approach to reduce rates of certain orthopedic injuries on the basis of the following studies on the effects of specific training regimens on young horses (Firth, 2004).

Evidence of Exercise-Induced Microdamage

Exercise will not necessarily result in positive adaptation. There is significant evidence that many fractures in equine athletes are preceded by accumulation of microdamage, which those responsible for developing training programs should attempt to minimize. These stress fractures are not associated with a single event, occur with repetitive loading, are often preceded by clinical signs (albeit sometimes subtle, temporary, or both), may present with a radiographically evident fracture line, and display fracture planes reminiscent of fatigue failure in engineering materials (Nunamaker et al., 1990). Stress fractures have been documented in the axial and appendicular skeleton of racehorses, with evidence of surrounding chronic pathologic change and identification of incomplete fractures in some horses at the known predilection sites (Riggs, 2002; Haussler and Stover, 1998; Stover et al., 1992a). Dorsal metacarpal disease (i.e., decreased bone stiffness in the dorsal Mc3 cortex associated with new periosteal bone formation) has been reported with an incidence of 24% to 70% in Thoroughbred racehorses in the United Kingdom and the United States, with approximately 12% going on to develop stress fractures at that site in subsequent racing (Boston and Nunamaker, 2000; Norwood, 1978; Nunamaker, 1996; Verheyen et al., 2005). Many life-threatening fractures in racehorses originate at articular surfaces and are considered stress fractures, including parasagittal fractures of the distal condyles of Mc3 and Mt3 (Parkin et al., 2006; Riggs, 2002). It has been hypothesized that exercise-induced sclerosis of the Mc/Mt condyles, but not the sagittal ridge, results in a stiffness gradient concentrating stress at the condylar–sagittal ridge junction and resulting in microdamage that weakens that site and predisposes it to fracture (Riggs et al., 1999a,b).

Microcracking of Cortical and Cancellous Bone

It is now generally agreed that fatigue of cortical bone can result in formation of small cracks in the matrix, or "microcracks," with a proposed equilibrium state existing between their formation and triggered repair by BMUs; the latter state is not supported by all researchers. The process of microcracking is dependent on strain magnitude, rate, and cycle number, at least in vitro (Burr et al., 1997). In vitro, in nonequine bone, there is a nonlinear relationship between the mechanical properties of bone and microdamage accumulation, with a significant reduction (degradation) in the elastic modulus only above certain levels of microstrain, in tension or compression (Pattin et al., 1996). The density of microcracks in bone with <15% degradation of elastic modulus is not significantly higher than in bone with no such degradation. However at >15%, microdamage accumulation appears suddenly; that is, the nature of

microdamage accumulation is threshold related (Burr et al., 1998). At that stage, very small amounts of damage have a surprisingly large effect on the mechanical properties of bone tissue (Burr et al., 1998). This behavior in response to fatigue loading is similar to nonbiologic composite materials.

Microcracks occur mostly in the older interstitial matrix between intact osteons and have a longer axis in the longitudinal axis than in the transverse axis of bone. There may be areas of "diffuse damage" containing many smaller cracks (approximately 1 μm in length) and cross-hatched patterns of intermediate-sized cracks (Taylor et al., 2007). There are technical issues in terms of identification of microcracks in bone specimens in vitro, as cracks can develop artifactually, even when tissue blocks are analyzed rather than sections, and there can be overanalysis of normal extracellular matrix spaces as "ultra-microcracks" (Boyde, 2003). Some researchers have interpreted diffuse areas of dye penetration into the matrix with or without association to visible microcracks as predisposing lesions, with correlation to elastic modulus degradation (Schaffler et al., 1996). In vitro, compressive cortices have fewer cracks compared with tensile cortices; cracks are less easily initiated in compressive sites, but their arrest by osteons is less efficient, and they are, therefore, longer (Carter and Hayes, 1977; Burr et al., 1998). Several models suggest that uniaxial tension on cracks in the tensile cortices tends to close the crack, reducing stresses at the crack tip and making it easier for a cement line to stop propagation.

It has been suggested that microcracking, when modest and limited in terms of numbers and lengths, is an adaptive response to exercise that increases resistance to catastrophic fracture. Microcrack formation may absorb strain energy and redistribute stress that would otherwise result in propagation of larger, catastrophic cracks (Firth, 2004; Reilly et al., 1997). Initially, the size of microcracks does not increase with repeated loading but there may be more of them. Results from in vitro studies using nonequine bone have indicated that longer fatigue life may be associated with higher, rather than lower, microcrack density, with the tissue eventually failing by propagation of just a few larger cracks (Sobelman et al., 2004). Mechanisms of microcrack propagation and toughening are complex and most likely differ between cortical bone and cancellous bone. In cancellous bone, microcracks are surface oriented rather than embedded in the matrix, and the fracture of a single trabeculum is unlikely to have as significant an effect as a propagating crack in cortical bone (Hazenberg et al., 2007). The control of the length of cortical microcracks appears to be essential in preventing the development of more serious damage and is controlled by (1) their formation in interstitial bone where cement lines of adjacent osteons limit propagation and (2) at least in some circumstances, partial removal by reparative cellular activity. In the bones of humans and some animals, there is an age-related increase in susceptibility to microcracking, but this has not been investigated in horses.

Microcracking and Targeted Remodeling

Some researchers believe targeted remodeling of bone stimulated by microcracking or other microdamage to be an important component of normal bone adaptation in addition to ongoing repair, and even propose that all cortical bone remodeling may be initiated by it (Martin, 2002; Mori and Burr, 1993). Because remodeling first involves removal of matrix, it reduces bone mass (increases porosity) and increases strain on the remaining matrix, predisposing to further microdamage if excessive loading continues. According to one theory, if the rate of increase in microdamage exceeds the rate of repair, then failure will occur unless adaptation is initiated to reduce the stress level.

There has been considerable focus on osteocytes as initiators of repair (Hazenberg et al., 2007). Osteocytes do become apoptotic near microcracks and areas of diffuse damage, and this might attract osteoclastic activity (Taylor et al., 2007). It has been suggested that in circumstances where shear forces are great enough across crack faces (in one model when >100 μm long), there could be disruption of osteocyte dendritic processes resulting in upregulation of RANK-L and secretion of macrophage colony-stimulating factor (M-CSF) (Hazenberg et al., 2006; Kurata et al., 2006). It is possible that BMUs serve only to control the sizes of microcracks, rather than to remove them completely; the lengths, and not numbers, of microcracks reduce in proportion to densities of resorption spaces in the human femoral bone (Martin, 2007). Perturbation of this remodeling process in some individuals, for example, acceleration with associated matrix weakening or reduction with failure to repair, could predispose to injury. In one study of military recruits, plasma hydroxyproline (HP) levels (a nonspecific marker of bone resorption) were higher in the first week of training in individuals who subsequently sustained stress fractures (Murguia et al., 1988). Markers of bone turnover did not, however, prove useful in predicting the likelihood of stress fracture in human track and field athletes (Bennell et al., 1998), but in a U.K. study, osteocalcin and ICTP levels in the early stage of training of 2-year-old Thoroughbred racehorses (n = 165) were higher in those that subsequently developed dorsal metacarpal disease (Jackson et al., 2005). Increased remodeling is known to occur in a number of clinical conditions in horses, including degenerative joint disease and navicular syndrome, in addition to stress fractures.

Despite the significant focus in the literature on the concept that bone repair and replacement is site-coupled to prior osteoclastic resorption of microcracked matrix, there is evidence that repair can occur directly. Bone cracks purposefully introduced during grafting procedures have been shown to be repaired by direct deposition of bone on the surfaces (Boyde, 2003). Cracks apparently healed by highly mineralized matrix have been identified in compact lamellar bone, particularly in interstitial regions of old osteonal systems, that is, at least some cracks may be repaired by infilling.

Does Microcracking Occur in Equine Bone?

Surprisingly few studies of equine bone have addressed the questions of whether (1) microcracking is an important fatigue event or (2) if microcracking is linked to remodeling preceded by resorption. Many previous studies of microcracking have involved analysis of older human bone, from which results cannot be directly extrapolated to horses. It is possible that in this highly specialized athletic species, the mechanisms of adaptation and microdamage repair in bone are not identical, with microcracking playing a lesser role. Equine distal limb bones experience long periods of continuous loading (in addition to high-speed locomotion) as horses spend only brief periods lying down. In fact, equine bone seems to have superior resistance to fatigue compared with that from humans and other species, and this may relate to differences in the microstructure, with residual strength not being

reduced in bone specimens subjected in vitro to loading equivalent to a "lifetime in racing" (Martin et al., 1997; Riggs, 2002).

Cyclical loading of milled equine tibial specimens in vitro at physiologic levels demonstrated expected loss of stiffness with increasing cycle number, and formation of microcracks centered on stress concentrators such as Haversian canals and macroscopic cracking shortly before final failure (Fleck and Eifler, 2003). "Unstained" cracks (<25 μm in length) associated with regions of woven bone in Mc3 specimens that underwent fatigue failure in vitro, appeared to be damaged Sharpey's fibers (perforating collagenous material connecting the periosteum to bone); their length was found to have increased after failure, but their density did not alter. These small cracks have not been identified in bone from nonequine species and were suggested to be a unique equine fatigue resistance mechanism (Martin et al., 1996). Specimens of Mc3 from exercised horses in the 18-week Bristol study had increased impact strength that was associated with the amount of microcracking produced (Reilly et al., 1997). A similar exercise-related effect was not noted in specimens from the radius of the same horses (Batson et al., 2000). In in vitro studies of bone specimens from the equine radius, the development of microcracks seemed to be strain driven, again with formation of dense microcrack clusters around small structures, including lacunae, remodeling cavities, and blood vessels (Reilly and Currey, 1999). It was suggested that in curved bones such as the equine radius that have compression and tension cortices, the matrix adapts to resist compressive and tensile microcracks, respectively (Reilly and Currey, 1999). Osteons in cortical bone may debond because of shear failure at or near the cement line and pull out from the interstitial matrix to control crack propagation; this has been supported by several in vitro studies of Mc3 specimens fractured in four-point bending with osteonal pullout at higher strain rates, and occurred at even higher strain rate values in compression cortex specimens with longer fatigue life (Hiller et al., 2003). These demonstrations of in vitro microcracking do not of course indicate the mechanisms at play in the live horse.

In one study, evidence of intense remodeling of the Mc3 dorsal cortex in Thoroughbred racehorses, associated with subtle matrix injury, was not strongly correlated with microcrack density or loss of osteocytes (Da Costa Gómez et al., 2005). Despite the very high strains experienced in this region of bone in the racehorse, only small numbers of linear microcracks were identified (Da Costa Gómez et al., 2003; 2005). In a study of specimens from the mid-shaft of Mc3 using thick slices, the basic fuchsin staining protocol, and motion parallax to display three-dimensional information, no microcracks were identified (Boyde, 2003). It has been hypothesized that the Mc3 actually increases resistance to fatigue through formation of secondary osteons, which are weaker than young primary osteonal bone but stronger than older bone that has accumulated microdamage. Secondary osteon area fraction and density in the Mc3 has been correlated with the logarithm of fatigue life, and with compliance (i.e., reduced elastic modulus) (Gibson et al., 2006). Secondary osteonal cement lines also more effectively serve as barriers to microcrack propagation. The importance of microcracking might vary with site; distal sesamoid (navicular) bones from horses with navicular syndrome contained significant evidence of remodeling, including intracortical cysts and multiple "tidemarks" in association with large numbers of microcracks, a low bone-volume fraction, and low density and connectivity of osteocytes, but this was not associated with larger numbers of resorption spaces (Bentley et al., 2007). One researcher used backscattered electron and scanning electron microscopy imaging to find microcracks that had developed in vivo in articular calcified cartilage and subchondral bone of the proximal phalangeal bones, Mc3, Mt3, and carpal and tarsal bones in young and old horses (up to 24 years), with evidence of repair by direct infilling with densely calcified matrix, as described above (Boyde, 2003). In one 18-month-old horse from one of the Bristol studies, there was evidence of repair of a trabecular fracture by encircling, local lamellar bone deposition in the absence of intervening osteoclastic resorption, with an outer woven bone microcallus (Boyde, 2003). This has also been observed in human cancellous bone and is termed a "bandaged microfracture."

Effects of Training on Equine Bone

It is not known how rapidly bone in horses can be adaptively modeled or remodeled in response to exercise of various types, durations, and frequencies. Detailed studies of individual bones and their responses to particular loading regimens have been undertaken in small laboratory animals but not in horses. Results from laboratory animal trials will not necessarily be directly applicable to equine athletes, as it is entirely possible that the training and competition environments of horses result in unusual bone growth and responses relative to other species. If an exercise regimen is osteogenic, bone strength might be increased in a number of ways, including altered bone geometry due to altered endosteal and subperiosteal surface apposition rates (Figure 13-17, *A*); increased mineralized mass; increased BMD of material or tissue; altered trabecular bone density and orientation (Figure 13-17 *B*); altered remodeling and increase in maturation of secondary osteons; altered collagen cross-link concentrations; and a change in the predominant direction of the collagen fibers (Firth, 2004).

Controlled Treadmill Exercise Studies

As a general rule, the most substantial exercise-induced increases in bone density measured have been in major load paths of epiphyses and cuboidal bone. In the 18-month and 18-week Bristol studies, the dorsal aspects of the radial and third carpal bones of horses subjected to high-speed treadmill galloping exercise (see Table 13-1) showed similar changes, with thicker cortices, thicker trabeculae, and resultant increased bone mass and vBMD (Firth et al., 1999a; 1999b). The density increase was almost 30% in some regions of the third carpal bone (see Figure 13-17, *C*). In horses exercised for 18 weeks, the vBMD increased in the distal aspect of Mc3, also due to an increase in bone volume fraction (Riggs and Boyde, 1999). In these trained horses, the main trabeculae in the epiphysis were predominantly sagittally oriented, thus imparting maximal strength in that plane, with significant mediolateral connections (Boyde et al., 1999). Mechanically tested mid-diaphyseal Mc3 specimens had a slightly higher toughness and impact strength associated positively with an enhanced ability to microcrack that was considered to increase resistance to fracture (Reilly et al., 1997). The impact strength was higher in the outer cortex, where bone had been more recently laid down, than in the inner cortex for exercised and control horses; however, this regional difference was more significant in the exercised group.

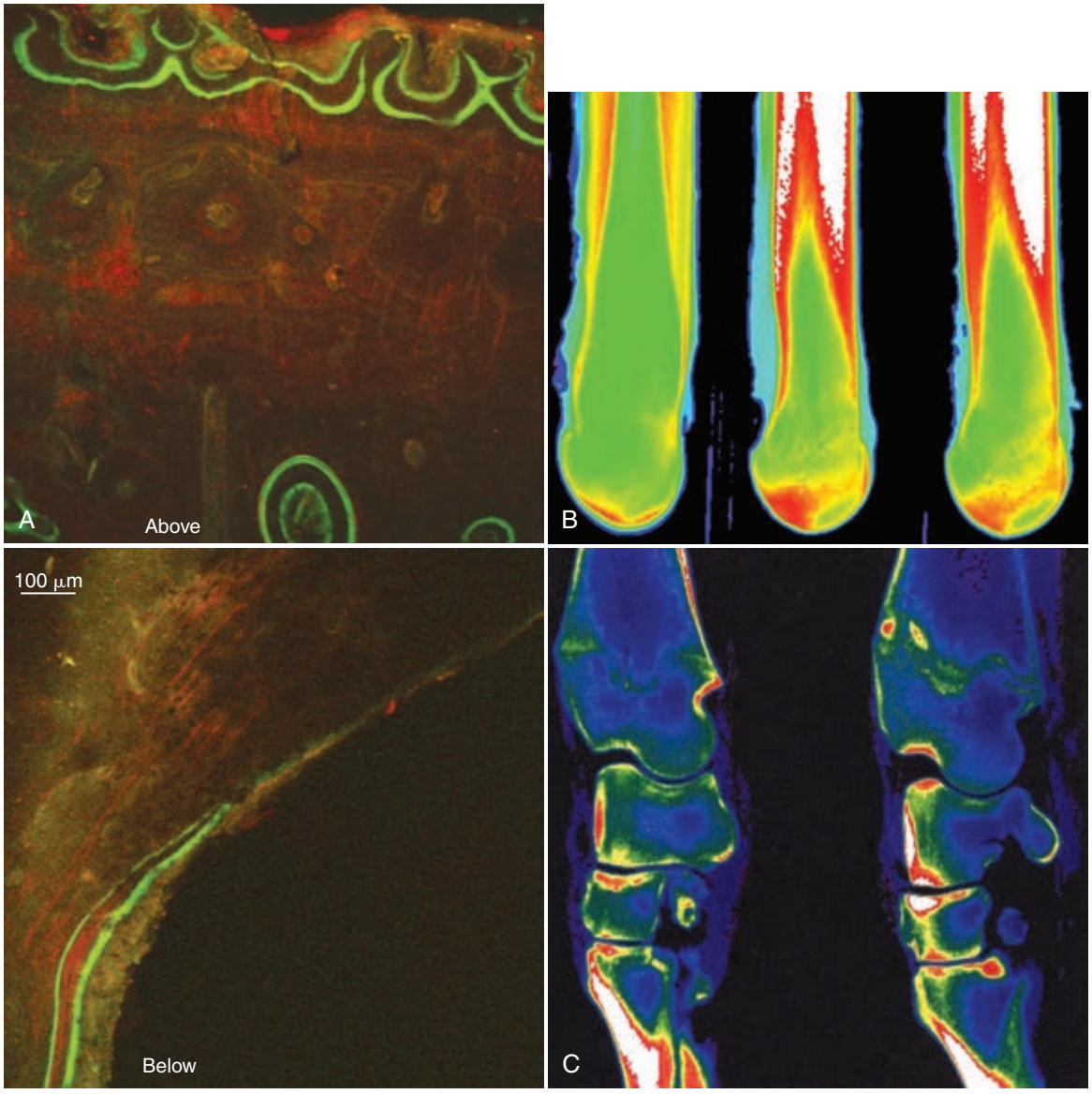

FIGURE 13-17 **A,** Bone labeling fluorogens (*green*) were intravenously injected in a 2-year-old horse 9 weeks after training began, and again 21 days later. The label accumulated at the site of new bone forming at the periosteal surface of the third metacarpal bone and in osteons in the cortex (*above*) and endosteally (*below*). The distance between the labels indicates the rate of bone formation during the 21-day interval between the label administration, and was 1-2 μm/day. The presence of label was highly regional. **B,** Sagittal computed tomographic (CT) images taken 2 mm abaxial to the parasagittal groove of the left third metatarsal bone (*left*), and right (*center*) and left third metacarpal bones of a 2-year-old Thoroughbred trained for 13 weeks. Plantar or palmar aspect is left, distal is below. Obvious are the thin cortical shell of the metaphysis, and the adaptive regional epiphyseal osteosclerosis, which extends from the palmarodistal to the dorsoproximal part of the epiphysis and has a volumetric mineral density (*red*) similar to cortical bone, some of which is more dense (*white*). The changes are more marked in the metacarpal epiphysis than in the metatarsal epiphysis. **C,** Sagittal CT slice of the carpus of trained (*right*) and untrained Thoroughbred horses. The effect on the limb of the increased forces associated with training is highly regional, and a clear load path can be identified in the dorsal part of the third carpal bone, radial carpal bone and into the radial epiphysis, physis, and dorsal diaphysis. The regional change in density is most obvious in the proximal aspect of the third carpal bone and distal and dorsal aspects of the radial carpal bone, where the white density values are similar to that in the cortex of the proximal part of the third metacarpal bone.

In 2-year-olds in a further study conducted by the Equine Orthopedic Research Laboratory at Colorado State University (see Table 13-1), horses that underwent high-speed treadmill exercise for 6 months showed increased radionuclide uptake and subchondral bone density (as measured by computed tomography [CT]) in their Mc3 condyles (Kawcak et al. 2000). Horses aged 16 months and trained for 14 weeks on a treadmill had a higher bone mineral content in the Mc3 diaphysis at 12 weeks, without an increase in CSA of the bone, although the ultrasound path may not have detected any dorsal cortical enlargement (McCarthy and Jeffcott, 1992; Sherman et al., 1995).

Controlled Racetrack Exercise Studies

While treadmill exercise studies have value because of the strict control of the exercise regimen, it is also important to acquire data from similar environments to those experienced by racehorses in practice, since the loads sustained in the two situations may be different. Findings in horses trained on racetracks have been similar, with comparable rapidity of bone responses. In the MUGES study, 2-year-olds galloped on grass and sand tracks showed slight but significant increases in vBMD in the diaphysis and much more obvious increases in the epiphysis of Mc3 and Mt3 bones, with greater bone size and bone apposition rates than in control animals (Firth et al., 2005). The mean tissue density of the Mc3 epiphysis of the exercised group was increased by 36.8%. In the diaphyses of Mc3 and Mt3, increased bone CSA made a greater contribution to increased bone strength than more modest increases in bone density (1.6%) (Firth and Rogers, 2005a; Firth et al., 2005). In this cortical bone, there were reductions in porosity (remodeling was inhibited) at lower (canter) speeds, followed by increased size and strength of the shaft by periosteal bone deposition at higher (gallop) speeds. Regional bone mass and density increases in epiphyses and in the third carpal bone (C3) were at least partially caused by the thickening of trabecular plates, with formation of columns oriented normal to the articular surface at nodes where the trabeculae joined. Deposition of bone between nodes with filling of intertrabecular spaces must have resulted in increased stiffness. CT scanning indicated that the increase in Mc3/Mt3 density began in the palmarodistal or the plantarodistal aspect, where forces through contact with the proximal sesamoid bones are apparently maximal (see Figure 13-17, *B*). The Mc3 and Mt3 epiphyses differed, with higher vBMD on the lateral side in the Mt3, where arthritis is known to be a frequent occurrence (Boyde and Firth, 2005). Similar observations of Mc3 diaphyseal enlargement and increased bone mineral content have been made in other treadmill-exercise studies and retrospective analysis of material from racehorses (McCarthy and Jeffcott, 1992; Sherman et al., 1995).

In the MUGES study, vBMD also increased with exercise in epiphyseal regions of the dorsodistal radius, and dorsal aspects of the radial and third carpal bones in addition to the palmarodistal subchondral bone of Mc3 (Firth et al., 2000). Trabecular vBMD was 26% greater in the dorsal aspect of the third carpal bone of trained versus untrained horses (Firth and Rogers, 2005b), which was a lesser response than that noted in the same site of treadmill-trained horses in the Bristol studies, presumably due to differences between the track and treadmill surfaces and the exercise regimen (Firth and Rogers 2005a; Firth et al., 1999a; 1999b). However, in the 18-week Bristol study, there was no evidence of exercise-induced subchondral remodeling in the third and central tarsal bones, as measured by magnetic resonance imaging (MRI) (Whitton et al., 1999). In a retrospective study of racehorses 2 to 3 years of age that had been in racing, training, or both, the bone area fraction (1-p where p = porosity) and the stiffness as measured by indentation were greater in the dorsal aspect of the radial facet of the third carpal bone compared with values from untrained horses (Young et al. 1991b).

The bone deposition in Mc3 condyles and carpal bones in trained 2-year-olds in the MUGES study was shown to occur by (1) deposition of lamellar bone on resting surfaces of trabeculae without prior osteoclastic resorption and (2) formation of strands and sheets of woven bone within marrow spaces, including incorporation of a newly developed vascular supply (Boyde 2003; Boyde and Firth, 2005). In the now-massive trabecular plates, some secondary osteons developed. This is not in agreement with the theory that resorption, as stimulated by osteocytic death, must precede bone formation and is proposed to allow rapid responses. Additionally, there was reduced osteoclastic activity at external growth modeling sites in comparison with controls. The increased deposits of bone increased the vBMD of the tissue as a whole, but the mineral density of the newly deposited matrix material was less than that of the pre-existing bone. Poorly mineralized lines separated pre-existing and new bone matrix. As a result, the material stiffness was most likely lower, but the overall structure stiffer due to filling of "non-bone" spaces. Similar deposition of less mineralized bone matrix was also identified in the distal Mc3 of trained horses from the 18-week Bristol study, that was more extensive in highly loaded distopalmar regions of medial and lateral condyles (Riggs and Boyde, 1999). It was suggested that radiography and CT imaging techniques that rely on normal mineralization will underestimate the deposition of this less mineralized new bone.

Track or treadmill surface characteristics are an important consideration when determining and analyzing effects of exercise on equine bone. This was demonstrated in a study of 2-year-old Thoroughbreds subjected to 5-month training regimens, with greater thickening of trabeculae (and lower porosity) in the proximal sesamoid bones of horses trained on less compliant tracks (Young et al., 1991a). Additionally, although only small numbers of controlled studies have been conducted, there is, unsurprisingly, evidence that the speed of the gait at which horses exercise is important. For example, the fracture-prone dorsal cortex of Mc3 has been shown to undergo significant remodeling in response to fast exercise of Thoroughbreds with complete replacement of primary cortical bone by secondary osteons (da Costa Gómez et al., 2005). There is significantly less modeling and remodeling of this area in Standardbreds, with a lesser minimum moment of inertia at the mid-diaphysis at maturity in this breed (Nunamaker et al., 1989). Compared with Thoroughbreds, Standardbreds train longer distances at slower gaits and have two feet (rather than one foot) on the ground at any support phase of the trot or pace; that is, the Mc3 dorsal cortex would be expected to experience lower strains. Horses trained at lower velocities than those at which they will eventually compete may, therefore, not experience sufficiently high strain magnitudes and rates for appropriate skeletal adaptation, in keeping with the mechanostat hypothesis (Frost et al., 2002). Although only small numbers of horses were analyzed in the MUGES study, individuals that only cantered during training had similar bone

density to those that had galloped, but their values for bone CSA, mineral content, periosteal circumference, and bone strength were similar to those of the controls; cantering apparently increased bone density by reducing porosity and galloping increased bone size (Firth et al., 2005). This coincided with the suggestion that a velocity of 12 m/s is required to induce an increase in Mc3 size and in the resistance of the dorsal cortex to fracture (Boston and Nunamaker, 2000; Davies et al., 1999). However, with training of greater intensity, there is the risk of pathologic change in the form of microdamage or frank lesions supervening.

Should Foals Be Trained from Soon after Birth?

During the early growth period, equine bone may be more responsive to exercise than in the latter stages of maturation when horses normally begin to train and race. In humans, early exercise, particularly around puberty, has been shown to enhance the mechanical competence of bone and reduce the later risk of stress fracture (Fredericson et al., 2005). Horses are genetically programmed for very rapid and early skeletal development with weightbearing occurring almost immediately after birth, and thus, the responses to exercise of bone tissue in young foals may actually be more comparable with the human adolescent. The only information relating exercise levels to bone structure in young foals unfortunately relates to the Dutch EXOC study, where there was no group of foals receiving high-intensity exercise in addition to free pasture exercise (see Table 13-2). In the JRA study, treadmill-trained foals also did not have free pasture exercise for the entire period, but no data on bone tissue from that study have been published. At 5 months of age, foals in the pasture group of the Dutch EXOC study had a significantly greater Mc3 cross-sectional area than those in the boxrest group (van Weeren and Barneveld, 1999). The BMD and levels of collagen cross-linking were reduced in the boxrest foals only, implying that short daily bursts of galloping exercise experienced by the boxtraining group were sufficient to maintain these parameters. In the medial aspect of the third carpal bone, the vBMD as assessed by dual x-ray absorptiometry was significantly higher in pasture and boxtrained groups than the boxrest group (Firth et al. 1999c). At 5 months of age, ALP levels were lower and TRAP levels higher in the subchondral femoropatellar specimens from boxtrained and boxrest foals, that is, bone resorption was increased by the relative immobilization (van de Lest et al., 2003); this difference was not present by 11 months. All the intergroup differences were no longer present at 11 months, following 6 months of free access of all groups to pasture. In the apical region of the proximal sesamoid bones (PSB), the trabecular vBMD was higher in boxtrained foals than in boxrest foals; however, by 11 months it was lower (Cornelissen et al., 1999). The trabecular bone of the medial aspect of the third carpal bone showed similar differences between groups at 5 months but not at 11 months. It was concluded that (1) immobilization by keeping foals in box stalls retarded normal bone development, with compensation once the foals were placed on pasture; (2) cancellous and cortical bones showed different responses; and (3) imposing galloping exercise on foals largely kept in box stalls led to overstimulation, with the result of less active long-term mineralization in some, but not all, skeletal sites. The lower vBMD in boxtrained foals at 11 months versus 5 months is not consistent with previous measurements from the Mc3 of pastured foals (Grace et al., 2003), which could relate to the unusual regimens or to the higher cortical density threshold used in analysis in the Dutch EXOC study that may have excluded newly formed (less mineralized) cortical bone. In another study, weanlings (5 months of age) given daily sprinting exercise for 5 days per week over an 8-week period appeared to have greater Mc3 cortical size and mineral content compared with foals confined in stalls or dry-lots (992 m^2) (Hiney et al., 2004). Pasture rearing or 12 hours daily turnout into pasture prevented loss of mineral in the Mc3 of Arabian weanlings as assessed radiographically (radiographic bone aluminum equivalence) (Bell et al., 2001).

In the GERA study, the vBMD increased most rapidly in the first few months, and a reciprocal relationship between vBMD and cortical bone area developed at older ages. Bone size and mineral content, not vBMD, played the main role in increasing resistance to deformation of the bone shaft, and the imposed exercise resulted in significant differences in most bone parameters of the proximal phalangeal bone but not the third metacarpal bone. In the epiphysis and cuboidal bones, the exercise imposed from a few weeks of age to 18 months did not result in significant differences in any of the measured bone parameters, indicating that epiphyseal and diaphyseal bones respond differently (Firth et al., 2011). In upper limb bones (radius and tibia), the circumference, bone area, and index of bone strength were significantly greater in the exercised group, and the diaphyseal strengthening was due more to increase in size than in mineral density or mineral content (Nicholson and Firth, 2010). A subgroup was trained as 2- and 3-year-olds with several months at pasture between the two training periods (Rogers et al., 2008). The bone shafts of horses previously exercised as foals were bigger and stronger at 18 months of age compared with those of foals not so exercised, and the intergroup differences were still present at the end of the study. Response of the diaphyseal bone parameters was most closely associated with cumulative workload at the gallop (Firth et al., 2012).

The question of whether to impose exercise on foals or not remains unsolved, since only one longitudinal study has been conducted (GERA) and its benefits were only moderate in terms of athletic performance. Larger scale epidemiologic studies would be required for determination of effect, but it is difficult to convince horse owners to accept counterintuitive evidence that early exercise appears harmless and may even be highly beneficial. The positive message is that exercising foals has, to date, produced no harmful effects (Doube et al., 2007b; Kawcak et al., 2010; Kim et al., 2009; Moffat et al., 2008; Nugent et al., 2004; Rogers et al., 2008; Stanley et al., 2008; van Weeren and Firth, 2008), but the concept obviously requires further study.

Can Training Increase Resistance to Fractures?

One of the ultimate aims of training horses is to increase resistance to musculoskeletal injury, but there are currently limited preventative strategies for stress-induced fractures. Prospective studies in humans have indicated lower risks of fractures in more active individuals but this does not specifically indicate that bone strength is responsible. Training also increases muscular strength and neural competence and has been shown to have positive kinematic effects in terms of the ability of horses to work at higher velocities (Rogers et al., 2005). There is very significant variation between individual

horses in amounts of bone mineral deposited in response to training, and in bone remodelling rates (Firth and Rogers, 2005a). Very small changes in bone structure can significantly increase resistance to fatigue, but they are not clearly defined for various injury-prone skeletal sites. Not all changes in response to training will necessarily be adaptive; for example, increasing bone diameter (increasing the moment of inertia) can markedly increase resistance to fatigue, but heavier bones are not desirable in terms of minimizing energy expenditure during locomotion. Sclerosis of the third carpal bone is frequently documented radiographically in sound as well as lame racehorses and has been demonstrated as an exercise-related change in Thoroughbreds and Standardbreds. In racehorses with pathologic lesions in the radial face of the third carpal bone, there were higher exercise-related area fraction and stiffness measurements (Young et al., 1991b). In the Colorado State University 2-year-old treadmill exercise study, exercised horses with higher Mc3 subchondral BMD were also significantly more lame (Kawcak et al., 2000). Additionally, in a study of Standardbreds, in which a radiographic grading system was used to analyze third carpal bone sclerosis in the first year of training, those horses with higher values were more likely to develop middle carpal joint lameness (Hopper et al., 2004). Metacarpal condylar fractures have been documented to occur in areas of high bone volume fraction (Whitton et al., 2010), although some had associated focal porosities, possibly related to greater amounts of microcracking in the sagittal groove as previously described (Muir et al., 2008). It had been proposed that extensive new bone formation in the Mc3 condyles, as documented in exercise studies, could concentrate strain at the condylar grooves and predispose to cracking (Riggs and Boyde, 1999) through calcified cartilage and subchondral bone. Poor mineralization of these tissues has been documented in the fracture predisposition site (Doube et al., 2007a; Firth et al., 2009) in horses before athletic training has begun, and the role of these early morphologic abnormalities in fracture risk requires further study. Some studies have indicated a positive effect of training on fracture rates, with reduced risk of dorsal metacarpal disease in 2-year-old racehorses in training in the United States if they had been subjected to short periods of training at high speeds (15–16 m/s) as opposed to those galloped at 11 m/s for longer periods (Nunamaker, 2002). This fits with current theory in that (1) bones should be subjected to levels of strain they will experience during athletic competition in order to adapt to it; and (2) only short numbers of high loading cycles are required per day to stimulate adaptive responses. Measurement of bone biomarker levels in the blood is one possible way of monitoring for risk of fracture. By using a multivariable logistical regression model, it was calculated that 2-year-old Thoroughbreds in training in the United Kingdom with ICTP>12365μg/L and older than 20.5 months were 2.6 times more likely to develop dorsal metacarpal lesions (Jackson et al., 2005). It may not always be necessary to directly monitor bone properties; epidemiologic studies are yielding information on effects of training practices. For example, in a 2-year study of young Thoroughbred horses in training for flat-racing in the United Kingdom (n = 335), there was a reduced risk of dorsal metacarpal disease with increasing cumulative exercise, but there was an increased risk if the exercise distances were increased over relatively short periods (up to 1 month). On the basis of these results, it was suggested that trainers should gradually introduce short periods of high-speed exercise and avoid large amounts of both cantering and high-speed work per week (Verheyen et al., 2005). This is consistent with current knowledge, that is, avoiding too many successive repetitive loading cycles that might lead to fatigue damage and an exaggerated orthopedic response. There is also some initial evidence that training foals at an early age does not induce damage and will delay the onset of orthopedic injury during subsequent racing careers (Rogers et al., 2008). Additionally, the chance of lifetime career success is significantly greater if racing for the first time occurs when the horse is a 2 rather than a 3 years of age, even if milestones of physical activity of lesser intensity have been achieved (Tanner et al., 2011).

CARTILAGE

Joints facilitate locomotion by allowing rotational or hinge movement and storage of elastic energy. Articular cartilage (AC), the highly specialized dense connective tissue lining the bone ends in synovial (diathrodial) joints, absorbs and transfers loads while protecting subchondral bone and enables smooth gliding of adjacent surfaces. Across all fracture categories in horses, most are osteochondral and originate within a joint; many major fractures originate in the deepest layer of the AC. Even excluding osteochondral fracture, AC is the musculoskeletal tissue most susceptible to injury, as evidenced by the high prevalence of osteoarthritis (OA) from a young age in domesticated (Neundorf et al., 2010) and wild (Cantley et al., 1999) horses; OA is probably the most common cause (30%–40%) of all lameness in horses (Rossdale et al., 1985; Todhunter and Lust, 1990). Training-induced osteochondral damage shows a predilection for certain sites including the metacarpophalangeal joint and the dorsal aspects of the radial, intermediate, and third carpal bone surfaces of the middle carpal joint. Significant cartilage damage has a grave prognosis, as this avascular tissue with lengthy extracellular matrix component turnover times has a poor regenerative capacity.

ARTICULAR CARTILAGE ANATOMY AND FUNCTION

The surface of normal adult hyaline articular cartilage (HAC) is white to slightly blue in color, smooth in appearance and on palpation, and covered in a lubricating film of synovial fluid. It can be up to approximately 2 mm thick in the distal femur of 2- to 5-year-old horses (Frisbie et al., 2006) but less in more distal joints, as measured under magnification. In some joints, a synovial fossa may be present, the surface of which is beneath that of the surrounding cartilage and is of a different color, usually dark blue or red, presumably because the vascular elements in subchondral bone and marrow are visible through the thinner cartilage of the fossa. The deepest layer, the articular calcified cartilage (ACC), is not grossly distinguishable from the underlying subchondral bone (SCB), the visible cartilage consisting only of soft cartilage or HAC. Thus, the "cartilage thickness" measured is usually that of HAC, which is logical, since blood vessels never normally enter it. Even in early life, the epiphyseal vessels, which pass through the ossification center of the foal epiphysis and traverse to the periphery of the unossified epiphyseal cartilage to end in looplike vascular arcades, do not enter the HAC. As the total cartilage (HAC plus epiphyseal cartilage) thickness in the young foal reduces, the SCB "plate" begins forming within a few months of birth, with

radiate trabeculae being superceded by bone deposited parallel to the joint surface.

The ACC has been studied in horses in their second year of life, at which stage the joint is still growing through the conversion of ACC into bone. The "tidemark" mineralization front marks the interface between the viscoelastic HAC and the ACC. This tidemark advances smoothly and uniformly outward into the HAC, and the ACC thickness is determined by (1) the rate of this advancement and (2) the resorption of ACC at its junction with SCB, a process which is localized and nonuniform (Doube et al., 2007a). The cement lines formed at the osteochondral junction each time chondroclastic resorption ceases and bone apposition occurs are the multiple "waves" seen in the ACC (Figure 13-18, *A*). Although the ACC appears to not have vascular communication with HAC, an electron microscopic study has shown large holes in the Mc3 ACC of 2-year-old horses (see Figure 13-18, *B*) (Boyde and Firth, 2004), indicating that the matrix of HAC is not as isolated as previously thought. In the adult horse the relatively thin HAC is intimately connected through the ACC with the SCB, with transmission of force from the extremely hard ACC into the superficial and deeper SCB and from there to the bulk epiphyseal trabecular bone.

Microanatomy and Matrix Composition

The cartilage matrix comprises a network of collagen fibrils enmeshed in a concentrated PG solution. The collagen and PGs interact to form a porous composite fiber-reinforced organic solid matrix that is swollen with water (Mow and Hung, 2001). Collagen is 15% to 22% of the wet weight, with 4% to 7% PG, and the remainder composed of water, inorganic salts, other proteins, glycoproteins, and lipids. The matrix is divided into theoretical compartments relative to the cells (chondrocytes): pericellular, territorial, and interterritorial. There are also four successive zones beginning at the articular surface that differ in terms of matrix composition, water concentration, collagen fibril orientation, and cellular alignment and morphology. These are termed *superficial*, *intermediate* (transitional or middle), *deep* (radial) and *calcified cartilage zones*. Despite the numerous textbook schematic diagrams, the collagen direction in the HAC matrix is only a resultant direction of what is effectively a three-dimensional "pseudo-random" intertwining of irregularly arranged and oriented collagen fibrils.

Collagenous Matrix

Collagen fibrils in the HAC of fetuses and neonates are small with a relatively uniform diameter (approximately 20 nm). In the adult, the fibrils have a highly variable diameter, from 25 nm to approximately 200 nm, dependent on their anatomic location. They are "heterofibrils" largely composed of type II collagen molecules ($[\alpha 1(II)]_3$), with incorporation of type IX molecules. The globular N-terminal noncollagenous domains of type IX molecules project from the fibril and may assist in anchoring it to the surrounding PGs. The other fibril-forming collagens were initially not thought to occur in cartilage; however, it is now known that type I may comprise as much as 10% of the collagenous matrix. Small amounts of collagen types III, V, VI, XI, XII, and XIV are also present (III and XI are fibril-forming collagens). Type X collagen occurs in the growth plates of fetal and juvenile long bones.

The arrangement of collagen fibrils varies between cartilage zones. In the superficial zone, representing 10% to 20% of total thickness, sheets of fibrils are randomly woven in planes parallel to the surface. In the intermediate zone (40%–60% of thickness), the fibrils are more loosely spaced and randomly oriented. Larger, radially oriented bundles of fibrils are noted in the deep zone (30% of thickness), which cross the tidemark. The direction of the fibrils is simplistically and repetitively depicted according to the arcade model of Benninghoff (1925), but the directionality may be different in different joints and regions of them and is affected by the loading regimen in early life (Brama et al., 2009; van Turnhout et al., 2008). Layers with differing collagen

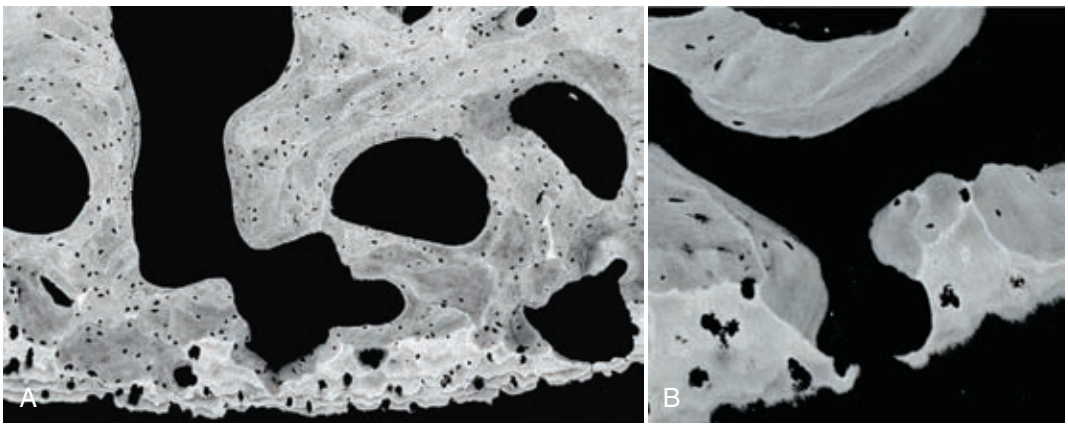

FIGURE 13-18 Backscattered electron microscopic images of the distal third metacarpal bone of an untrained Thoroughbred horse. **A,** There are tidemarks in this image because of the progressive growth of calcified cartilage, and various gray shades in the subchondral bone contain different concentrations of mineral. Cutting cones are in the subchondral bone and the calcified cartilage. The border of the calcified cartilage is intact and does not communicate directly with the overlying soft cartilage (*below, but not visible having been removed in processing*). **B,** The calcified cartilage layer is not continuous, and there is communication between a marrow space in the subchondral bone and the hyaline cartilage matrix (*below, but not visible*). Such communication has been observed in both trained and untrained horses, and its significance is not known.

arrangement appear to function to distribute stress more uniformly across loaded regions of the joint tissue. The collagen content is highest in the superficial zone and remains relatively constant in the deeper zones. The pericellular matrix is essentially fibril-free, containing a fine mesh of type VI collagen and appearing to attach directly to chondrocyte membranes; surrounding this is an envelope of territorial matrix containing thin collagen fibrils that form a basket-like network around the cells. The interterritorial matrix is the largest matrix compartment, containing larger diameter fibrils. As with type I collagen, type II molecules are released extracellularly as procollagen molecules; the disintegrin and metalloproteinase with thrombospondin-like motifs (ADAMTS)-3 cleaves the N-propeptide and bone morphogenic protein-1 cleaves the C-propeptide (CPII). In healthy adult articular cartilage, CPII levels are low and relatively constant. Collagen turnover times in articular cartilage are thought to be very long, and have been estimated at 200 to 400 years in humans (Maroudas et al., 1992).

Noncollagenous Matrix

Proteoglycans are in highest concentrations in the intermediate zone and lowest in the superficial and deep zones. Small PGs comprise less than 10% of the total PG content in cartilage matrix. Most are aggrecans (large PGs) with approximately 150 GAG chains (chondroitin sulfate and keratan sulfate [KS]) and both O-linked and N-linked oligosaccharides attached. The GAGs are heterogeneously distributed along the protein core, with CS-rich and KS-rich regions, respectively. The protein core itself is heterogenous, with three globular regions. Aggrecans vary significantly in length, molecular weight, and composition, with the amounts of KS-rich molecules and ratios of chondroitin-6-sulfate to chondroitin-4-sulfate increasing throughout development and aging. Most aggrecans in cartilage are attached to a hyaluronan (HA) molecule via a globular (HABR) region, with this binding stabilized by a link protein. Several hundred aggrecans attach to a single HA core molecule, the latter being a nonsulfated disaccharide chain up to 4 μm in length. The HA-aggrecan macromolecule has a "bottle-brush" appearance when visualized by electron microscopy. In situ, negative charges of sulfate and carboxyl groups on the CS and KS chains create strong intramolecular and intermolecular repulsive forces that are diminished by "clouds" of counterions and co-ions (sodium [Na^+], calcium [Ca^2+], and chloride [Cl^-]). The charge–charge repulsive forces extend and stiffen the PG macromolecules into spaces between surrounding collagen fibrils. As in other tissues, PGs can closely associate with collagen fibrils and are thought to be involved in their structural organization, contributing little to cohesive strength while being central in maintaining compressive stiffness (Broom and Silyn-Roberts, 1990).

Collagen-binding by cartilage matrix oligomeric protein (COMP), fibromodulin, decorin, biglycan, matrilins-1, matrilins-3 has been reported in cartilage matrices. COMP is a prominent component of equine HAC, and in carpal bone sites, it is located diffusely in the matrix of superficial and deep zones but primarily in pericellular and territorial matrix compartments of the intermediate zone (Murray et al., 2001b). Fibronectin is also important in binding to PGs and attaching chondrocytes to the extracellular matrix, and in equine carpal articular cartilage, it is most prominent in the pericellular and interterritorial compartments of the superficial, deep, and calcified zones (Murray et al., 2000).

Water is the most abundant component of articular cartilage, with highest concentrations near the surface. Approximately 30% of the water content is closely associated with collagen fibrils, but most is freely mobile within the interfibrillar spaces. The extracellular fluid contains large amounts of cations and is essential for gas, nutrient, and waste product movement between this avascular tissue and the synovial fluid.

Biomechanical Properties

Structural and Material Properties

In considering the biomechanical properties of cartilage tissue, it is treated as a biphasic material consisting of (1) an interstitial fluid phase and (2) a porous-permeable solid phase. Forces may vary from almost zero to more than 10 times the body weight, with considerable and complex variation in the site and size of contact areas in the different joints. Under physiologic loading conditions, cartilage is a highly stressed material. As in bone, compression, tension and shear loading must be considered, and the tissue is anisotropic, that is, the material properties vary with the direction of loading. Under tension, this anisotropy is thought to relate to varying collagen fibril arrangements in the structural zones as well as to variations in collagen–proteoglycan interactions. Cartilage is also viscoelastic, with this behavior in compression primarily caused by the flow of interstitial fluid and associated frictional drag (biphasic viscoelastic behavior) and in shear by motion of long polymer chains, including collagen and PGs (flow-independent) (Ateshian et al., 1997). Interstitial fluid pressure supports more than 90% of a load applied to a cartilage surface immediately following loading, lasting sometimes >1000 seconds and shielding the matrix and chondrocytes.

Compressive, Tensional, and Shear Loading

As the loading environment of cartilage is very complex, it is difficult to measure the mechanical properties of specimens in vitro. An explant loading configuration, known as *confined compression*, has been used; in this, a cylindrical specimen is positioned in a confining ring that does not allow deformation or fluid flow in the radial direction. Under loading, fluid flow occurs vertically through a porous platen (Mow and Hung, 2001). Constant compressive stress can be applied, with the tissue being allowed to creep to its final equilibrium strain by fluid exudation, which can involve up to 50% of total fluid content under high loads (>1 MPa) and is fully recoverable. The time it takes to reach creep equilibrium varies inversely with the square of the thickness of the tissue (Mow et al., 1980). Because the rate of creep is determined by the rate of fluid exudation, this can be used to determine the permeability coefficient. Permeability is a measure of the ease with which fluid can flow through a porous material, being inversely proportional to the frictional drag experienced (Mow and Hung, 2001). At equilibrium, no fluid flow occurs, and the deformation at this point can then be used to measure the intrinsic compressive modulus of the collagen-PG solid matrix (H_A) (Armstrong and Mow, 1982). Measured values for H_A and permeability in articular cartilages of middle carpal joints have varied significantly (0.2–1.5 MPa; 0.9–5 $m^4/Ns \times 10^{-14}$), this most likely being caused by differences in the exact sampling sites, ages, and exercise histories of the horses used, as well as the methodology (Murray et al., 1998; Palmer et al., 1995b). The

intrinsic compressive modulus varies directly with PG content and inversely with water content. Values of 1.6 to 2.4 MPa, as measured for various sites on equine femoral condyles, showed a strong correlation with sulfated GAG content (Changoor et al., 2006).

Stress relaxation occurs in cartilage; first, a constant compression rate is applied (compression phase) until a certain deformation and associated stress level is reached (σ_0), this being associated with forced fluid exudation from the matrix and compaction of matrix near the surface. That deformation is then maintained and the stress decays to an equilibrium level ($\sigma\infty$) due to fluid redistribution within the matrix. This equilibrium is reached when compressive stress within the matrix equals the stress generated by the intrinsic compressive modulus of the solid matrix. This phenomenon is protective in cartilage as it prevents maintenance of excessive stress levels; inhomogeneity of H_A within the depth of the cartilage (0.079 to 2.10 MPa in bovine articular cartilage) has been shown to result in faster relaxation (Schinagl et al., 1997).

The behavior of cartilage under tension depends on the nature of the collagenous matrix and the direction of loading. There is significant heterogeneity associated with depth (as mentioned above), with specimens from the collagen-rich superficial layers tending to be stronger and stiffer. Viscoelastic behavior is also seen in tension. At physiologic strain levels (<15%), the modulus of elasticity is between 5 MPa and 10 MPa. Few such measurements of equine cartilage have been made; an average modulus of 5.2 MPa was calculated for osteochondral units from the femoral trochlear ridge of young adult horses (3–5 years) (Gratz et al., 2006). Testing cartilage in tension and compression involves volumetric changes resulting in fluid flow and viscoelastic effects, so only equilibrium intrinsic properties of the collagen–PG matrix can be measured. Dynamic shear testing at low strains does not result in this interstitial fluid flow. The dynamic shear modulus is a measure of the total resistance offered by the viscoelastic material. Shear moduli of 0.6 to 0.7 MPa were measured in articular cartilages of middle carpal joints of 2-year-old horses (Murray et al., 1998). Measurements made in various shear studies have indicated that shear stiffness is provided by the collagen network alone, collagen–PG interactions, or both (Mow and Hung, 2001).

Direct measurements of in vivo stresses in articular cartilage are not currently possible. Some estimates have been made of peak contact stresses in various weightbearing joints of humans, in the range of 6 to 9 MPa, which is three to four times less than the threshold for permanent damage (Buckwalter et al., 2006). For some activities and joints (e.g., in the hip joint of a person rising from a chair), higher and very rapid stresses of 10 to 20 MPa have been calculated (Hodge et al., 1986). It is not known if equine articular cartilage has as high a "safety factor," and it seems likely that higher stresses are experienced in the joints of horses during high-speed locomotion, and perhaps in highly localized sites of some joints at even low levels of exercise undertaken spontaneously at pasture.

Mechanics at Ultrastructural and Molecular Levels

When articular cartilage is loaded, collagen–PG networks and the interstitial fluid function together to protect tissue against the high levels of stress and strain. Aggregation of large PGs immobilizes them within the collagen network, promoting structural stability and rigidity of the matrix. The strong repulsive forces between GAG chains, which are hydrophilic because of their negative charge, and charge shielding by counter-ions and co-ions attract water and result in a swelling pressure, which imbues the HAC with its compressive stiffness. This swelling pressure is resisted and balanced by tension in the collagen fibril network, which confines the PGs to approximately 20% of their potential free solution domain; that is, the swelling pressure subjects the collagen fibrils to significant "pre-stress" before external loading is even applied. The Donnan osmotic pressure theory has been used to calculate these swelling pressures in articular cartilage tissue.

As detailed above, large amounts of water between collagen fibrils can move when load or pressure is applied (approximately 70% of total water). When a compressive load is applied to the articular cartilage matrix, the internal pressure exceeds the swelling pressure and causes fluid to flow out of the tissue. The normally large area of solution occupied by the PG macromolecules ("solution domain") is now reduced, increasing the charge density and intermolecular repulsive forces and bulk compressive stress until they reach equilibrium with the external stress. The ability of PGs to resist compression is therefore related to both the Donnon osmotic swelling pressure (0.05–0.35 MPa) and the bulk compressive stiffness of the collagen–PG solid matrix (0.5–1.5 MPa) (Mow and Hung, 2001).

Topographic Variations

Topographic variation in loading within joints is thought to be responsible for the well-documented heterogeneity of matrix composition and synthesis, morphology, and mechanical properties in AC. The load-bearing regions of cartilage are thicker, mechanically stronger, and have higher PG concentrations compared with non–load-bearing regions in the same joint. Neonatal equine metacarpal articular cartilage does not show topographic variation in water, deoxyribonucleic acid (DNA) or GAG content, collagen content, or post-translational modifications of the collagen network (the "blank joint"); however, such differences develop during the first 5 months of weightbearing and growth under mechanical influences (Brama et al., 2000a; 2002). Articular surfaces experience complex mechanical environments, and strains under an applied surface load have, for example, been shown to vary significantly within different zones, to be highest in the superficial zone, and to correlate with differences in chondrocyte biosynthetic activity (Wong et al., 1997). Marked site-associated variations in cartilage biomechanical, structural, and biochemical properties, including water, DNA, collagen, and GAG contents and levels of hydroxylysylpyridinoline and pentosidine collagen crosslinks, have been documented in the metacarpophalangeal joints of 18- to 24-month-old Thoroughbreds or older horses (Brama et al., 1999; Brama et al., 2000b; Nugent et al., 2004). Although there was topographic variation in the biochemical characteristics of the collagen network in the proximal articular cartilage of the proximal phalangeal bone (P_p), no age-related changes were measured other than increased nonenzymatic glycation (Brama et al., 1999). Similar variations have been measured in carpal joints, in which the mechanical environment has been relatively well defined; injury-prone dorsal sites of carpal joints are known to experience high but intermittent loads, whereas palmar sites are more consistently loaded at lower levels and have differences

in HAC and ACC thickness (Firth and Rogers 2005b; Murray et al., 1999b). In one study of young adult Thoroughbred horses, dorsal carpal bone HAC had a higher collagen and DNA content and a higher chondrocyte density but a lower GAG content compared with palmar cartilage (Murray et al., 2001a). In dorsal locations, fibronectin occurred in deep and calcified zones, with very low levels in superficial and transitional zones; however, palmar locations showed more diffuse fibronectin expression with the exception of the injury-prone palmar aspect of the medial third carpal bone (Murray et al., 2000).

Significant variations in thickness, mineralization density, tidemark count, and tidemark linear accretion rate were documented in the ACC of the distal Mc3 of 18-month-old Thoroughbreds (Doube et al., 2007a). Regions with thinner ACC had greater linear accretion rates; lower mineralization and slower chondroclastic resorption of bone are responses to high loads. It should be noted that as AC, SCB, and metaphyseal trabecular bone are closely associated and functionally interdependent, they should not be considered in isolation. In one study of the proximal phalangeal bone, topographic differences in hydroxylysine concentration, hydroxylysylpyridinoline cross-links, and (for the bony components) calcium content were similar in all three of these tissues (van der Harst et al., 2004). In the distal Mc3 of 18-month-old Thoroughbreds, thick and poorly mineralized ACC tended to be situated on high volume fraction bone; that is, both tissue types responded to high load (Doube et al., 2007a).

Fewer measurements have been made of biomechanical properties, but site-related differences in aggregate modulus of the third carpal AC have been reported (Palmer et al., 1995b). In another study using automatic indentation apparatus, significant variations in aggregate modulus, Poisson's ratio, permeability, and cartilage thickness were documented between test sites in the AC of third, radial, intermediate, fourth, and ulnar carpal bones from six male adult horses (2–7 years) that had not been raced or trained for racing (Murray et al., 1995). AC from dorsal sites, which are more injury prone and experience higher loads (as above), was softer, thinner, and, in some limbs, less permeable than that in central test sites. In the metacarpophalangeal joint, compared with the dorsal aspect, the palmar aspect AC was thinner, had a thinner superficial zone and lower indentation modulus, and was more vulnerable to severe impact-induced damage, which may contribute to the more severe and prevalent palmar osteochondral pathology observed (Turley et al., 2012).

CELLULAR BASIS OF CARTILAGE MATRIX MAINTENANCE AND RESPONSES TO EXERCISE

Chondrocytes

Chondrocytes account for less than 10% of tissue volume and do not proliferate in normal adult cartilage other than in late-stage degenerative lesions. Chondrocytes are responsible for synthesis, organization, and maintenance of the organic component of the extracellular matrix. The chondron is defined as a microanatomic unit comprising a chondrocyte and the pericellular microenvironment (Poole, 1997). The morphology and alignment of chondrocytes varies with their depth in the HAC: In the superficial zone, they are elongate and flattened, with their major axis parallel to the surface; in the intermediate zone, the chondrocytes are more spherical with greater volumes of intracellular synthetic organelles (endoplasmic reticulum, Golgi apparatus, and mitochondria); and in the deep zone, the cells have a similar appearance but are aligned in columns. There are associated differences in matrix composition, including variation in collagen fibril arrangement, as detailed above.

Chondrocytes reside in a relatively hostile and changeable environment. Large amounts of negatively charged GAGs in the cartilage matrix influence the ionic composition of the extracellular fluid, with high concentrations of mobile cations and a resultant osmolarity of 350 to 450 milliosmoles (mOsm); osmolarity is a powerful regulator of chondrocytic activity (Urban et al., 1993). The oxygen tension in cartilage is very low, and this appears to assist in maintaining chondrocytic phenotype in vitro. It is thought that relatively high constitutive levels of expression of hypoxia-inducible factor-1α activity are critical for the survival of chondrocytes in these conditions (Pfander and Gelse, 2007). Chondrocytes largely use glycolytic pathways to produce adenosine triphosphate (ATP), extruding lactate ions and hydrogen (H^+) ions into their extracellular environment to produce a pH of at least 6.9.

Cellular Responses to the Mechanical Environment

In cartilage explant model studies, stimulation of matrix production depends on frequency, amplitude, and wave forms of fluctuating loads, whereas static pressure differentially alters synthesis of aggrecan, link protein, and hyaluronan (Kim et al., 1996). Motion is important, in addition to direct loading, with even small amounts reducing PG depletion and stimulating anabolic pathways in unloaded joints (Deschner et al., 2003). Dynamic loading of cartilage deforms cells and matrix, causes cyclical fluctuations in hydrostatic pressure, and results in fluid movement (Wilkins et al., 2000). Static loading expresses fluid, concentrates matrix macromolecules, reduces extracellular pH, and increases extracellular osmolarity. The end result appears to be alterations in chondrocyte membrane permeability that influence intracellular pH and ionic strength, rates of glycolysis, and gene expression patterns. Fluid flows within the matrix generate currents and electric streaming potentials but may also alter transport and availability of soluble factors, nutrients, and growth factors (Mow et al., 1999; Vincent et al., 2004).

The forces experienced at a cellular level in cartilage are thought to be significantly less than those experienced by the tissue as a whole because of the pericellular matrix (Millward-Sadler and Salter, 2004). The composition of the matrix, as determined by magnitudes and types of previous loading and including collagen orientation and depth-dependent fixed charge densities, in turn, determines the forces that the chondrocytes will experience (Korhonen et al., 2008). At dorsal and palmar margins of the Mc3 and corresponding P_1 articular cartilages, subjected to intermittent but heavy peak loads during athletic activity, GAG content is lower, but collagen and HP cross-link levels are higher than those in the more central regions of the joint that experience continuous but lower-level loading during weightbearing (Brama et al. 2000b). Chondrocytes also seem to vary in their biosynthetic responses, depending on their zonal location; for example, chondrocytes in superficial layers have a faster rate of PG turnover and a slower rate of PG synthesis than those from deeper layers. As in other connective tissues, the mechanical signals are thought to be mediated by integrins and by the release of soluble factors that may act in an autocrine or paracrine fashion (e.g., prostaglandins,

cytokines, growth factors, and nitric oxide). A maximal response to mechanical stimulation in the form of membrane hyperpolarization has been shown when chondrocytes are adhered to fibronectin via the $\alpha_5\beta_1$ integrin (Millward-Sadler and Salter, 2004). Other potential mechanoreceptors include stretch-activated ion channels, CD44 (hyaluronan receptor), and anchorin II (collagen type II receptor). Chondrocytes must act as sensors and effectors; in contrast to the linked cellular networks in tendon and bone, chondrocytes exist largely as individual cells. Gap junctions (Cx43) are important during development of cartilage, but in the adult are mainly expressed by superficial, flattened chondrocytes (Schwab et al., 1998). Apparently central to the mechanotransductive capabilities of AC, like other tissues, are the primary cilia of chondrocytes (McGlashan et al., 2010).

FUNCTIONAL ADAPTATION OF ARTICULAR CARTILAGE

Few studies investigating effects of exercise specifically on equine AC have been performed. The results of such studies have been difficult to interpret in terms of determining if adaptation, or conversely, damage has occurred. This is for several reasons, including the very limited understanding in any species of the physiology and pathophysiology of this tissue, the difficulty in separating exercise-related effects in AC and the immediately underlying SCB, the fact that alterations in SCB induced by exercise can affect cartilage quality and integrity (discussed below), and the difficulty in examining AC in vivo, which is now being overcome by sophisticated imaging, spectroscopic, and other techniques.

Evidence of Exercise-Induced Microdamage

As in tendon and bone, repetitive microtrauma can result in subclinical changes that accumulate and may eventually result in osteochondral failure and osteoarthritis (OA). Subclinical lesions, including surface roughening, mechanical softening, chondral or osteochondral fragmentation, and cartilage wear lines, have been documented at common sites of clinical injury in equine AC (Firth and Rogers, 2005a; Nugent et al., 2004) (Figure 13-19). However, a direct relationship between these lesions and eventual development of OA has not been proven, and some precede significant participation in training or racing (Kim et al., 2009). Epidemiologic studies of human populations have indicated that moderate levels of running exercise are not associated with development of OA, but high levels of exercise are a risk factor. The exact relationship among exercise intensity, frequency, and type on the development of pathology at certain sites in horses has not been clearly defined. Of the exercise-induced changes documented in equine AC, many have occurred only or maximally at sites predisposed to clinical lesions. This suggests that physiologic thresholds may be more likely to be exceeded at those sites because of training-induced variations in loading within susceptible joints.

Fatigue wear of cartilage surfaces initially results from cumulative damage under repetitive stressing (as in other connective tissues), not from surface-to-surface contact. The direct relationships between cartilage matrix damage in various sites in equine joints and the frequency, magnitude, and periods over which various loads are applied have not been defined. There are several possible mechanisms by which accumulation of fatigue damage could occur: (1) disruption of the collagen–PG matrix (e.g., tensile failure of collagen fibrils, or disruption of PG-PG interaction sites); (2) PG "wash out" due to repeated massive imbibition and exudation of the interstitial fluid; and (3) rapid application of high loads that do not allow time for fluid to redistribute to compacted regions, again resulting in collagen–PG matrix damage (Mow and Hung, 2001). Structural defects that result include "fibrillation," where the surface matrix splits vertically and eventually extends throughout the full thickness of the cartilage, and erosion ("smooth-surfaced destructive thinning"). Loosening of the collagen network allowing abnormal PG expansion and tissue swelling is thought to be a key factor in the pathogenesis of OA (Mow and Hung, 2001).

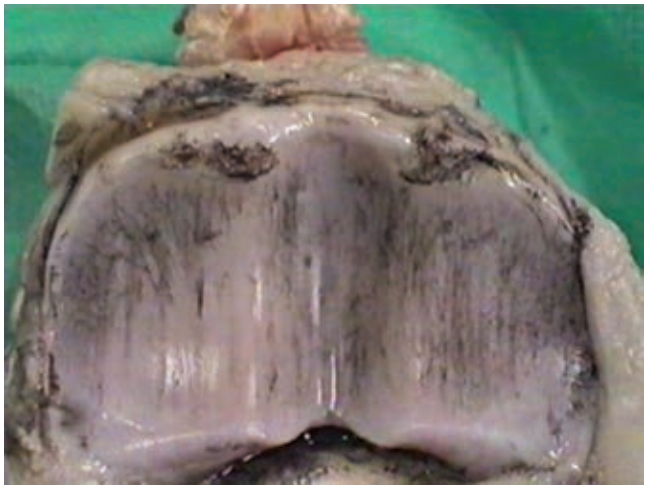

FIGURE 13-19 En face view of the proximal surface of the proximal phalangeal bone of a 2-year-old horse that had been trained for 13 weeks on soft tracks and was entirely asymptomatic in the metacarpophalangeal joint involved. Cohort horses that had not been trained showed similar wearlines, so it is not clear how the parallel lines made obvious by staining with India ink (wearlines) are related to the osteochondral change in the medial and lateral eminences of the bone.

Excessive mechanical stress is also known to alter chondrocyte function, including the balance between synthetic and degradative activity. Stress-induced degenerative changes in their surrounding matrix can significantly increase stress and strain experienced by chondrocytes; that is, a vicious cycle of damage can develop. Overloading of AC has been shown to disrupt the collagenous network, cause chondrocyte-mediated loss of PGs, upregulate matrix-degrading enzymes, and result in chondrocytic necrosis and apoptosis (Chen et al., 2003). Chondrocytes are not replaced when they are lost from adult cartilage. There is also evidence that following injurious compression, particularly at high strain rates, the remaining chondrocytes are less able to respond to lower, normally stimulatory loads (Kurz et al., 2001). In one study of Standardbred horses, 12 animals were subjected to a moderate, graded exercise program (3–4 days per week of up to 4000 m at 8 m/s), followed by 17 weeks of moderate or strenuous exercise, and then a 16-week rest period (Little et al., 1997). There were no histopathologic changes in the articular cartilage of the third carpal bone or alterations in PG content; however, in animals that experienced the strenuous exercise regimen, there was a significant reduction in aggrecan synthesis and a concomitant increase in decorin synthesis in the dorsal radial facet. This was interpreted as a disturbance of PG biosynthesis in a cartilage region subjected to

high contact stresses that had persisted over the subsequent 16-week rest period.

In the 18-week Bristol study, although exercise-induced changes in the ACC and SCB of carpal articular surfaces were interpreted as potentially adaptive, deterioration was noted in the HAC tissue, which, in trained horses, showed increased clustering of chondrocytes and fibrillation (Murray et al., 1999b). The dorsal radial carpal bone cartilage and the third carpal bone cartilage from exercised horses were less stiff and had reduced superficial toluidine blue staining. Injury-prone sites also showed significant exercise-related reductions in collagen content (Murray et al., 2001a). In the deep layer of the articular cartilage of carpal bones, exercised horses showed positive immunolabeling for COMP in the interterritorial matrix only, compared with diffuse expression in the matrix of this region in control horses (Murray et al., 2001b). Only small numbers of horses were analyzed; however, there was greater loss of COMP in exercised horses from more highly loaded (dorsal) sites in these articular cartilages, which was suggested to represent acceleration of a normally age-related change. At the dorsal distal radial carpal bone site, fibronectin immunoreactivity was higher in exercised horses in the superficial zone, where the matrix was softened and showed evidence of increased superficial PG loss (Murray et al., 1998; 2000). Fibrillation was associated with increased COMP and increased fibronectin immunoreactivity (including intracellular accumulation) around the fissures.

In the MUGES study, grossly observable HAC lesions of the proximal surface of P_1 (as observed by India ink staining) were more severe in exercised horses (Firth et al., 2004b), with increased water content and a sharp reduction in collagen HP cross-linking that was correlated with the presence of wear lines (see Figure 13-19)(Brama et al., 2000b). There was loss of site-specific differences in collagen parameters present in the control horses. The changes in the collagen network, which would be expected to have very limited capacity for repair and remodeling given the normally lengthy turnover times, were interpreted as microdamage. This predilection for pathologic change in the metacarpophalangeal joint was previously noted as an age-related change in wild horses and as an exercise-related change in horses that underwent 6 months of treadmill training (Cantley et al., 1999; Kawcak et al., 2000); that is, such lesions may represent acceleration of aging as in other connective tissues, or they may be caused by the effect of normal activity on subtle, already existing developmental lesions (Boyde and Firth, 2008).

The health and integrity of HAC is dependent on the properties of the ACC and the underlying bone. It has been proposed that stiffening of subchondral bone in response to exercise may alter the stress distribution between cartilage and bone, initiating and progressing AC damage (Radin and Rose, 1986). Sclerosis of SCB has been frequently reported, but its exact effect on the stiffness gradient of ACC and SCB in response to exercise has received little attention. Impact severity below the level required to cause intra-articular fractures will result in substantial microdamage, including thin and irregular ACC, SCB microfractures, cartilage fissures, chondrocyte death, and PG release. Condyles from the Mc3 and Mt3 of racehorses with metacarpophalangeal joint arthrosis associated with overloading showed evidence of microcracking, microfractures, activated remodeling, and bone collapse, with extension into the ACC in some horses being associated with articular cartilage indentation ("traumatic osteochondrosis"; Norrdin and Stover, 2006; Norrdin et al., 1998). Traumatic osteochondrosis is often asymptomatic but, when severe, has been associated with lameness.

Effects of Training on Equine Articular Cartilage

Controlled Treadmill Exercise Studies

In the 18-week Bristol study, the depth of the middle carpal ACC was increased in four dorsal sites of three bones of the middle carpal joint without alteration in HAC thickness and was greater in both exercised and control groups in dorsal compared with palmar sites (Murray et al., 1999a). Subchondral bone at high load sites was thicker in the exercised horses, with increased bone modeling and reduced resorption; in the absence of changes in material stiffness, this would be associated with increased structural stiffness of the subchondral tissue (Murray et al., 2001c). The increased ACC thickness was proposed to be a response to maintain the articular surface stiffness gradient in the face of the exercise-induced alterations in the SCB. However, it was also stated that the SCB thickening that occurred in injury-prone sites might alternatively have predisposed to cartilage damage had the horses continued to train. In the MUGES study, in which horses were trained on sand and grass tracks, the HAC of the third carpal bone was thicker in the exercised group, with no histologic lesions and no such change in the thickness of the ACC (Firth and Rogers, 2005b). Similar positive responses were not noted in the metacarpophalangeal joints in the same horses (Brama et al., 2000b). The differences in cartilage response between the MUGES and 18-week Bristol studies might reflect differences in number and position of sites studied in the carpus, pretraining exercise histories, and a more active exercise regimen for the control group in the Bristol treadmill study.

In the metacarpophalangeal joints of the horses in the 18-week Bristol study, there were exercise-induced increases in PG synthesis that were only statistically significant in the right hindlimb and were interpreted as adaptive (Bird et al., 2000). Similarly, less strenuous exercise of horses of various ages (30 minutes, 3 times a week, for 6 weeks) resulted in increased PG synthesis by articular cartilage of the third carpal bone with an increase in the matrix permeability constant that indicated an increased capability for fluid movement (Palmer et al., 1995a; 1995b); in that study, however, the horses were housed in box stalls rather than having free pasture access to allow normal activity levels. Additionally, exercise caused a reduction in cartilage thickness that could be interpreted as maladaptive (see below). Load-induced changes in GAG synthesis by equine chondrocytes may be at least partially regulated by alterations in levels of various factors in the synovial fluid during exercise (van de Lest et al., 2000).

Should Foals Be Trained from Soon after Birth?

Mechanical stimulation resulting from normal activity is known to be necessary for development of equine AC from the biochemically "blank" structure in neonates. In the Dutch EXOC study (see Table 13-2), foals in the boxrest group showed retarded AC development, with failure to develop load-matched topographic heterogeneity. In terms of collagen characteristics, this was not compensated for by subsequent exercise (Barneveld and van Weeren, 1999). In the boxtrained group, there was a long-term inability to increase PG synthesis in response to stimulation and a long-term effect on chondrocyte viability (Barneveld and van Weeren, 1999; van den Hoogen et al.,

1999). Normal development of the supporting SCB was also negatively affected by box stall confinement in normally loaded regions of proximal P_1, including post-translational collagen modifications and topographic variation (Brama et al. 2000a). As for the bone and tendon analyses of these animals, these results indicated that normal pasture activity is necessary for normal connective tissue development, and that subjecting foals without that access to strenuous training has long-term negative effects on adaptive cellular activity.

However, as for tendon and bone, it is possible that during the period of postnatal growth, when cellular activity is significantly modeling the locomotor tissues in response to mechanical stimuli, there may be a greater potential for adaptation to training resulting in increased injury resistance. This could be particularly important for AC, as modeling of the collagen network appears to occur during only the first 6 months post partum (Brama et al., 2000a). In the GERA foal study (see Table 13-2), there were no detectable exercise-related effects on indentation stiffness, collagen content, or water content in the distal Mc3 articular cartilage at 18 months of age, with no increase in subclinical degenerative lesions (Nugent et al., 2004). The mean percentage and number of viable chondrocytes in distal Mc3 and Mt3 cartilage were greater in exercised horses, as were PG scores determined by histochemical staining (Safranin O/Fast Green); these were interpreted as beneficial exercise effects in these sites (Dykgraaf et al., 2008). However, in AC of the proximal surface of P_1 of the right hindlimbs, there were lower GAG and collagen levels in exercised (CONDEX) animals but higher levels of hydroxylysine, hydroxylysylpyridinoline cross-links, and pentosidine cross-links (van Weeren et al., 2008). Most of these differences were detected only in the central fovea (an area of continuous but relatively low-level weight-bearing). This was proposed to be caused by the relatively low speed at which these foals were exercised over the first few months. As in the forelimb metacarpophalangeal joints, no increase in degenerative change was noted. The overall effects were interpreted as an acceleration of changes that normally occur during maturation; however, although this could be a positive effect allowing athletic use at an earlier age, it might also prematurely curtail the collagen remodeling period (van Weeren et al., 2008). In the middle carpal joint, in some of the same 18-month-old animals, at the osteochondral lesion-prone site at the distal aspect of the radial carpal bone and the proximal aspect of the third carpal radial facet, there was thickened calcified cartilage, microcracking, and increased subchondral vascular channel area without evident HAC abnormality (Kim et al., 2012). This was the site where contiguous slices with thickened ACC had a significantly higher mean swelling strain than sites with ACC of normal thickness; it may indicate possible slight collagen matrix disruption already present in the HAC and, in turn, indicate the earliest evidence of mid-carpal osteochondral disease in lesion-prone sites, in horses that have had only mild exercise imposed or had only spontaneous exercise at pasture.

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CHAPTER

14 Age and Disuse in Athletes: Effects of Detraining, Spelling, Injury, and Age

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Exercise is an acute perturbation, a stress on the physiologic systems of the body that chronically strive to maintain homeostasis. The stress of exercise evokes a coordinated response from multiple organ systems to physiologically support the increased demand in work. If repeated, exercise stimulates an adaptive (training) response that results in a significant increase in functional capacity, thus lowering the relative stress of future bouts of submaximal exercise. For example, a pretrained 2-minute mile may represent a 90% maximum effort, whereas after training, this same speed may represent a 70% maximum effort.

The stimulus to evoke a training adaptation involves the frequency (days/week) of exercise, its duration (minutes), and intensity (% of max). These three variables are manipulated to focus the training response specifically on the physiologic systems that are primary in supporting the activity for which the horse is being trained. Training must also take into consideration genetics. The equine athlete is typically bred for a specific type or work. Thus, physiologic enhancements are not only dependent on the training program but on the breed of horse as well. The caveat is that the breed must be taken into account, as most research into the effects of training and detraining in horses has focused on middle distance and endurance athletes. However, regardless of breed, the end result of training is an enhancement of physiologic function.

Increases in physiologic capacity come with a high metabolic cost, and this upregulation in metabolic capacity cannot be maintained without continually scheduled stimulation (exercise). The cessation of exercise results in a downregulation in metabolic capacity and in the physiologic systems involved in work. Thus, when a horse stops training because of injury or even during a long winter season when exercise cannot be performed on a regular basis, there is a general reversal of training adaptations. Activity may also become limited as a horse ages, resulting in the same loss in work capacity that occurs with detraining in young athletes. However, in the aging, detrained equine athlete, it is sometimes difficult to separate the decreases in performance that are caused by the aging process from those caused by inactivity.

DETRAINING AND DISUSE

The well-known increase in maximal oxygen uptake ($\dot{V}O_{2max}$) that occurs with training is reversed relatively quickly when injury or other reasons dictate prolonged rest (Poole and Erickson, 2008). In humans, the cessation of training can result in significant decreases in $\dot{V}O_{2max}$ within a matter of 2 to 3 weeks. However, in the horse, this decline appears to be slower, and it has been reported that extended stall rest or turnout, following cessation of a 9-month training program, will cause significant decreases in $\dot{V}O_{2max}$ within 12 weeks, with a much longer period required to return to pretraining levels (Tyler et al., 1996). The increase achieved in cardiac output with training is the first adaptation to reverse itself with detraining (Poole and Erickson, 2008). Maximal end-diastolic volume (EDV), ejection fraction (EF), and, therefore, stroke volume (SV) return to pretraining values within the first 2 weeks of detraining in humans; however, the changes seen in horses appear to take longer, declining between 4 and 12 weeks of deconditioning (N. Kriz et al., 2000). Without the regular occurrence (training) of cardiovascular stress, there is no need for the heart to maintain its left ventricular hypertrophy. Since this hypertrophy is caused by a mere stretching of the walls of the left ventricle and does not involve any protein synthesis of new tissue, the return to pretrained EDV is rapid.

Peripheral changes due to extended rest include a decrease in capillary density (Poole and Erickson, 2008) and over the long term (>12 weeks), a decrease in mitochondrial density with an associated decrease in oxidative (citric acid cycle) enzymes (Poole and Erickson, 2008). Again, without frequent and regular stress, there is no need for the cardiovascular system to maintain an elevated capacity for the delivery of oxygen to the muscle. Therefore, the increase in muscle capillarization that occurs with training will be lost (Poole and Erickson, 2008).

Training adaptations in the muscle itself involve the synthesis of new intercellular structures or enzymes. These positive peripheral changes are more enduring with detraining than are central changes. Mitochondrial density, oxidative enzyme concentration, and myoglobin will diminish with detraining. However, studies have shown that even after 6 months of detraining, up to 50% of the gains made in these parameters remain in place (Rivero and Piercy, 2008). Detraining also results in a decrease in muscle glycogen synthesis and, thus, a decrease in muscle glycogen stores (Rivero and Piercy, 2008).

Other detraining changes related to energy or metabolism include a decrease in lipolysis during exercise and a decrease

in lactate threshold (OBLA) (Rivero and Piercy, 2008). The increased energy demand of exercise is met, in part, by an improved ability to mobilize and metabolize fat during higher intensities of work. When this demand is absent (i.e., stall rest), this adaptation is diminished and eventually will return to its pretraining capacity (Rivero and Piercy, 2008). Similarly, the training-improved mechanisms responsible for counteracting exercise acidosis will return to their pretraining capacities during detraining. This loss is best illustrated by the occurrence of OBLA at a lower oxygen consumption (milliliters per kilogram per minute [ml/kg/min]) following detraining (Rivero and Piercy, 2008).

Skeletal muscle atrophy is an apparent consequence of detraining. Training-induced protein synthesis results in an increase in myofibrils inside the cell. In particular, there is a significant increase in myosin heavy chain proteins (Rivero and Piercy, 2008). Along with this increase is a hypertrophy of the cell itself. With cessation of training, there is an associated loss of myosin heavy chain proteins, and thus, cellular atrophy occurs (Rivero and Piercy, 2008).

Detraining and disuse can also alter the strength and integrity of bones, ligaments, and tendons. Although longitudinal data on the effects of detraining on bone in horses are lacking, there is an abundance of information demonstrating that there is a substantial decline in bone mineralization and strength with casting, weightlessness, bedrest deconditioning, and hindlimb suspension. Clinical reports involving horses have suggested diminished bone strength during periods of casting or suspension for the care of animals with severe orthopedic injuries (Hutchins et al. 1987; Nunmaker, 2002). More information is needed to determine if long-term stall housing and lack of activity alter bone, ligament, and tendon strength in the horse.

In summary, without the regular occurrence of exercise-induced stress (training), there is neither a structural need nor a metabolic need for the central or peripheral systems associated with performance to exceed their normal, untrained capacities. However, the equine athlete, unlike its human counterpart, has a relatively high capacity ($\dot{V}O_{2max}$) for work, even in an untrained state. The ability of the horse to immediately contribute its splenic reserve to active circulation with the onset of exercise, gives it an incredible ability to perform at high intensity. Although the sustainability of high-intensity work is lengthened with training or shortened with detraining, this remarkable capacity still resides in the horse.

EFFECT OF AGING ON EXERCISE CAPACITY

The number of horses over 20 years of age is increasing every year, and many of these animals are still performing various athletic activities (Hintz, 1995; McKeever, 2002; Rich, 1989). As with older humans, the reasons older horses are able to continue to compete include genetics, better general health care, and continual improvements in nutritional management (Hintz, 1995; Malinowski et al., 2002; McKeever, 2002; McKeever and Malinowski, 1997; Ralston and Breuer, 1996; Ralston, 1992; Rich, 1989). Studies of older humans show that overall working capacity decreases with age. The associated declines in aerobic capacity and muscular strength have been attributed, in large part, to the effects of aging on overall physiologic function. However, there is debate in the literature on how much of the age-associated decline in physical performance is due to actual physiologic aging versus disease processes related to inactivity. Research points to the fact that some of the decline in exercise capacity in older humans is related to a general decline in physical activity rather than to physiologic aging (Dempsey and Seals, 1995; Haskell and Phillips, 1995; Holloszy, 1993; Holloszy and Kohrt, 1995). Studies of older humans have shown that both dynamic and resistance exercise training forestalls or even reverses the decline in cardiopulmonary performance and muscular strength (Dempsey and Seals, 1995; Ekelund et al., 1988; Haskell and Phillips, 1995; Holloszy, 1993; Holloszy and Kohrt, 1995; Lakatta, 1995; Raven and Mitchell, 1980; Stamford, 1988). Such results base a strong argument in favor of the preventability of age-related decreases in performance that are rooted in disease or inactivity.

Limited data have been published regarding the exercise capacity of the aged horse (Betros et al., 2002; Horohov et al., 1999; Lehnhard et al., 2004; Lehnhard et al., 2001; Malinowski et al., 2002; McKeever, 2002; McKeever et al., 2002; McKeever and Kearns, 2001;; McKeever and Malinowski, 1999; McKeever et al., 1998; McKeever and Malinowski, 1997). Existing studies have focused on establishing a physiologic profile of the older equine, which may or may not have included a single bout of exercise performance. There has been, and continues to be, extensive research to determine the nutritional needs of the older horse (Ralston, 1992; Ralston and Breuer, 1996) however, there have been few studies, to date, to determine physiologic *adaptations* to training in horses 20 years of age or older.

AGING-INDUCED CHANGES IN RESPIRATORY FUNCTION THAT MAY IMPACT EXERCISE

In humans, aging appears to have a significant effect on lung function during exercise (Davies et al., 1988). A review of the literature (Dempsey and Seals, 1995) suggests that there are multiple alterations in pulmonary function that can limit respiratory capacity in older individuals. Studies reporting flow–volume loops suggest that an expiratory flow limitation occurs at lower work intensities in older individuals. Dempsey and Seals (1995) also pointed out the fact that the elastic recoil of the lung is altered with aging, a change reducing expiratory flow rates. Older humans also have a greater dead space that increases the dead space to tidal volume ratio. All together, these age-related changes affect the work of breathing during exertion. Lung hemodynamics are also affected by aging-induced decreases in arteriolar compliance, a detrimental change that may lead to capillary stress failure. If this decrease in compliance should occur in the horse as well, it would have significant implications, since capillary stress fractures are part of the etiology of exercise-induced pulmonary hemorrhage. Despite all of these age-related changes in human lung physiology, Dempsey and Seals (1995) reported that alveolar to arterial gas exchange and pulmonary vascular hemodynamics are only slightly modified.

In the horse, there is a void of research regarding the effects of aging by itself on the respiratory response to exercise. It is known that the normal young equine lung is not large enough to handle the demand of high-intensity exercise (Lekeux and Art, 1994). It is also well recognized that factors affecting pulmonary health can have a cumulative

effect in the horse. A lifetime of exposure to pathogens and allergens can lead to small airway disease (Lekeux and Art, 1994). Pathologies such as hyper-reactive airway disease, chronic obstructive pulmonary disease (COPD), and exercise-induced pulmonary hemorrhage tend to be more prevalent in older animals (Lekeux and Art, 1994). Such conditions can negatively impact respiratory function during exertion (Lekeux and Art, 1994). However, data are needed to determine the specifics of *aging-induced* respiratory changes in the horse and their similarity or dissimilarity to humans.

AGE-RELATED CHANGES IN THE CARDIOVASCULAR RESPONSE TO EXERCISE

Aging has profound effects on the cardiovascular system. In and of itself, the aging process results in a decreased maximal heart rate (HR_{max}), decreased vascular compliance, changes in baroreceptor sensitivity, and the possibility of concomitant hypertension in such species as rat, dog, and human (Dempsey and Seals, 1995). Functional studies in the horse have demonstrated a similar age-associated decrease in HR_{max} with a related decrease in $\dot{V}O_{2max}$ (Betros et al., 2002; McKeever, 2002; McKeever and Malinowski, 1997). This overall loss in working capacity is similar to that observed in older humans (Haskell and Phillips, 1995; Holloszy, 1993; Holloszy and Kohrt, 1995; Lakatta, 1995; Raven and Mitchell, 1980; Stamford, 1988).

In humans, decreases in HR_{max} with age appear to be caused by several factors, including changes in the number of pacemaker cells in the sinoatrial (SA) node, increases in elastic and collagenous tissues in all parts of the conduction system, and the deposition of adipose tissue around the SA node (Lakatta, 1995). In humans, aging also alters the autonomic tone, with a resulting downregulation of sensitivity to the sympathetic nervous system. This appears to influence the ability to increase HR during exercise (Lakatta, 1995). The horse appears to use autonomic mechanisms in the same way as do humans for the control of HR. Indirect evidence suggests that the horse may also undergo the same aging-induced changes in the neuroendocrine control of cardiovascular function (Goetz and Manohar, 1990; McKeever and Malinowski, 1997; McKeever and Hinchcliff, 1995).

A decrease in HR will result in a decrease in cardiac output (Q) unless compensated by an increase in stroke volume (SV) ($Q = HR \times SV$). Data, however, are mixed regarding the effect of age on SV. In unfit older humans, studies have demonstrated a decline in maximal SV, whereas their fit counterparts have maintained, or even increased, this function (Dempsey and Seals, 1995; Ekelund et al., 1988; Haskell and Phillips, 1995; Holloszy and Kohrt, 1995; Holloszy, 1993; Lakatta, 1995; Raven and Mitchell, 1980; Reaven, 1995; Seals, 1993; Seals and Reiling, 1991; Seals et al., 1984; Stamford, 1988). SV is a function of EDV and EF ($SV = EDV \times EF$). A greater filling volume of the left ventricle (EDV) results in a greater myocardial preload (stretch). This greater preload produces a greater myocardial force of contraction, resulting in a greater EF and, thus, a greater SV. Left ventricular hypertrophy (increased EDV) is a well-documented adaptation to endurance training in humans. Studies suggest that older humans are still able to achieve this beneficial training adaptation, compensating, to some extent, for the age-associated decrease in HR_{max} and, thus, better able to maintain Q. However, in very old individuals, there appears to be both a decrease in HR_{max} and SV that certainly results in a decline in maximal cardiac output (Dempsey and Seals, 1995; Holloszy and Kohrt, 1995; Lakatta, 1995; Raven and Mitchell, 1980; Stamford, 1988).

Until recently, data on the effects of age on central cardiovascular function, cardiac output, HR_{max}, and SV have been lacking in horses. However, a recent study by Betros et al. (2002) attempted to determine if any of the age-related declines in cardiovascular function were reversible with training. It was found that HR_{max} (218 versus 213 beats per minute [beats/min]), $\dot{V}O_{2max}$ (116 versus 109 mL/kg/min), maximal oxygen pulse (OP_{max}) (0.55 versus 0.52 mL/kg/beat) velocity at HR_{max} (9.0 versus 9.3 meters per second [m/s]) and velocity at $\dot{V}O_{2max}$ (8.8 ± 0.2 m/s versus 8.8 ± 0.2 m/s) were similar in young and middle-aged horses (Betros et al., 2002). However, there appeared to be a breakpoint once a horse was over 20 years of age. Old horses had a lower HR_{max} (193 beats/min), $\dot{V}O_{2max}$ (95 mL/kg/min), and OP_{max} (0.43 mL/kg/beat). In addition, the older horses reached their maximal values at lower velocities compared with young and middle-aged horses (Betros et al., 2002). Interestingly, the authors found that training resulted in substantial improvements in $\dot{V}O_{2max}$ and OP_{max} but did not alter HR_{max} in young, middle-aged, or old horses (Betros et al., 2002). First, the results of this study support the occurrence of an age-related decline in HR_{max} and maximal SV in the horse. This is important for horse owners who use HR to judge the intensity of the work their horses are performing. Second, these results document that training can partially reverse some of the decline in cardiovascular function that occurs in the older horse (Betros et al., 2002).

Since $\dot{V}O_{2max}$ is the product of the ability to deliver oxygen to the body's tissues (Q) and their ability to utilize it (a-vO_2 difference), the decline in HR_{max} (and, therefore, maximal cardiac output) most likely contributes to the decline in $\dot{V}O_{2max}$ (and, therefore, working capacity) seen in older humans and horses. Data are mixed, however, regarding the relative contribution of central versus peripheral factors toward the decline in exercise performance seen in horses, dogs, and humans (Betros et al., 2002; Dempsey and Seals, 1995; Haidet and Parsons, 1991; Holloszy and Hohrt, 1995; Lakatta, 1995; McKeever and Malinowski, 1997; Raven and Mitchell, 1980). In humans and other species, an age-related decline in central cardiovascular function accounts for some of the observed decrease in $\dot{V}O_{2max}$. However, decreased muscle mass, alterations in muscle capillary density, and decreased vascular compliance may also limit exercise capacity by limiting blood flow to working muscles (White, 1995). Thus, some of the decline in aerobic capacity is also caused by changes in peripheral mechanisms affecting the ability to utilize oxygen. Unfortunately, these data have been extrapolated from submaximal studies of humans, and the debate whether age-related declines in cardiovascular capacity in humans are predominated by central or peripheral mechanisms continues.

Older horses undergo a decline in $\dot{V}O_{2max}$ as also seen in healthy older humans. This decline was first documented in horses over 20 years of age by McKeever and Malinowski (1997). More recent studies have shown that the decline in $\dot{V}O_{2max}$ appears to start at 18 to 19 years of age (Walker et al., 2010) (Figure 14-1). In horses over 20 years of age, a lower

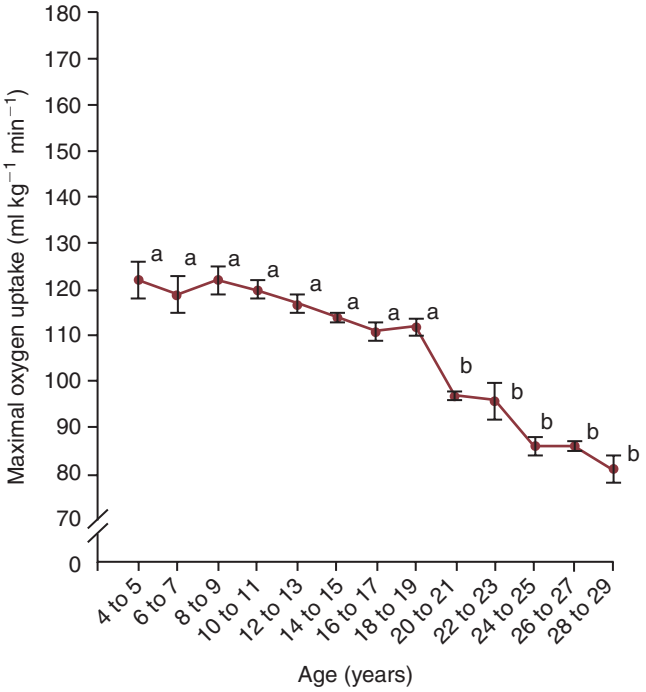

FIGURE 14-1 Mean (± SE) maximal oxygen uptake ($\dot{V}O_{2max}$) presented in 2-year intervals. Data are obtained from 50 unfit Standardbred mares. Points marked with different letters are statistically different. *(From Walker A, Arent SM, McKeever KH: Maximal aerobic capacity ($\dot{V}O_{2max}$) in horses: a retrospective study to identify age-related decline. Cambridge University Press,* Comparat Exerc Physiol *6(4):177–181, 2010.)*

$\dot{V}O_{2max}$ was achieved during an incremental exercise test compared with their younger mature counterparts. Although submaximal oxygen consumption appeared to be similar in young and old horses, the workload needed to reach $\dot{V}O_{2max}$ was lower in older horses (McKeever and Malinowski, 1997). These observations were similar to well-documented, aging-induced decreases seen in humans (Dempsey and Seals, 1995; Holloszy and Kohrt, 1995; Raven and Mitchell, 1980; Stamford, 1988).

Interestingly, when compared with their human counterparts (60 to 80 years of age), the older mare (>20 years) has a tremendous innate aerobic capacity (McKeever and Malinowski, 1997). For example, $\dot{V}O_{2max}$ in moderately fit, healthy, postmenopausal women averages 22 mL/kg/min (Holloszy and Kohrt, 1995; Raven and Mitchell, 1980), whereas, young, elite, female athletes typically have capacities ranging from 60 to 80 mL/kg/min (Haskell and Phillips, 1995). Although the average $\dot{V}O_{2max}$ of 90 mL/kg/min seen in old mares is well below their younger fit equine counterparts (145–200 mL/kg/min), it is still above the level reported in elite human athletes (McKeever and Malinowski, 1997).

Besides the ability to accomplish greater workloads, a second benefit of a higher $\dot{V}O_{2max}$ is a delay in the need to increase the rate of glycolysis to fuel higher-intensity exercise (McKeever and Malinowski, 1997). Younger horses must work harder to reach their "anaerobic threshold." This is the point where one observes the onset of blood lactate accumulation (OBLA) conventionally marked by a blood lactate concentration of 4 millimoles per liter (mmol/L) (McKeever and Malinowski, 1997). At this point, there is a curvilinear increase in blood lactate concentration, indicating that lactate production by the working muscles has exceeded lactate utilization throughout the rest of the body (McKeever and Malinowski, 1997). This variable is important, as the velocity to produce a blood lactate concentration of 4 mmol/L (V_{LA4}) coincides with changes in several important physiologic processes. Older horses appear to reach V_{LA4} at both a lower speed and at a lower percentage of their $\dot{V}O_{2max}$, which suggests a possible central limitation on the ability to perform work (McKeever and Malinowski, 1997). However, the older mares were also not able to run as long or as hard (V_{max}) before reaching fatigue, which suggests a reduction in factors affecting the peripheral mechanisms associated with general exercise tolerance.

The decline in aerobic capacity in older horses is accompanied by a decline in their capacity to tolerate high-intensity ("anaerobic") work as well, resulting in an overall decline in general working capacity. As with older humans, aging equine athletes (>20 years) have the ability to continue to perform. However, given age-associated changes in the cardiovascular system, training protocols employed for younger animals may not be appropriate for the older equine athlete.

AGE-RELATED CHANGES IN THERMOREGULATION AND FLUID AND ELECTROLYTE BALANCE

The athletic (work) capacity of the horse can be considered elite among mammalian species. However, this capacity is, in part, governed by the animal's ability to dissipate the body heat generated during exercise. Unfortunately, in the horse, this ability is limited because of a relatively small ratio of surface area to mass (see Chapter 8). During high work intensities, the rate of heat production of the horse can exceed basal levels by 40-fold to 60-fold. If the excess metabolic heat generated during exercise is not dissipated, life-threatening elevations in body temperature may develop, and the horse's athletic performance will be adversely affected (see Chapter 8). The adverse effects of hyperthermia on the health and performance of the horse can develop during all exercise intensities and weather conditions. Failure to dissipate metabolic heat can cause a continuous and excessive rise in internal (core) body temperature (Geor et al.,, 1995). If heat loss mechanisms are impaired by aging, then the only way that a horse would be able to decrease its body temperature is to decrease the rate of heat gain by decreasing the intensity of the exercise (work).

Studies comparing the thermoregulatory responses of older and younger men and women during exercise in the heat have shown that age influences thermoregulatory function (Armstrong and Kenney, 1993; Kenney, 1995; Kenney and Zappe, 1994). Suggested reasons for this age-related decline in humans in their ability to efficiently thermoregulate include lower cardiovascular capacity due to the age-related decrease in cardiac output, alterations in the mechanisms associated with the control of skin blood flow, and a possible state of hypohydration in older adults (Kenney, 1995; Kenney and Zappe, 1994). Data on the role of each of these factors alone or in combination in exercising older humans are mixed. Human studies have found lower SVs and cardiac outputs in older men compared with younger men, during upright exercise. These differences are also present when skin venous

pooling is augmented by the imposition of an additional heat stress (Kenney, 1995; Kenney and Zappe, 1994). In studies of older men and women, it has been demonstrated that older individuals have lower cardiac outputs compared with younger subjects, even though they were exercising at the same absolute low-intensity workload (Kenney, 1995; Kenney and Zappe, 1994; Seals et al, 1984).

Data on thermoregulation in young horses are plentiful (Geor et al, 1995; Nieman, 1995). However, only a few studies have addressed the effects of age on the thermoregulatory response to exercise in the horse. Exercise is a costly endeavor in terms of energy. The generation of adenosine triphosphate (ATP) fuels work but also generates a great deal of heat that must be dissipated. If two horses are the same weight and have similar mechanical efficiencies, then the metabolic cost for any given submaximal activity should be similar, as should the amount of heat that must be dissipated. This assumption was tested by McKeever and coworkers (2010) in young horses versus old horses. Both age groups were exercised at the same submaximal absolute work intensity of 1625 watts until they reached a core temperature of 40°C. The older horses in the study reached this core temperature in almost half the time required by the younger horses, and their HRs were substantially greater. However, both groups had similar HRs and core temperatures by 10 minutes after exercise. These results suggest that during submaximal exercise, the older mares in this study were not able to thermoregulate as effectively as the younger mares could. The greater HR seen in the older mares suggests that they required a greater cardiac output to accommodate the combined blood flow demand of the organs, working muscles, and skin (thermoregulation) (McKeever et al., 2010). Even at a higher cardiac output, the older horses were still unable to dissipate the excess heat during exercise as quickly as their younger counterparts were able to.

Human studies may shed light on the mechanisms causing the impairment of thermoregulation occurring with age. During exercise, the delivery of oxygen to active muscle involves a local decrease in vascular resistance, which, in turn, creates a challenge to blood flow delivery that is met by both increases in cardiac output and adjustments in vascular resistance in nonactive tissues. Increasing cardiac output during exercise helps meet the dual demand for increased blood flow to the working muscle and to skin, especially when coupled with the redistribution of blood flow from visceral organs to augment the perfusion of skin and active muscle vascular beds. In aged horses and humans, cardiac output is limited, and thus, there is insufficient blood flow to support both the metabolic demand of the working muscles and the thermoregulation demand of skin. This leads to a compromised ability to dissipate heat and defense against hyperthermia (Kenney, 1995; Kenney and Zappe, 1994).

Age-related alterations in fluid and electrolyte balance can also impair thermoregulatory capacity in older humans, who commonly have lower total body water, plasma volume, and reserves of fluid for sweating. Data, however, as to whether older humans are chronically hypohydrated are mixed (Kenney, 1995; Kenney and Zappe, 1994). Interestingly, the same markers of fluid status in young and old horses show shifts in similar relative magnitude (McKeever et al, 2010). However, older horses have a substantially lower pre-exercise plasma volume compared with younger animals (McKeever and Kearns, 2001; McKeever et al., 2010) (Figure 14-2). Thus, while the relative reduction in plasma volume during exercise was similar, the older horses started off with a significantly lower absolute plasma volume. This, of itself, would be cause for a lower venous return, SV, and cardiac output, leading to a compromise of thermoregulatory stability. In younger humans

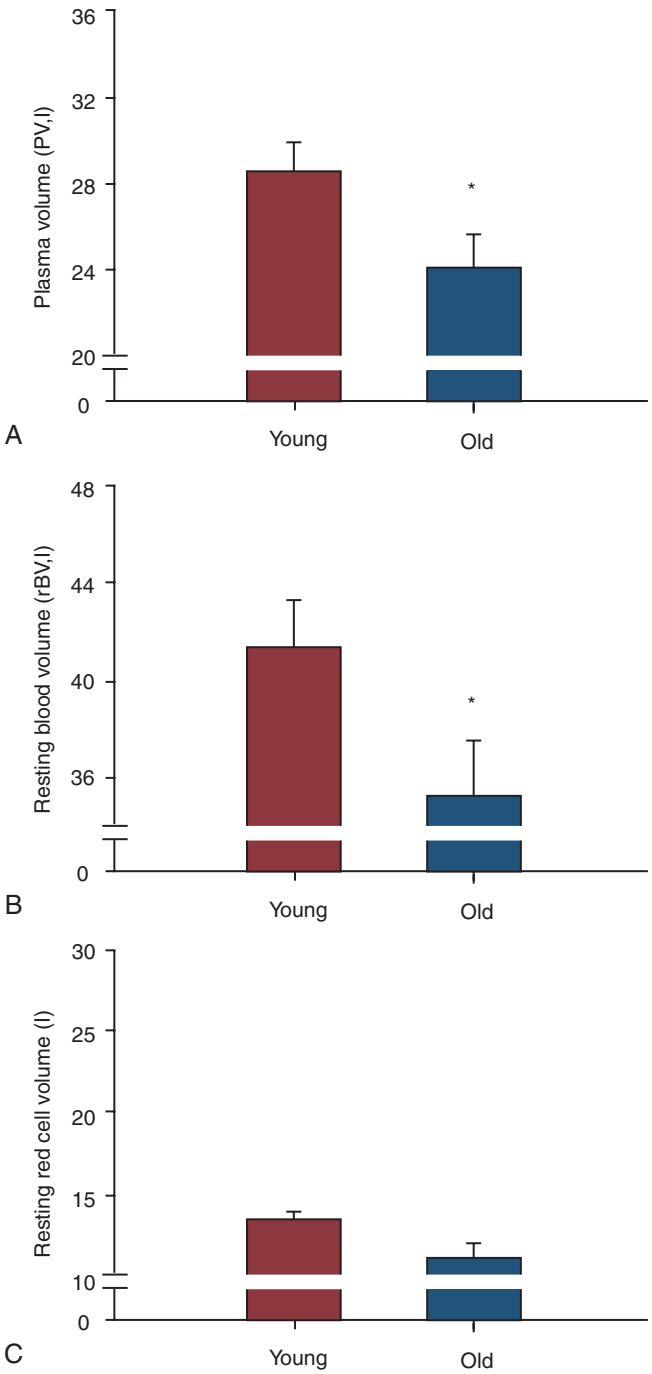

FIGURE 14-2 Mean (± SE) plasma volume (PV), resting blood volume (rBV), and resting red cell volume (liters) in young and old mares. Asterisk (*) indicates a difference ($p < 0.05$) between the means for young and old horses. *(From McKeever KH, Eaton TL, Geiser S, et al: Aging related decreases in thermoregulation and cardiovascular function,* Equine Vet *J 42(S38):220, 2010.)*

and horses, the demands of repeated acute exercise result in a substantial expansion of plasma volume. In the horse, this increase in total body water is mediated by aldosterone-induced retention of sodium and water by the kidneys (McKeever et al., 2002). As with humans, the ability to reverse the apparent inadequate fluid reserve in response to training appears to be impaired in older horses. The mechanism behind this age-induced lack of a hypervolemic response to training is not known; however, it could be due to impairment of neuroendocrine mechanisms associated with a change in cardiopulmonary baroreceptor sensitivity.

Skin blood flow was not measured in the above-mentioned horse studies; however, studies of humans have demonstrated that aging impairs the skin blood flow response to exercise (Ho et al., 1997; Kenney, 1995; Kenney and Zappe, 1994). The paradoxical inability to keep cool despite an increase in the sweat rate in older horses is consistent with an impairment of skin blood flow as observed in humans. Although it is pure speculation, the mechanism for an age-related decline in skin blood flow during exercise may involve alterations in the sensitivity of mechanisms affecting vascular tone.

Recognition of the fact that older horses have a decreased ability to thermoregulate during exercise should lead to closer monitoring for heat stress during equine athletic activities. The increased susceptibility of older horses to overheating should cue veterinarians, owners, and riders to identify the older horse as being more likely than others to become symptomatic of hyperthermia (heat stress) during exercise.

EFFECTS OF AGE ON BODY COMPOSITION AND MUSCLE FIBER TYPE

The older horse also undergoes significant changes in body composition, with some older horses exhibiting an obese phenotype and some having a thin "old horse" appearance. Importantly, the thin old horses have a smaller muscle mass, a pattern that is also similar in aged humans, whereas the obese old horses tend to have 50% more fat mass compared with their younger counterparts (Lehnhard et al., 2001; Lehnhard et al., 2004). However, as in the case of other organ systems, the question remains as to whether this is caused by inactivity or aging. Interestingly, old horses, like old humans, undergo a change in muscle fiber type distribution away from a more aerobic profile that fits with their less active lifestyle and decline in aerobic capacity (Lehnhard et al., 2001; Lehnhard et al., 2004).

Another measure that may have an important bearing on the ability to perform exercise is a horse's body composition and, more importantly, total fat-free mass (FFM) (Kearns et al., 2002a; 2002b; 2002c). Older humans exhibit substantial decreases in muscle mass and, in many cases, increases in fat mass that affect the ability to perform exercise (Forbes and Reina, 1970; Kohrt et al., 1993; Kohrt et al., 1992; Stamford, 1988). Data from recent work in horses suggest that they may undergo similar aging-induced changes. Lehnhard and colleagues (2001; 2004) found that old horses could be divided into two groups by appearance. Some horses were either very lean or very fat. The thin old horses (Figure 14-3) had significantly smaller rump fat thickness, lower percent body fat, and less fat weight compared with the fat old horses (Figure 14-4) as well as the young horses (Lehnhard et al., 2001; Lehnhard et al., 2004). The thin old horses had significantly less body weight compared with the fat old horses but not the young horses. They also had greater lean body weight compared with the young horses, but there was no significant difference in this parameter when compared with the old fat horses (Lehnhard et al., 2001; Lehnhard et al., 2004). In turn, the old fat group had a significantly larger rump fat thickness, percent body fat, and fat weight compared with the young horses (Lehnhard et al., 2001; Lehnhard et al., 2004; Malinowski et al., 2002). These data suggest that as mature horses gain weight, they gain fat mass at a rate faster than FFM. This is indicated by an increase % fat per given body weight.

The larger fat mass seen in some older horses may be detrimental to performance and may lead to other complications in older horses such as insulin resistance (Malinowski et al., 2002). The observed morphometric differences may have resulted from some of the same factors at play in aged humans (Forbes and Reina, 1970; Kohrt et al., 1992; Kohrt et al., 1993; Stamford, 1988). For instance, aged horses exhibit similar clinical metabolic and endocrine disorders as those seen

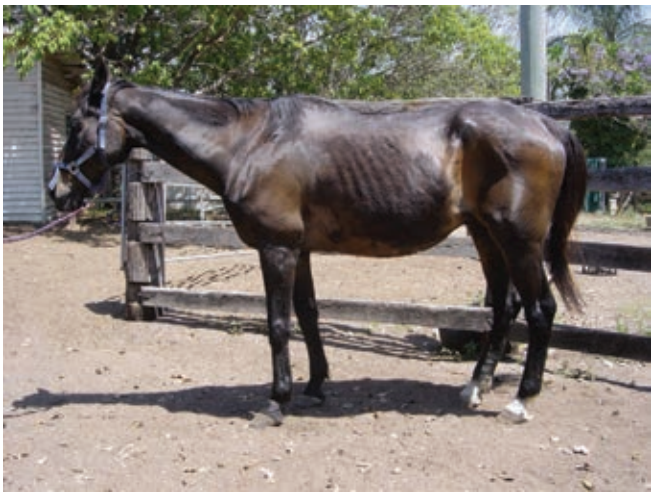

FIGURE 14-3 Example of a thin old horse demonstrating reduction in fat-free body mass

FIGURE 14-4 Example of a fat old horse where percentage of fat is greater than fat free body mass.

in aged humans, including hyperinsulinemia and insulin resistance, thyroid adenomas, Cushing's syndrome or pituitary pras intermedia dysfunction (PPID), decreased somatotropin concentrations, and so on (Malinowski et al., 2002; McKeever, 2002). Although old horses appear to retain muscle mass, the question is: Is that functional muscle mass? Many studies have documented muscle fiber type, enzymatic activity, and substrate storage and utilization patterns in the horse (see Chapter 12). However, only a few studies have examined the peripheral changes associated with aging in the horse, and even those studies have focused only on changes in fiber type associated with aging (Lehnhard et al., 2001; Lehnhard et al., 2004; Rivero et al., 1993). It appears that only two studies have attempted to present data grouped by age (Lehnhard et al., 2001; Lehnhard et al., 2004; Rivero et al., 1993). Rivero (1993) examined muscle fiber type distribution in a number of horses; however, the mean age of the oldest age group in that study was only 15 years, an age at which many horses are still in their prime athletically and physiologically, analogous to >40-year-old humans (Rivero et al, 1993). A more recent study that compared young and very old horses, however, found that older horses had fewer type I and IIA fibers compared with young mares (Lehnhard et al., 2001; Lehnhard et al., 2004). Old horses had more type IIX fibers compared with young horses (Lehnhard et al, 2001; Lehnhard et al, 2004). On a functional level, this suggests that the older horse undergoes a switch in fiber type population away from that which would be favorable to endurance exercise. This may, in part, explain the decrease in $\dot{V}O_{2max}$ documented in other studies (McKeever and Malinowski, 1997).

On a cellular level, aging appears to alter muscle structure and function (Andersen et al., 1999; Coggan et al., 1992; Coggan et al., 1993; Davies et al., 1988; Geor et al., 1995; Lehnhard et al., 2004; Roneus and Lindholm, 1991). Studies of other species (rat and human) demonstrate that connective tissue content in the muscle is altered with evidence of significant amounts of collagen, changes that would tend to interfere with normal contractile function (Cartee, 1994; Cartee and Farrar, 1988; Stamford, 1988). Other studies have reported changes in skeletal muscle blood capillarity and decreases in blood flow in older men (Holloszy and Kohrt, 1995). Oxidative capacity, glucose utilization, glucose transporter type 4 (GLUT-4) activity, insulin insensitivity, insulin-regulatable glucose transporter, and glycogen depletion–repletion patterns in skeletal muscle appear to be altered by age in rats and humans (Cartee, 1994; Cartee and Farrar, 1988; Coggan et al., 1992; Davies et al., 1988; Holloszy and Kohrt, 1995; Tankersley et al., 1991). Unfortunately, no data that have examined the effect of exercise on muscle enzyme concentrations or glycogen depletion and repletion patterns in the old horse have been published.

AGING-RELATED ALTERATIONS IN THE ENDOCRINE RESPONSE TO EXERCISE

Exercise involves the integration of multiple organ systems which communicate via neural and endocrine pathways. Humans undergo substantial alterations in neural control mechanisms, with primary alterations in sympathetic nervous system responsiveness (Holloszy and Kohrt, 1995; Kohrt et al., 1993; Lonnqvist et al., 1990; Seals, 1993). No such information exists for the horse. Aging also alters the endocrine response to exercise with reported changes in hormones associated with the control of cardiovascular function, stress hormones, and endocrine or paracrine factors related to the control of metabolic function and substrate utilization (Holloszy and Kohrt, 1995; Lonnqvist et al., 1990; Seals, 1993).

A recent study reported on the effect of aging on four of the hormones related to the control of cardiovascular function in the horse. McKeever and Malinowski (1999) reported similar resting concentrations of the various hormones involved in the control of cardiovascular and renal function, including atrial natriuretic peptide (ANP), arginine vasopressin (AVP), plasma renin activity (PRA), aldosterone (ALDO), and endothelin-1 (ET-1) in healthy old and young horses. Similar observations have been reported for normal healthy humans and other species (Kenney, 1995; Kenney and Zappe, 1994; Wade et al., 1989). Old and young horses had directionally similar exercise-induced alterations in PRA, ANP, AVP, and ALDO. However, the major finding of their investigation was an aging-related change in the magnitude of the response to exertion (McKeever and Malinowski, 1999). Although old horses had different concentrations of these hormones, the observed concentrations were still within the range of normal for maximally exercised horses and other species (Kenney, 1995; Kenney and Zappe, 1994; Reaven, 1995; Wade et al., 1989). Interestingly, plasma concentrations of the vasoconstrictor ET-1 were not affected by exercise in either group of horses, a phenomenon previously reported in young horses and humans (McKeever, 2002). Age-related differences in PRA and the plasma concentrations of ANP, AVP, and ALDO may reflect differences in sensitivity in the regulation of blood pressure and blood flow during exertion. Younger horses were observed to have greater plasma concentrations of the vasodilator ANP. Functionally, this ANP-induced vasodilation would aid in the redistribution of blood flow during exercise (Reaven, 1995; Wade et al., 1989). ANP also inhibits PRA and the production and release of antagonistic hormones such as AVP and ALDO (Reaven, 1995; Wade et al., 1989). Thus, greater concentrations of plasma ANP in younger animals may enhance the ability to optimally vasodilate blood vessels in the periphery, especially in the working muscles.

McKeever and Malinowski (1999) also reported that older horses had greater PRA at speeds eliciting $\dot{V}O_{2max}$. This change may be physiologically important as part of the defense of blood pressure during exercise and the defense of fluid and electrolyte balance after exercise. Increases in circulating plasma angiotensin I and II aid in the vasoconstriction in nonobligate tissues that is part of the redistribution of blood flow. Furthermore, increases in PRA and angiotensin also act to stimulate thirst and cause an increase in the synthesis and release of aldosterone. Mediation of this neuroendocrine defense of blood pressure involves the integrative actions of both the low and high pressure baroreceptors (Reaven, 1995; Wade et al., 1989). However, aging appears to alter baroreceptor sensitivity and the normal feedback loop that integrates cardiovascular control (Seals, 1993). One might speculate then that reported differences in ANP and PRA may reflect an age-related difference in baroreceptor sensitivity to the challenge of exercise. It may also reflect differences in autonomic control and input from the sympathetic nervous system and other stimuli that affect the control of central and peripheral cardiovascular function. This may be important as increases in renal sympathetic nerve activity and

the stimulation of the juxtaglomerular apparatus is the primary stimulus for renin release during exertion. A recent study of humans suggests that aging-induced changes in PRA may also affect renal function and may indirectly contribute to possible alterations in skin blood flow (Kenney, 1995; Kenney and Zappe, 1994). The later speculated aging-induced change could be important as it potentially could alter the ability to thermoregulate in humans. It would also be important to the horses as well as they are the only other athletic species that thermoregulates principally by sweating (Reaven, 1995). Any age-related difference in blood volume, HR_{max}, and vascular tone may have also influenced the integrative neuroendocrine defense of blood pressure.

Surprisingly, younger horses appear to have a greater vasopressin response during exercise. Plasma AVP concentration increases when the cardiopulmonary baroreceptors sense that cardiac filling pressure is inadequate, when the high pressure baroreceptors sense that mean arterial blood pressure is too low, when hypothalamic osmoreceptors sense that plasma osmolality is too high, or both. Vasopressin causes vasoconstriction in nonobligate tissues during exercise. It also facilitates the uptake of water and electrolytes from the large intestine, another important action during exercise. Postexercise AVP causes retention of solute-free water by the kidney and stimulates thirst and drinking (Reaven, 1995; Wade et al., 1989). Studies are needed to explore the role of aging in these later functions, as some studies have suggested that older humans do not drink as much and often are hypohydrated (Kenney, 1995; Kenney and Zappe, 1994). Suppression of thirst and drinking is an important consideration for those concerned with postevent care of the horse.

It is well recognized that older horses have lower plasma concentrations of the thyroid hormones, somatotropin (ST), and insulin-like growth factor-I (IGF-I) compared with young animals, which suggests an age-related decline in the somatotropic axis in horses similar to that observed in other mammalian species (Malinowski et al., 1996). This may be linked to aging-induced changes, including decreases in cardiopulmonary function, decreased aerobic and exercise capacity, decreased immune function, impaired nutrient utilization, decreased nitrogen retention, and decreased lean body mass (Malinowski et al., 1996). Similar changes have been observed in other species, and comparative physiologic data from studies of rats, dogs, and humans (Haskell and Phillips, 1995; Holloszy and Kohrt, 1995; Stamford, 1988) have shown that there is a causal relationship between plasma concentrations of somatotropin and what has been termed the "aging phenotype."

Endorphins and cortisol have been utilized as markers of the degree of physiologic stress during exercise (Malinowski et al., 2006). The release of these two hormones is a normal response to exercise; however, the direction and the magnitude of their response distinguish what can be considered a normal response to the physiologic challenge of exertion and a true stress response. Increases in these hormones are linked to exercise duration and intensity, and their release may provide protection from the physiologic challenge of exertion (Malinowski et al., 2006; Mehl et al., 2000). β-endorphin functions as a natural opiate, forestalling the central mechanisms that would induce fatigue. Cortisol functions as a metabolic hormone during exercise and influences glucose metabolism. After exercise, cortisol exerts anti-inflammatory and immunosuppressive activity, possibly aiding in the repair of tissue altered by exertion and protecting against the inflammation associated with overexertion (Malinowski et al., 2006; Mehl et al., 2000).

Only one study seems to have attempted to determine if training and age affect the plasma β-endorphin, cortisol, and immune function (see below) responses to acute exercise in unfit Standardbred mares (Malinowski et al., 2006). Unfortunately, β-endorphin and cortisol were measured at rest and at 5, 10, 20, 40, 60, and 120 minutes following an incremental exercise test (GXT) but not during exercise. The authors reported that cortisol rose by 5 minutes following GXT in young and middle-aged mares before and after training. However, there was no rise in cortisol in the old mares following GXT either before or after training. Before training plasma β-endorphin increased by 5 minutes after exercise in all mares. After training, β-endorphin was higher compared with pretraining levels in all three age groups; however, the peak in the old mares occurred later than in the other groups (Malinowski et al., 2006).

Aging substantially alters metabolic function in humans (Cartee, 1994; Reaven, 1995; Seals, 1993) and is frequently associated with glucose intolerance and insulin resistance in both humans and horses (Ralston, 1992; Reaven, 1995). Participation in regular exercise activity may elicit a number of favorable responses that contribute to healthy aging (Reaven, 1995). Information on the effects of aging and training on the glucose and insulin response following acute exertion in horses is limited (Malinowski et al., 2006; Liburt et al., 2012). The primary findings of those studies indicated that unfit old horses require greater concentrations of insulin to successfully manage their response to an oral glucose tolerance test (OGTT). The authors also reported that there was an age-related effect on the glucose and insulin responses to acute exercise both prior to and after 12 weeks of exercise training (Malinowski et al., 2006; Liburt et al., 2012). Interestingly, exercise training resulted in a post-GXT increase in insulin sensitivity (Malinowski et al., 2006) in older animals, and this may have improved the ability to replenish muscle glycogen (Malinowski et al., 2006; Liburt et al., 2012). More recent studies have demonstrated that exercise training improves insulin sensitivity and glucose homeostasis in both old and young horses (Liburt et al., 2012). Changes in the hypothalamo-pituitary-adrenal axis (HPAA) and the glucose–insulin response to acute exertion were interpreted to be related to an improvement in insulin sensitivity in the older animals. The exact mechanisms to explain the age-related difference in glucose and insulin metabolism are unknown, but published papers from studies of rats and humans suggest that it may be related to differences in fuel utilization, mitochondria respiration rates, and the skeletal muscle content of GLUT-4 transporters (Cartee, 1994; Cartee and Farrar, 1988; Coggan et al., 1993; Coggan et al., 1992).

Endocrine-based research has demonstrated that insulin-like growth factor (IGF-I) concentrations decline with age in the horse (Malinowski et al., 1996). Aging also appears to alter metabolic control, immune function, and endocrine function in horses, both at rest and following exercise (Betros et al., 2002; Horohov et al., 1999; Lehnhard et al., 2001; Lehnhard et al., 2004; Malinowski et al., 2002; McKeever, 2002; McKeever and Kearns, 2001; McKeever and Malinowski, 1997; McKeever and Malinowski, 1999; McKeever et al, 1998; McKeever et al., 2000; McKeever et al., 2002).

ALTERATIONS IN THE IMMUNE RESPONSE TO EXERCISE

Studies of humans have demonstrated that aging alters the immune response in general and, more importantly, the immune response to the challenge of exercise (Fiatarone et al., 1989; Nieman, 1995). Two studies have reported on the effect of aging on the immune response to acute exertion in horses (Horohov et al., 1999; Malinowski et al., 2006). Horohov and coworkers (1999) reported that there were differences in the immune system of young and old horses both before and after exertion. Interestingly, in the study, acute exercise caused a decrease in the lymphoproliferation response in the younger horses but not the old mares (Horohov et al., 1999). Old horses also exhibited a lower proliferative response to mitogens suggesting an aging-related alteration in T-cell mediated function and an immunosenescence that the authors suggested may have been related to a lower cortisol response to exertion in the old horses (Horohov et al., 1999). A more recent experiment examined the effects of aging and training by measuring immune responses to a graded exercise test performed before and after 12 weeks of training (Malinowski et al., 2006). The older horses had lower monocyte counts following GXT after training (Malinowski et al., 2006). Age also affected the lymphocyte response to acute exercise before and after training, suggesting a degree of immunosenescence (Malinowski et al., 2006). Together, these studies suggest that special preventive care may be needed for the older athletic horse.

RENAL, GASTROINTESTINAL, AND OTHER SYSTEMS

Research on humans has suggested that many of the observed changes in renal function seen with aging are the combined effect of pathologies coupled with the aging process. Only limited data on the effect of aging on the renal response to acute exercise in humans seem to be available. Those studies have primarily focused on the effect of aging on the normal reduction in blood flow seen with acute exertion. Functionally, older individuals have smaller reductions in renal blood flow and smaller increases in skin blood flow compared with younger individuals (Farquhar and Kenney, 1999; Kenney, 1995; Kenney and Zappe, 1994). This may alter renal function as well as thermoregulatory capacity because the redistribution of blood flow away from nonobligate tissues toward the working muscles and skin are important responses to exertion. No work has been performed in the horse. More work is also needed to determine if aging alters mechanisms affecting the glomerular filtration rate and tubular function both during and after exercise. The latter may be significant, as long-term control of total body water, plasma volume, and fluid and electrolyte balance appear to be altered in humans and horses.

In other species, the gastrointestinal tract, bones and ligaments as well as the integumentary system are altered with age (Holloszy and Kohrt, 1995; Holloszy, 1993). Changes in the gastrointestinal tract—from wear down of teeth to decreased absorptive capability—influence the uptake of water and nutrients and have the potential to alter the ability to perform exercise. Bone pathology, ranging from osteoarthritis to demineralization, certainly can alter the ability of old horses to perform exercise. Changes in skin have the potential to alter sweating and thermoregulation. No data on the effects of aging on these organ systems in the horse seem to be available.

CONCLUSION

Surveys indicate that in the United States, the equine population over 20 years of age is growing larger (Hintz, 1995; McKeever, 2002; Rich, 1989). As with humans, these geriatric equine athletes have the ability to continue to perform in athletic events. Unfortunately, many horse owners continue to train their active older animals using exercise training protocols that, although appropriate for a younger animal, may not be appropriate for the older equine athlete. Studies of older humans have led to a fine-tuning of exercise prescription for the older human athlete to prevent the adverse and potentially dangerous effects of excessive work. Published results have led to new and improved programs to promote fitness for the growing population of older adult humans. Future studies of the effects of aging on exercise capacity in equine athletes should have similar goals.

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CHAPTER 15

Conformation

BRONWYN GREGORY

Conformation can be defined as the "formation of something by appropriate arrangement of parts or elements: an assembling into a whole" (Webster's dictionary, 1976) and equine conformation appraisal is traditionally based on the external appearance of the body shape, form or outline of the animal. This evaluation may be regarded as the front line for judgments when selecting horses for specific intended tasks, including breeding selection. Prepurchase recommendations and perceived animal value rest highly on this assessment. There is wide variation of conformation between and within different breeds, the significance of which requires expert understanding of optimal breed characteristics and potential effects on soundness or performance. The success of a horse in any equine discipline or industry is not dependent on "perfect" conformation, as this does not guarantee performance or soundness, and "imperfect" conformation does not necessarily exclude a horse from performing at elite levels. Other factors such as human management, environmental conditions, genetics, nutrition, temperament, training, and the health status of the horse will also have a large bearing on ultimate performance. Conformation can, therefore, only be considered an indicator for future athletic potential. Nonetheless, conformation can assist prediction of possible musculoskeletal strengths and weaknesses, possible predisposition to injury, or both, based on known etiology and pathophysiology of musculoskeletal disorders.

Despite considerable anecdotal information, there is still a considerable lack of evidence-based quantification of conformation assessment and the relationships among conformation, performance, and orthopedic health. Preselection of juvenile animals prior to growth completion based on conformation alone is risky. Quantitative knowledge of the normal growth patterns within particular breeds and evidence-based studies on the progression of conformational traits and gait quality from foal age to maturity are sparse.

The conformation or inherent anatomic structure of the horse is an integral part of the equine musculoskeletal constitution and will influence the quality of dynamic performance. The skeletal format will affect such factors as joint range of motion, limb arc and hoof flight patterns, and weight distribution in motion, with subsequent effects on coordination of movement (including limb interference), balance, power (propulsion, impulsion, and collection), agility, and endurance. Conformation will, therefore, partially dictate the relationship between form and function, thus modifying the potential for biomechanical efficiency, superior performance, musculoskeletal durability, and perhaps even longevity (Wallin et al., 2001). As some conformational traits are dynamic and will only be apparent during ambulation, the traditional emphasis of conformational assessment as a pure description of static external appearance has been extended to include a more functional assessment of conformation during unridden and ridden gaits in some of the studies cited in this chapter.

There is a great need to clarify and standardize the descriptive terminology of joint alignments, as most conformational traits are described using multiple traditional and variable nonscientific terms, rather than by defining anatomic configuration. For example, a caudal deviation at the radiocarpal or metacarpal joint complex (knee) may be described as "back at the knee," "calf knee," or "carpal hyperextension," none of which describes the precise origin of segmental misalignment. Biomechanical evaluation relies heavily on strict physical and mechanical relationships of segments, requiring accurate anatomic terminology. Yet, most studies have employed generalized or horsemanship terms in describing conformational traits. The lack of anatomic precision, documentation, or both limits the interpretation of some studies. The literature presented in this chapter will follow the terminology appearing in the research papers. Some common terms describing conformational alignments are defined anatomically in Table 15-1 and illustrated in Figure 15-1 and Figure 15-2.

ASSESSMENT OF CONFORMATION

All assessment of equine conformation should be conducted with the horse standing squarely (loading all limbs symmetrically) on a level surface. The stance of the horse has been identified as a major source of error in conformation assessment, as small changes in limb placement and weight distribution can introduce significant variation in segmental alignment. When assessing deviation of the limb from the vertical, Weller et al. (2006a) found measurement variations in stance within one horse to be almost as large as between horses, thus

TABLE 15–1

Anatomic Description of Commonly Used Conformational Terms

Common Term	Anatomic Description
Base narrow	Distance between the forelimbs is greater at the chest than feet, the limb sloping medially
Back at the knee/calf knee	Carpal hyperextension due to a caudal displacement of the proximal row of carpal bones, the radiocarpal joint being <180 degrees (Ross, 2003). An upright pastern is often also related to this conformation (Ducro et al., 2009a)
Forward at the knee/bucked knee/over at the knee/sprung knee	Radiocarpal joint angle >180 degrees or lack of full carpal extension causing a flexion moment
Offset knee/bench knee	Traditionally described as the metacarpus laterally deviated relative to the carpus; however, the displacement is usually in the radiocarpal joint (Ross, 2003)
In at the knee/knock knee	Carpal valgus
Tied in below the knee	Distinct notch distal to the accessory carpal bone on the palmar aspect of the limb causing the circumference of the leg below the carpus to be less than that above the metacarpophalangeal joint (fetlock)
Upright pastern	Metacarpophalangeal and proximal interphalangeal (pastern) joints have a straight appearance
Toed out feet	Metacarpophalangeal valgus
Toed in feet	Metacarpophalangeal varus
Uneven feet	Forefeet differ in size, shape, or both, causing variable hoof–ground angles
Sickle hock/curby hock	Tibiotarsal (hock) angle 53 degrees or less (Holmstrom et al., 1990)
Straight behind	Tibiotarsal angle >170 degrees (Marks, 2000), usually due to a more upright tibia
Cow hocked/in at the hock	Either a rotational change in the hindlimb or tarsus valgus >180 degrees

highlighting the importance of standardized repeatable positioning of the horse.

Conformation assessment should be a systematic and organized process incorporating a general overall observation of size, symmetry, musculature, posture, balance, and demeanor, followed by a more specific evaluation of conformational traits of the body, individual limbs, and feet. Briefly, relevant body observations should include head shape and size; height at the withers and croup; body length; neck length; shoulder length (top of the withers to point of the shoulder); pelvic length (tuber coxae to tuber ischii); scapular and humeral inclination; pelvic and femoral inclination; and chest width. From these observations, an overall proportioned symmetry in lengths and heights is desirable, both left to right and fore to hind. Congruent sloping angulation of the shoulder and hip is also desirable, with a proportional length of individual limbs in relation to the height and size of the body (Figure 15-3). The segment lengths of specific long bones of limbs should also be noted at this time. Establishing the exact source of the alignment deviation is imperative; for example, does a laterally pointing hoof, commonly described as toed out, originate from an externally rotated limb or from a particular distal joint? Cranial, caudal, and lateral views are needed to determine limb deviations in the sagittal, coronal (frontal), and transverse planes (see Figures 15-1 and 15-2).

When examining the conformational traits of individual limbs, a plumb line approach is useful in identifying angular or torsional deviation of segments from the vertical or horizontal at each joint level (Figure 15-4). In horses with "ideal" conformation, a visualized vertical plumb line dropped from the tuberosity of the scapular spine should bisect the longitudinal axis of the forelimb to the metacarpophalangeal joint (MCPJ or fetlock) and fall 5 cm behind the heel in the lateral view. A line dropped from the cranial aspect of the greater tubercle of the humerus (point of the shoulder) should bisect the forelimb in the cranial view. In the hindlimb, a plumb line dropped from the ischial tuberosity should touch the point of the calcaneous (prominent caudally in the tarsus or hock), follow the plantar metatarsal surface to the metatarsophalangeal joint (MTPJ or fetlock), and fall 7.5 to 10 cm (Ross, 2003) caudal to the heel in the lateral view. The entire hindlimb should be bisected evenly in the caudal view (see Figure 15-4). When assessing foal conformation, limbs can also be viewed from above at the shoulder and hip (skyline view).

Particular attention is warranted in evaluation of distal limb alignment, hoof quality, size, and balance due to the concentration of locomotive stresses in this area. Although different breeds will have feet of different shapes and sizes, it is universally and anecdotally desirable to have balanced feet positioned symmetrically under the central limb axis with a straight hoof–pastern axis (the dorsal surface of the hoof wall lies parallel to the dorsal surface of the pastern region) (see Figure 15-3 and Figure 15-5). The constant growth of the hoof creates a dynamic relationship between the digital axis and dorsal hoof wall, which suggests that completely straight hoof–pastern axes cannot exist over time without natural wear or appropriate trimming (Moleman et al., 2006).

After assessment, overall observations can be related to desirable or "benchmark" breed-specific conformational characteristics and judgment made on the horse's suitability to a given career. Notably, the definition and number of traits evaluated, the point scale scoring system of conformational traits, and the image of an ideal phenotype varies greatly

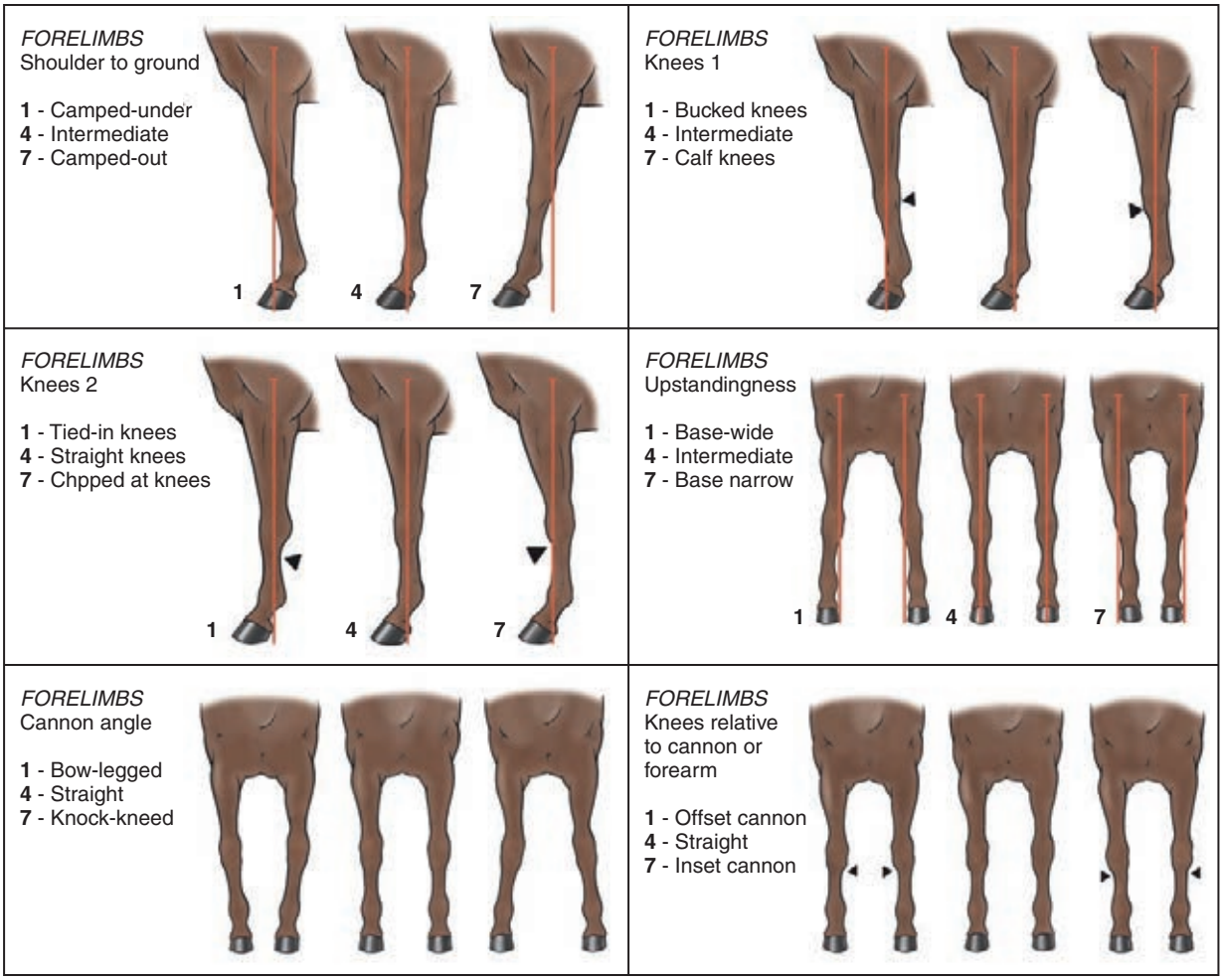

FIGURE 15-1 Illustrations of some common conformational defects of the forelimbs (see Table 15-1 for description). *(From Mawdsley A, Kelly EP, Smith FH, Brophy PO: Linear assessment of the thoroughbred horse: an approach to conformation evaluation,* Equine Vet J *28:461, 1996.)*

among registries, organizations, and countries; therefore, specific classification is essential for comparative evaluations. Selection of a horse in the presence of a less-than-desirable conformation is not always considered unwise. A study on Thoroughbred racehorses highlighted that variation in horses and performance is not fully explained by a few underlying conformational components but is a result of a complex interaction of all conformational parameters (Weller et al., 2006b). However, certain conformational faults such as extreme tarsal angulation (large or small) and tarsal valgus are almost certainly predisposing to injury or lameness in racing events and are best avoided. Veterinarian conformational assessment should particularly focus on the presence of any such faults and the relationship of these faults to existing or potential pathologic conditions (Rossdale and Butterfield, 2006). In many instances, coexisting conformational anomalies will be present, at times allowing biomechanical compensation and at other times exacerbating musculoskeletal stresses during locomotion.

SUBJECTIVE ASSESSMENT OF CONFORMATION

The evaluation of conformation has traditionally been subjective or empirical and remains the primary method of assessment. Visual appraisal of defined criteria (the outlines and axes described above) and manual palpation of specific bony landmarks have been the basis of assessment, giving the examiner multiple three-dimensional images over a period. The combinations of joint configurations and segment lengths are infinite and multifaceted, so the resulting judgment is variable and directly dependent on the individual expertise and personal "ideal" of the practitioner. Magnusson (1985) showed less variance among judges on overall impressions and type traits. However, opinions concerning segment lengths, joint angles, and skeletal inclinations were largely discrepant. This finding was supported by a study comparing radiographic and visual assessments of hoof–pastern conformation in Warmblood foals (Kroekenstoel et al., 2006).Visual assessment was only in agreement with radiologic evidence in 6 of 92 (6.5%) evaluations. Weller et al. (2006c) also suggested that variability in judgment is affected by the limited repeatability of measurement techniques due to inaccurate identification of anatomic landmarks and inconsistent positioning of the subject. Some studies and studbooks have used a system of linear scoring in an attempt to quantify the repeatability of subjective evaluation (Dolvik and Klemetsdal, 1999; Koenen et al., 1995; Mawdsley et al., 1996). This

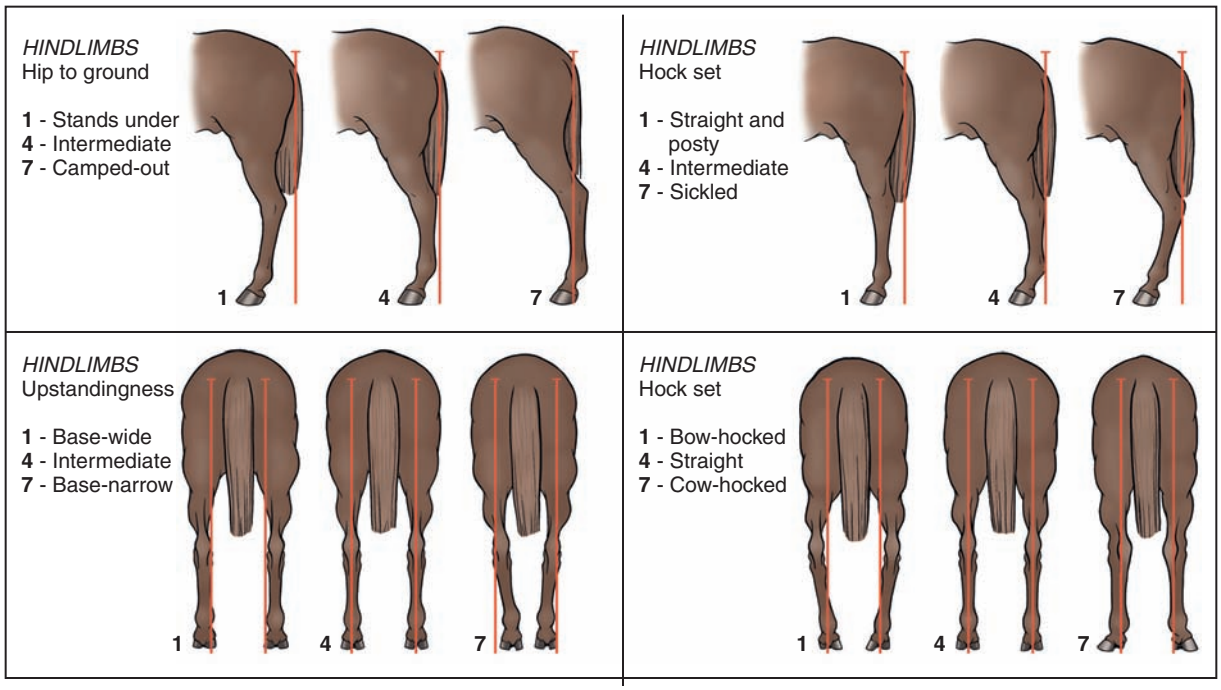

FIGURE 15-2 Illustrations of some common conformational defects of the hindlimbs (see Table 15-1 for description). *(From Mawdsley A, Kelly EP, Smith FH, Brophy PO: Linear assessment of the thoroughbred horse: an approach to conformation evaluation,* Equine Vet J *28:461, 1996.)*

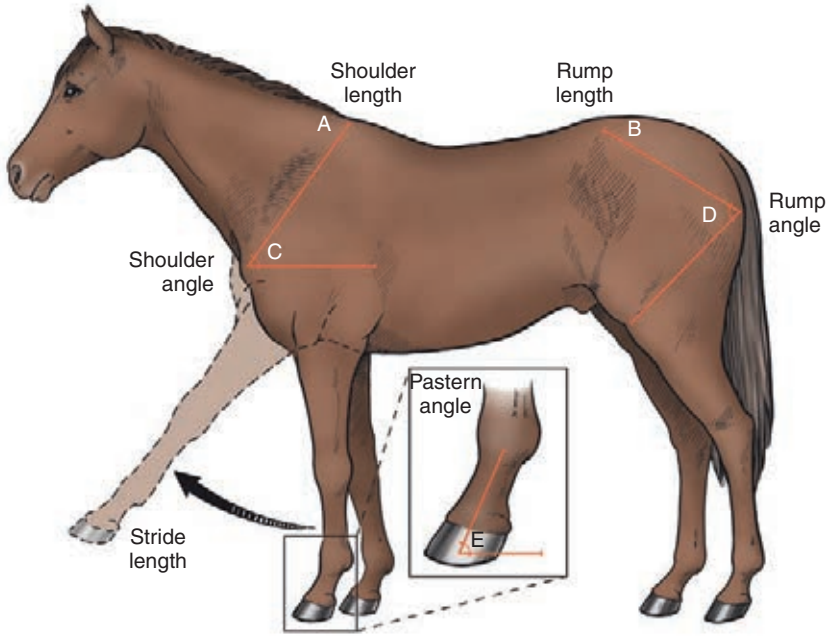

FIGURE 15-3 Measurement of shoulder length (*A*), rump length (*B*), shoulder angle (*C*), and rump angle (*D*). The pastern angle (*E*) should be equal to the shoulder angle.

method of assessment employs a numeric scale to describe defined conformational traits across the entire spectrum of possible configurations, one biologic extreme to the other. Although meeting with some success, 6 of 21 traits were classified unacceptably low in repeatability (Mawdsley et al., 1996). These traits were hoof–pastern axis in both forelimbs and hindlimbs, head size, and vertical alignment of the forelimbs and hindlimbs, all having a coefficient of variation greater than 10%. Despite these limitations, subjective evaluation can be easily and quickly performed by an experienced evaluator, expediting the assessment of large numbers of horses within a short time frame. The absence of standardized evaluation standards, lack of centralized training programs internationally, and a large source of error introduced by

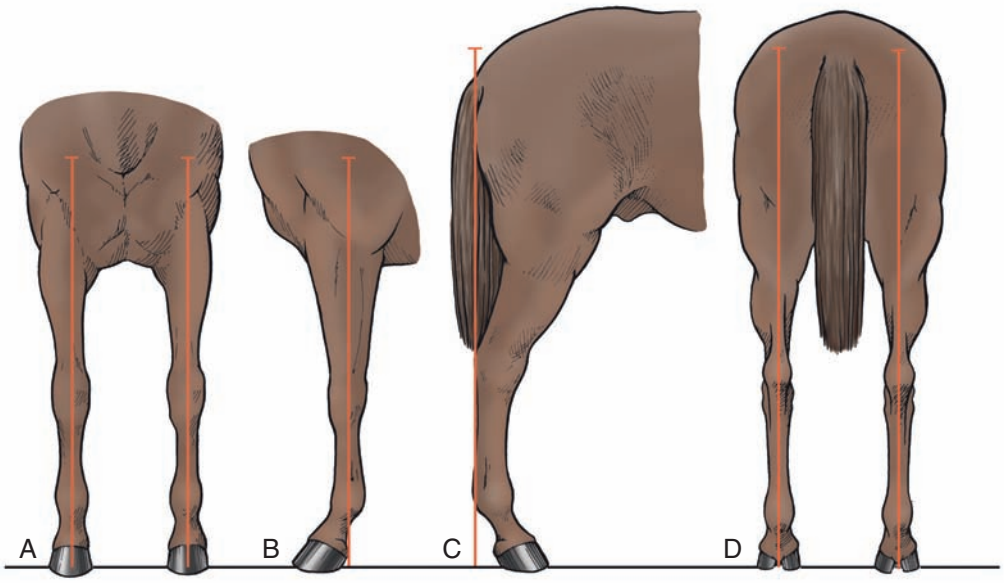

FIGURE 15-4 In horses with "ideal" conformation, a visualized vertical plumb line dropped from the tuberosity of the scapular spine should bisect the longitudinal axis of the forelimb to the metacarpophalangeal joint (fetlock) and fall 5 cm behind the heel in the lateral view. A line dropped from the cranial aspect of the greater tubercle of the humerus (point of the shoulder) should bisect the forelimb in the cranial view. In the hindlimb, a plumb line dropped from the ischial tuberosity should touch the point of the calcaneous (prominent caudally in the tarsus or hock), follow the plantar metatarsal surface to the metatarsophalangeal joint (MTPJ or fetlock) and fall 7.5 to 10 cm caudal to the heel in the lateral view. The entire hindlimb should be bisected evenly in the caudal view. *(From Ross MW: Conformation and lameness. In Ross MW, Dyson SJ, editors:* Diagnosis and management of lameness in the horse, *Philadelphia, PA, 2003, WB Saunders, p 21.)*

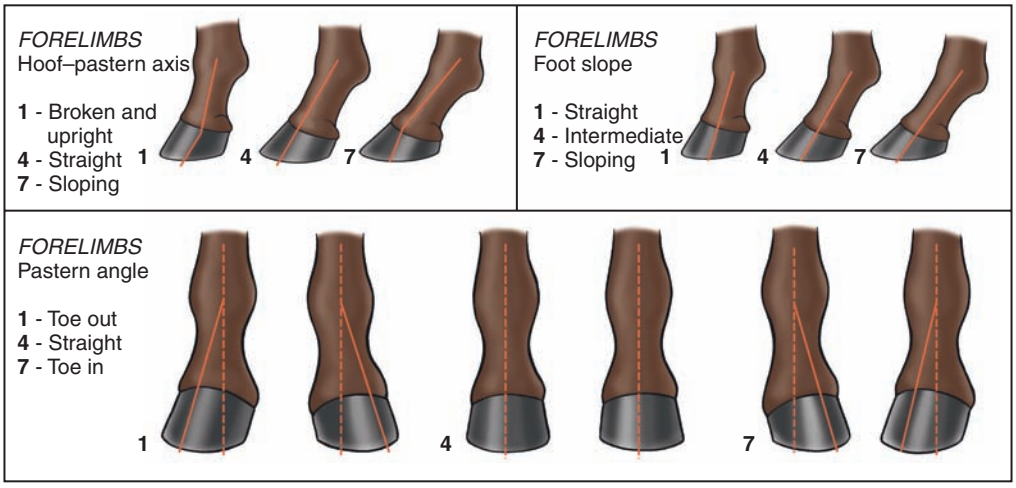

FIGURE 15-5 Illustrations of some common conformational defects of the hooves (see Table 15-1 for description). *(From Mawdsley A, Kelly EP, Smith FH, Brophy PO: Linear assessment of the thoroughbred horse: an approach to conformation evaluation,* Equine Vet J *28:461, 1996).*

subjective assessment precludes sole use of this method to compare results between studies or substantiate the more complex relationships among conformation, performance, and soundness. For these, quantitative conformational assessment, in addition to these traditional judging methods, has been suggested to improve predictive capability (Holmstrom and Philipsson, 1993).

OBJECTIVE ASSESSMENT OF CONFORMATION

Initial attempts to provide absolute values in conformation assessment have used the tools listed in Table 15-2 in combination with a reference marker system. A founding study by Magnussen (1985) described the comprehensive set of landmarks listed below, and many research studies have followed this protocol or a derivative of it.

TABLE 15–2
Tools of Conformation Measurement

Tool	Measurements Taken
Goniometer (see Figure 15-3)	Joint angles Scapular/pelvic inclinations
Tape measure	Height at withers Length of croup and back Width of chest and mandible Circumference of girth; neck at poll and withers (Mawdsley et al., 1996); carpus; the third metacarpal/metatarsal; girth
Box level +/– crossbar	Height at withers, back, and croup Length of head, body, limbs Depth of chest Width of breast and pelvis
Calipers	Width of head and third metacarpal/metatarsal Width of chest and pelvis

Neck and Forelimb

1. Cranial end of the wing of atlas
2. Proximal end of the spine of the scapula
3. Caudal part of the greater tubercle
4. Transition between the proximal and the middle thirds of the lateral collateral ligament of the elbow
5. Lateral tuberosity of the distal end of the radius
6. Space between the fourth carpal, the third metacarpal, and the fourth metacarpal bones
7. Proximal attachment of the lateral collateral ligament of the fetlock joint to the distal end of the third metacarpal bone
8. Dorsal edge of the coronary band

Hindlimb

1. Proximal end of the tuber coxae
2. Center of the anterior part of the greater trochanter of the femur
3. Proximal attachment of the lateral collateral ligament of the stifle joint to the femur
4. Attachment of the long lateral ligament of the tibiotarsal joint to the plantar border of the calcaneus
5. Space between the fourth tarsal, the third metatarsal, and the fourth metatarsal bones
6. Proximal attachment of the lateral collateral ligament of the fetlock joint to the distal end of the third metatarsal bone
7. Dorsal edge of the coronary band

The major disadvantages in using these methods are the possible errors introduced by marker placement on skeletal landmarks, particularly in the proximal skeleton, the consequent reliability of findings, and the time required to perform the measurements (Weller et al., 2006a). Radiography has also been used to measure joint angles and segment lengths. However, this requires expensive equipment, has the health and safety implications of possible radiation exposure to personnel involved, and is very sensitive to subject positioning (Barr, 1994; White et al., 2008).

Advancing technology has allowed more objective, quantitative evaluation of conformation amenable to statistical analysis and aims to find evidence-based relationships among conformation, performance, and soundness. This has resulted in verification of some traditional empirical ideals and refuting of others, though results are often conflicting. For global advancement in this area of study, it is clearly imperative to use universally comparative methodology, which is somewhat lacking. Objective conformational evaluation provides a useful adjunct to subjective assessment by quantification of some conformational traits; however, it must be remembered that not all conformational aspects can be measured objectively. Aesthetic factors such as athletic elegance, suppleness, overall balance and harmony, jumping style, and movement symmetry are necessarily subjectively based.

Photography or Videography and Digital Photography

Photographic analysis employing reference skin markers has been used widely to assess whole animal linear and angular conformation. The advantage of this technique is the ability to assess large numbers of horses quickly. However, several potential sources of error are introduced: distortion of measurements due to the lens angle and distance of camera placement relative to the horse; geometric error when a three-dimensional object is assessed in two dimensions; and limited accuracy of repeatable manual placement and identification of markers. Intra operator repeatability for manual identification of markers on photographs in one study was below 1% for length measurements but was around 10% for segment inclinations (Weller et al., 2006a). The largest interoperator variations were found at the carpus (length) and inclinations related to the tuber sacrale, humerus, and stifle markers. In addition, whole animal assessment requires a minimum of four photographs (left and right lateral, cranial, and caudal) which is difficult to attain in uniform stance without subject movement. When using this technique, the camera should ideally be directed at the center of the horse's thorax, and a reference frame should be included in the camera view to scale and validate the measurements taken (Figure 15-6).

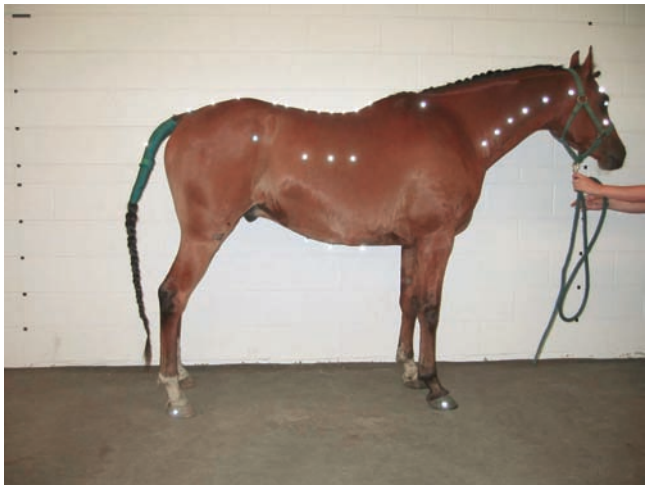

FIGURE 15-6 Photographic analysis using a reference marker set. *(Photograph courtesy of Hilary Clayton, Mary Anne McPhail Equine Performance Centre, School of Veterinary Science, Michigan State University)*

In contrast to whole animal assessment, digital photography was compared with radiographic imaging in a small population and found to be a precise, easily applied method for objective external assessment of equine foot conformation both in clinical and research settings (White et al., 2008). This technique is dependent on having a horse compliant enough to stand stationary on low wooden blocks for long enough to complete image acquisition. In contrast, Weller et al. (2006a) compared two-dimensional digital photography with three-dimensional motion analysis and showed the biggest intraoperator (hoof angles) and intrasubject (heel measurements) errors during foot conformation assessment. A possible explanation for this finding is geometric error caused by the camera angle, as the lens was directed toward the tuber olecrani rather than specifically at the hoof in the latter study.

Motion Systems

The sources of error using photography are primarily overcome when conformational parameters are measured by using three-dimensional computerized motion analysis systems, although similar experimental flaws with marker placement reliability and stance of the horse will occur. The major advantages of using motion analysis include the facility to acquire rapid, repetitive data without subject movement, the relative independence of camera angle relative to the horse, and the redundancy of postacquisition identification of markers by an operator. Studying whole animal conformation requires a minimum of four (ideally eight for bilateral data acquisition) video cameras, custom-written software, and an appropriate examination area, so current use is mostly limited to research and educational facilities.

INFLUENCES ON CONFORMATION

Before evaluating the current literature in this area, it should be mentioned that most studies either compare elite performance horses with inferior performers or evaluate only elite horses and so may not be representative of all horses. Though significant conformational differences are apparent between these groups, horses with severe conformational anomalies are unlikely to be represented by this research as they will have been rejected from studbook admission or withdrawn from selection for performance. The effect of this is to skew the populations and limit broad application of the results.

GENETIC INHERITANCE

Heredity influence is considered an important part of the jigsaw of conformation and the desirability of pedigree-based traits is dependent on the intended use of the athletic horse. Quantification of heredity influence is often expressed using either:

1. A heritability index (h^2): a statistical expression of the relative contribution of genetic factors to the total variance of a particular conformational trait within a population under specific conditions. The heritability index ranges from 0 (no involvement of genetics in conformation) to 1 (conformational traits entirely determined by genes). This index gives an impression of the extent of environmental influence on the expression of genetic properties.
2. A genetic correlation: quantifies the proportion of variance that two traits share due to genetic causes.

These objective measures provide a standardized platform for comparing studies. Although some trends do appear to be emerging, differing populations and methodology will still preclude evidence-based conclusions and agreement as to the absolute relationship between genotype and conformation.

In a study of Dutch Warmbloods, Ducro et al. (2009a) found a significant heritability estimate for height at the withers ($h^2 = 0.67$) and a moderate heritability estimate for foot conformation traits ($h^2 = 0.16–0.27$). These foot conformation traits (pastern angle, heel height, and hoof shape) had a moderate genetic correlation with each other. A moderate genetic correlation between bone circumference and hoof shape was also reported (a larger bony circumference was associated with a broader hoof shape). Other studies (Dolvik and Klemetsdal, 1999; Love et al., 2006) have shown the conformational traits of back at the knee and tied in below the knee to have breed-dependent, strong heritability ($h^2 = 0.19–1.00$). Toeing in, toeing out, offset knees, sickle hock, and straight behind are also documented as being highly heritable, often presenting in both the foal and dam (Love et al., 2006; Ross, 2003). Carpal conformation of yearling Thoroughbreds has been associated with both sire and dam carpal conformation, though no such association was found for the metacarpophalangeal joint (Leibsle et al., 2005; Santschi et al., 2006). The 8% occurrence of offset carpus reported by Santschi et al. (2006) may be genetically linked, although heavy birth weights are also strongly associated with this conformational trait (Leibsle et al., 2005). In Spanish Purebred horses, the maximum hoof height in the forelimb, range of stifle angle, elbow angle, and minimum angle of the carpus showed high heritability ($h^2 = 0.88$; 0.74; 0.86; 0.86, respectively), although tarsal angles showed only medium levels of heritability ($h^2 = 0.57$) (Valera et al., 2008). Similarly, Molina et al. (1999) reported a medium to high heritability of seven zoometric properties in Spanish Purebred horses: (1) height at withers, (2) height at chest, (3) body length, (4) chest width, (5) girth circumference, (6) carpus circumference, and (7) third metacarpal (MCIII) circumference.

Although not a conformational trait per se, gait quality may be inherited as the locomotive pattern of a foal is very predictive for the mature gait of the individual horse (Back et al., 1995). It has also been proposed that natural "leggedness," or laterality, may be inherited, as 8-month-old trotting Standardbred colts had developed this trait before training commenced (Drevemo et al., 1987). A laterality preference may have implications for training or athletic performance and may also be useful in selection for an intended career. For example, if a horse is to be used in dressage or cutting, where balance requirements are vital, a lack of laterality bias would be preferable. It is apparent that this research arena offers a wide scope for further investigation.

The elusive relationship between genetics, conformation, and performance has been discussed in the literature, with little consensus of opinion and sparse evidence. Variable results have been reported when using the overall subjective trait of conformation grade to define this relationship. Ducro et al. (2009a) found conformation grade in Dutch Warmblood horses to have a moderate heritability ($h^2 = 0.30$), accompanied by a high genetic correlation with dressage ranking ($h^2 = 0.67$). Other authors have reported the genetic correlations between conformation score and competition results in dressage or showjumping to be moderate at best (Koenen et al., 1995; Wallin et al., 2003).

In evaluating more specific descriptive conformational traits, Ducro et al. (2009a) found moderate genetic correlations between height at the withers, neck length, limb quality, and dressage performance in competition (h^2 = 0.32–0.36), although genetic correlations for foot conformation and dressage or jumping ranking were only low to moderate. These results are possibly caused by the increasing influence of environmental and human factors (such as training, rider, farriery) with increasing age and competition experience. Conformation, inherited racing ability (largely stemming from the sire pedigree), and reduced Thoroughbred performance were significantly related in horses with "back at the knee" conformation (Love et al., 2006). After including the sire as a cofactor in the analysis, this result became nonsignificant, and the authors suggested that other inherited traits such as size, muscle fiber type, or cardiorespiratory function may have been responsible for reduced performance. In this study, "turned in" or "turned out" feet were also associated with reduced racing performance, although these trends were only statistically significant for animals with the most severe defect score and only weakly significant for inheritance. Therefore, although pedigree was important, conformational defects had a limited effect on racing performance. These authors did caution against extrapolating these findings to all Thoroughbred racehorses, as severe conformational defects were unlikely to be present in the population studied.

AGE

The shape, structure, and biochemical composition of bone, tendon, and articular cartilage adapt in response to the direction, magnitude, and repetition of biomechanical loading with form following function (see Chapter 13). Tissue components such as subchondral bone mineral density and the proteoglycan or collagen network of articular cartilage have been shown to be influenced by normal loading and activity in foals during early development. The distal phalanx has also been shown to be undergoing rapid bony absorption and remodeling during this period (Kroekenstoel et al., 2006). Any conformational defects appearing in the young horse may cause a consistently abnormal loading pattern, which may disturb normal cellular activity and result in mechanical weakness, loss of energy absorbing properties, or both in these structures (Brama et al., 2009a; 2009b). Although foals are susceptible to adverse influences on musculoskeletal development in their early life, the regenerative capacity is high, even in tissues with limited repair capacity in the mature individual (Barneveld and van Weeren, 1999).

A link between age and conformation is frequently stated in the literature, although this is largely unsupported by research, particularly for foals. As early (preweaning and yearling) selection is common for breeding and performance horses, a substantiated knowledge of the normal growth pattern and the effect of age on developmental conformation, or the lack thereof, would be very advantageous for prediction of adult performance. Young sporting horses frequently have one or more conformational deviations, which may or may not persist into adult life, making appropriate early selection for performance more an intuitive art form than a science. This is well illustrated by a study in which subjective evaluation was used to compare carpal conformation in Thoroughbred foals from birth to 525 days (Santschi et al., 2006). Comparisons (cf) of the respective prevalence of carpal deviations within a month of birth and at 525 days were combined carpal deviations 54% cf 48%; carpal valgus 94% cf 21%; outward carpal rotation 54% cf 56%; and offset carpus 8% cf 68%, respectively. The marked reduction (73%) in the incidence of carpal valgus over time indicates a natural correction of the deviation, possibly related to the distal growth physis of the radius. The 60% increase in offset carpus is not easily explainable though a true increase with age is apparent. Perhaps these two traits are biomechanically related, with a decreasing carpal valgus influencing the medial orientation of the distal carpus, thereby increasing the occurrence of offset carpus. Anderson and McIlwraith (2004) also described an increasingly offset carpus over time and a progressive change from carpal hyperextension to slightly over at the knee from weanling to 3 years. They suggested that carpal hyperextension will probably reduce with age and that selection of a yearling over at the knee is inadvisable. These authors also found an expected increase of body segment length during the same period, with an associated strong relationship between long bone length and height at the withers. This is in accordance with Mawdsley et al. (1996), who found six traits significantly linked with age which are attributable to growth and maturation of the Thoroughbred horse 2 to 3 years old. The most significant traits ($p < 0.001$) were height, neck circumference at the withers or manubrium, back length, and shape of the withers. In the distal limb, Kroekenstoel et al. (2006) showed a marked radiographic change in Warmblood conformation between 27 and 55 weeks of age, with a decreasing dorsal angle of the hoof and an increase in parallelism of the hoof wall to the distal phalanx. Radiographic findings demonstrated all these young horses to be broken backward during this period. Using photographic evaluation, Anderson and McIlwraith (2004) found a similar decrease in the dorsal angle of the hoof of the juvenile Thoroughbred. However, they assessed the hoof–pastern angle to be broken-forward in weanlings, straight in yearlings, and broken-back at both 2 and 3 years of age. More clarification of the developmental aspects of distal limb conformation in young horses of different breeds is necessary to draw accurate conclusions from prepurchase radiographic assessments.

Uneven feet are defined when a significant observable difference in the size and shape of the right and left forefeet is present. The proximal interphalangeal joint appears to remain aligned in both forefeet, the dorsal hoof wall angles are variant, and the center of pressure in the foot with the lower hoof angle is moved caudally (van Heel et al., 2006). A lower hoof angle creates a broken-back hoof–pastern angle, which has been shown to increase the extension moment of the distal interphalangeal joint with subsequent increased force on the navicular region and increased tension in the deep digital flexor tendon (Eliashar et al., 2004; Moleman et al., 2006). The other more upright hoof may predispose the distal limb to suspensory ligament injury, as elevated heels have been shown to increase tension in the suspensory ligament (Lawson et al., 2007) (Figure 15-7). The conformational trait of asymmetrical or uneven feet is initially observed and scored at studbook admission (foal age) and appears to increase in prevalence with age. Ducro et al. (2009a) studied a large population of Warmbloods over 12 years and found the incidence of uneven feet increased from less than 4.5% at year 3 to greater than 8% at year 10 of the recording. It is uncertain whether this trend is distorted by

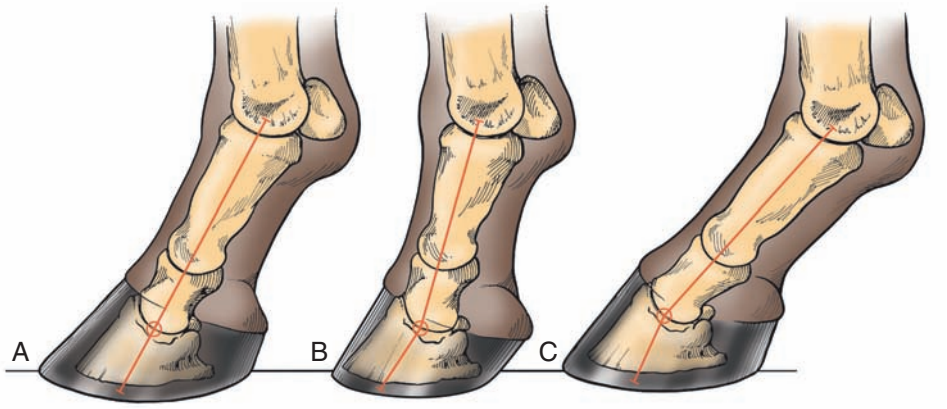

FIGURE 15-7 Ideal (*A*), broken forward or upright (*B*) and broken-back (*C*) hoof–pastern axis.

either corrective trimming masking the defect at the original scoring or a more stringent subjective evaluation occurring with increasing age and performance requirements. Although juvenile horses with uneven feet are commonly sound, all these interrelated structures maintain digit equilibrium, and the altered biomechanical loading and stimulation of the rapidly developing musculoskeletal system may have a negative effect on tissue architecture and integrity predisposing the horse to injury or reduced performance in later life (Kroekenstoel et al., 2006). Uneven feet are not necessarily a criterion for rejection in performance horses, as the long-term clinical implications of this fault are unclear. Varying effects of uneven feet on performance have been distinguished between disciplines and levels of excellence. A marked decrease in the probability of survival and significant reduction in the median duration of competitive life was found in elite show jumpers with uneven feet (Ducro et al., 2009b). The effect on dressage performance was less compelling in this study, showing only a trend for reduced competition life in elite dressage horses. A definitive quantification of the degree of unevenness distinguishing natural variation of hoof angles from pathologic variation has yet to be determined. Early preselection in the presence of this defect has, nevertheless, been discouraged by some authors (Ducro et al., 2009a; 2009b).

Conformational traits consistent over time may have some predictive power in early selection of performance horses. Mawdsley et al. (1996) showed the Thoroughbred to have little change in head shape, neck shape, and croup length with increasing age, indicating these traits in a juvenile to be prognostic for the mature horse. Although angular joint conformation at the elbow and stifle have been shown to be significantly influenced by age in Spanish Purebred horses (Cano et al., 2002; Valera et al., 2008), the slight influence of age on other joint angles, temporal and linear variables may enable early phenotypic preselection of horses. Other studies have suggested that dynamic conformational traits unaffected by age may assist in early determination of biomechanical aptitude. For example, there have been significant correlations shown between foals and mature horses in maximum flexion of the hock during stance (Holmstrom and Drevemo, 1997), kinematics of the hindlimbs (Back et al., 1995), and jumping capacity (Bobbert et al., 2005).

The relationship among age, conformation, and performance is difficult to quantify because of the increasing influence of environmental, experiential, and other nonconformational factors over time (Wallin et al., 2003). Performance is frequently evaluated using lifetime earnings, starting status, win percentages, best racing time, and duration of competitive life which cannot be determined until later in the sport horse's career and do not solely reflect the performance potential of the horse (Weller et al., 2006c). Patterns of locomotion will evolve over time, particularly in the dressage and showjumping horse. More starts necessarily means an older horse, and increasing numbers of starts appears to improve all performance parameters in the Thoroughbred racehorse (Weller et al., 2006c). Further research ascertaining the complex relationship among performance, existing or developing conformational characteristics, and the effect of age, if any, is needed to validate basic selection criterion. It is prudent to note here that when studying biomechanics and the subsequent performance of young horses, any interpretation of this relationship may be confounded by such factors as the underdeveloped power, balance, and fatigability of the immature musculoskeletal system (Ducro et al., 2007). For example, when rigorous training is superimposed on the immature skeleton of the young Thoroughbred and Quarterhorse, dorsal metacarpal disease (bucked shins) may develop from fatigue failure and inadequate remodeling of the MCIII. The incidence of dorsal metacarpal disease may range between 30% and 90% (Nunamaker, 2003), which will undoubtedly affect performance results. As this condition is thought to be caused by changing inertial properties of the MCIII during growth and training, differentiation of the effect on performance of age, conformation, and orthopedic health as independent components is difficult, thus illustrating the complexities involved in this area of research.

SEX

There appears to be significant differences in conformation between mares and stallions across most documented breeds, the gelding mean values falling somewhere in between. These differences are summarized in Table 15-3.

BREED AND PERFORMANCE CRITERIA

The success or failure of a breed of horse in any chosen endeavor has traditionally been attributed, at least in part, to its conformation, yet most objective studies across different

TABLE 15–3

Summary of Conformational Differences between Stallions and Mares in Different Breeds

Breed	Stallions	Mares
Cold blooded trotters (Dolvic and Klemetsdal, 1999)	Higher at withers Greater cannon and carpus circumference Wider breast	Larger girth circumference
Warmbloods	Males considerably higher scores for canter, type, and total conformation Higher performance level than mares	Shorter at withers Shorter limb lengths Longer bodies Narrower metacarpi Smaller hock joint angles Larger hind fetlock joints (Holmstrom et al., 1990)
Standardbred trotters	Higher and broader at withers Greater width and circumference of cannon and carpus Flatter croup Smaller hip angle Outwardly rotated limb axes	Greater width of pelvis Longer body Longer distance between the last rib and the pelvis (Magnussen, 1985)
Thoroughbred (Mawdsley et al., 1996)	Increased head and neck shape Increased neck circumference at the poll/larynx and withers/manubrium of the sternum Less upright hoof pastern axis in the forelimb	
Banei draft racehorses (Kashiwamura et al., 2001)	Greater chest width Greater cannon bone circumference	Greater hip width, croup width, and rump length

breeds have demonstrated only a weak correlation between conformation and performance (Dolvic and Klemetsdal, 1999; Holstrom and Philipsson, 1993; Love et al., 2006). Some of these results indicate previously "undesirable" conformations may, in fact, be within normal limits or even advantageous to performance. As "ideal" conformational attributes have historically been empirically selected and bred in or out by human design, rather than by natural selection, engineered development of a "type" best suited for performance in a discipline may not have been in the horse's best musculoskeletal interests. The demand for high-speed locomotion may have compromised the structural stability of musculoskeletal tissues, challenging the narrow safety margins for tissue failure. The variations of selected vocations within a specific breed will also affect the desirable conformation because of the sport-specific challenges on the musculoskeletal system. A thorough knowledge of the demands of each particular sport is essential to determine the compatibility of conformation and intended use, possible contraindications for selection as well as allowing for early recognition of injury manifestation. The controversial relationships among conformation, injury, and performance will be illustrated by a review of the evidence-based literature concerning the "ideal" conformation of Thoroughbred racehorses, both in flat and National Hunt disciplines. The significant animal welfare and economic issues associated with wastage in Thoroughbred racing warrant this particular attention. For more information on other breed and sport-specific conformational requirements, refer to Chapters 20-27.

Height at the withers is a standard conformational selection criterion which may be used to predict the general skeletal format of the horse. All circumference measurements and the majority of length measurements are significantly correlated with wither height in Thoroughbreds, so a taller horse may be expected to have longer lengths of neck, back, and limb segments; a larger chest circumference; and broader hooves (Weller et al., 2006b). There was no such correlation found between joint angles, inclines, or deviations in this study, so biomechanical integrity cannot be determined from wither height alone. There is some evidence that increased height improves Thoroughbred flat racing performance, as measured by lifetime earnings, starting status, and win percentages (Smith et al., 2006). Other factors may also be relevant in determining the optimal wither height for this discipline. Larger animals have an associated faster growth rate, and this may be an intrinsic factor shaping structural limb quality, perhaps weakening mechanical durability, and predisposing taller horses to conditions such as osteochondrosis (Barneveld and van Weeran, 1999; Ducro et al., 2009b). Supporting this are the findings of a radiographic study by Stock and Distl (2006), which showed increased development of osseous fragments in the fetlock and hock with increased wither height. Additionally, longer bones have an increased mass and are subject to higher moments of inertia, increasing the applied bending moments, tensile and compressive loads, and metabolic cost of locomotion. As it is generally accepted that wither height is unrelated to stride length or frequency and increased limb mass has been shown to decrease the maximal velocity of limb movement (Dellanini et al., 2003), the biomechanical advantage of a taller horse is somewhat debatable in flat racing, and the balance between performance and orthopedic health must be seriously considered. In

contrast, Thoroughbreds competing in National Hunt may benefit from extra height and longer body segments, as this has been shown to improve performance within National Hunt competitors (Weller et al., 2006c) and in the jumping performance of other breeds (Holmstrom et al., 1990).

It is commonly thought that a straight forelimb conformation is desirable for racing performance in the Thoroughbred. Recent studies appear to challenge this belief, as the average racehorse was found to have carpal hyperextension (back at the knee) and a carpal valgus deformation of around 5 degrees (Weller et al., 2006b). Although these results indicate that these deviations may be considered a normal occurrence in Thoroughbreds, there is some controversy among authors about the possible effect on performance and soundness. It is anecdotally accepted that carpal hyperextension will create high stress levels in the carpus and may contribute to fractures of the dorsal carpal bones, especially in the fatigued Thoroughbred (Johnston et al., 1999). This idea was refuted by Barr (1994), who maintained that carpal hyperextension was unlikely to play a major role in the etiopathogenesis of carpal chip fractures. Although carpal hyperextension appears to increase in a linear manner with increasing speed (Burn et al., 2006), it has not been established if there is a causal relationship between the degree of hyperextension and bony or connective tissue injury. Weller et al. (2006b; 2006c) suggest carpal valgus is detrimental to racing and jump performance, risk of injury due to an increase in loading of the superficial digital flexor tendon, or both. Interestingly, Anderson et al. (2004) showed that carpal effusion and incidence of fracture in 3-year-old Thoroughbreds decreased as the carpal valgus increased, suggesting that a slight valgus may be an important protective accommodation in racehorse conformation. However, the perennial problem of lack of uniformity among research papers makes it difficult to draw definitive conclusions.

Similarly, a straight hindlimb conformation has been sought after, especially in relation to the shock absorbing tarsal joint. An "ideal" tarsal angle has yet to be established in Thoroughbreds. However, extreme joint angulation may not optimize muscular leverage and the ensuing power generation, so this may be an important consideration in the racing conformation. Small tarsal angles (sickle-hocked) or large tarsal angles (straight-behind) have been implicated as risk factors in the etiology of tarsal disease, with consequent loss of performance or useful career lifetime in other breeds. Axelsson et al. (2001) used radiography to show a significant relationship between a small tarsal angle and prevalence of degenerative joint disease in the distal tarsus of Icelandic horses. In a mixed population of horses, Gnagey et al. (2006) found that a large tarsal angle absorbed less energy (concussion) compared with a small tarsal angle during the impact phase at trot, perhaps predisposing to arthritic changes. This study also identified that horses with large tarsal angles had reduced tarsal flexion and slower angular velocity during weightbearing with a trend toward less energy generation during pushoff at the end of stance. It would also be interesting to investigate whether tarsal angle has any effect on the integrity or efficiency of the reciprocal apparatus—the coupling mechanism that links the stifle and tarsus, providing a single functional lever arm in the tarsometatarsal joint.

It is suggested that toeing out of the hindlimbs is not only a common but desirable conformation for the successful racing Thoroughbred (Anderson et al., 2004; Love et al., 2006). Whether this appearance is related to externally rotated hindlimbs or mean pastern angles greater than 180 degrees in the sagittal plane is not always clearly defined in the literature. Mawdsley et al. (1996) found the hindlimb pastern angle to cause a toed out conformation in the entire population of 120 superior Thoroughbreds between 2 and 3 years of age, concluding that this conformation is not rejected during selection for performance. Love et al. (2006) found a much lower incidence (30%) of turned out feet in yearling Thoroughbred horses; yet, in contrast, they found a tendency for lower national mean lifetime ratings with this conformation. Another study found no such hindlimb deviation in 5-year-old National Hunt Thoroughbreds and proposed that this was a reflection of the age of the horse assessed (Weller et al., 2006b). The authors suggested that a straight leg may be necessary to endure the intensive demands of training and racing, so horses with inferior conformation may have already been withdrawn from the starting line.

Other conformational traits appearing to have a positive effect on Thoroughbred performance in National Hunt racing include the following (Figure 15-8):

1. *Increased intermandibular (IM) width* (Cook, 1988; Delahunty et al., 1991; Weller et al., 2006c). It is possible that an increased IM width may have a positive effect on respiratory function because of reduced airway resistance. There is also a questionable relationship between a smaller IM width and recurrent laryngeal neuropathy (Marks, 2000), which is quite prevalent in National Hunt Thoroughbreds (Dixon et al., 2001).

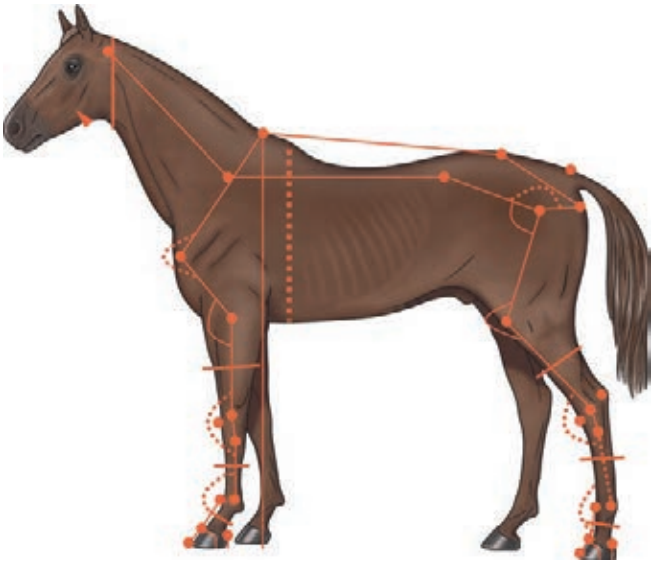

FIGURE 15-8 Schematic drawing of a horse with marker positions, segment lengths, joint angles, and circumference measurements (*bold lines*) used in the study of Weller et al. (2006c); conformational parameters that contributed significantly to the regression models for performance or musculoskeletal injury (*dotted lines*). *(From Weller R, Pfau T, Verheyen K, et al: The effect of conformation on orthopaedic health and performance in a cohort of National Hunt racehorses: preliminary results,* Equine Vet J *38:622, 2006. Reproduced with permission).*

2. *Increased flexor angle of the shoulder* (Weller et al., 2006c). A smaller flexor angle was shown to be detrimental for performance in this study, which infers a larger flexor angle would be positive for performance. In terms of biomechanics, a more acute (smaller) scapulohumeral joint angle may structurally limit the range of forelimb protraction, thereby affecting stride length.
3. *Large lateral coxal angle—the angle between ischium and ilium* (Weller et al., 2006c). An increased coxal angle showed both a lesser risk of pelvic fracture and increased performance in this study. A possible explanation for these findings are that a closer approximation of the axis of the pelvis to the alignment and direction of contraction of gluteal musculature may help in force transmission and provide a mechanical advantage for muscular contraction, while reducing the bending moment applied to the ilium.

Clinical experience based on qualitative observation often associates a relationship between faulty conformation and musculoskeletal disease, lameness, or both in Thoroughbreds, yet very few objective studies support these observations. Table 15-4 summarizes the conformational traits anecdotally considered detrimental to performance or predisposing to lameness, as well as the possible biomechanical and orthopedic ramifications of the misalignments. Some references are cited for research-based studies or controversial findings.

TABLE 15–4

Conformational Traits, Applied Biomechanics, and Consequent Orthopedic Risks in Thoroughbreds

Anatomical Region and Conformational Trait	Implications for Biomechanics	Potential Orthopedic Outcome
RADIOCARPAL (RC)/ METACARPAL (MC) COMPLEX		
Back at the knee	Increases tendency of carpus to extend	Carpal lameness
Carpal valgus	Weight concentrated on medial aspect of carpus and proximal MC region	Carpal chip or slab*
Offset knee	Joint compression increases distally	SDF injury; Carpal lameness; Splints
Tied in behind	Diameter of flexor tendons is less proximally than distally	Splints; Injury +/– lameness in all distal limb joints; Tendonitis SDF/DDF
METACARPOPHALANGEAL (MCP) COMPLEX		
MCP valgus	Moves the ground reaction force vector away from the sagittal plane	SDF injury; Interference; Sub solar bruising medial heel
Short upright pastern	Increased concussion; Shorter strides	SDF tendonitis (Weller et al., 2006a); Interphalangeal joint disease +/– lameness
Long sloping pastern	Increased tension in palmar structures: SDF, DDF, suspensory ligament	SDF tendonitis; Weak significance for carpal chip* (Barr, 1994; Anderson et al., 2004); Proximal sesamoid* ; Proximal phalanges*; Osteoarthritis of the MCP joint
TIBIOTARSAL (TT)/ METATARSAL (MT) COMPLEX		
Straight behind	Increased stifle angle and reduced metatarsophalangeal angle	Stifle lameness Upward fixating patella Proximal/high suspensory desmitis Osteoarthritis of metatarsophalangeal joint
Sickle hock	Increases the extensor moment and vertical impulse in stance; Loads distal plantar aspect of hock	Distal tarsal disease Plantar desmitis (Gnagey et al., 2006) Collapse middle tarsal bone
Tarsal valgus	Asymmetrical loading of the hindlimb in a lateromedial direction.	Increased risk of pelvic* Increased occurrence of digital tendon sheath effusion (Weller et al., 2006c)
TRUNK		
Large girth	Big body mass increases peak limb forces and energy cost of locomotion (Weller et al., 2006c)	Increased risk of limb injury in general

*, fracture; *DDF,* deep digital flexor; *SDF,* superficial digital flexor.

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CHAPTER

16 The Biomechanics of Equine Locomotion

BRONWYN GREGORY

Historically, the equine locomotor system has had demands placed on it for military, agricultural, and transport purposes. This required a robust and functional, but not necessarily refined, musculoskeletal system. In more recent times, the emergence of the horse as a competing athlete in a diverse range of equine sports and leisure pursuits has placed different and often higher demands on the equine locomotor system. This has stimulated more scientific research into equine gait and biomechanics. There is enormous wastage in racing and equestrian sports because of lameness (Jeffcott et al., 1982; Kobluk, 1998; Rossdale et al., 1985), which can be primarily attributed to orthopedic conditions resulting from repetitive stress injuries, spontaneous soft tissue injury (ligamentous, muscular, or tendinous), or blunt trauma. Blunt trauma is mostly related to unpredictable circumstance; however, repetitive stress and spontaneous musculoskeletal injuries could be avoided. Evidence-based research that is able to objectively define normal locomotion, improve diagnosis or recognition of locomotor deficiencies, or identify predisposing factors for injury is important for injury prevention or at least in the prevention of deterioration in certain conditions. Ultimately, the development of appropriate sports-specific training programs based on such research will facilitate reduced wastage and consequent economic losses associated with lameness and result in improved equine welfare.

The reasons equine gait analysis is undertaken are multifold, including the following:

1. To systematically investigate, characterize, assess, and quantify normal gait patterns enabling numeric comparisons between different populations
2. To identify typical features of specific gait abnormalities
3. To understand potential mechanisms of injury consequent to various work demands and conformational traits
4. To predict performance and soundness
5. To evaluate lameness
6. To evaluate neurologic conditions with or without lameness
7. To design and evaluate medical, surgical, training, shoeing, and rehabilitation interventions

This chapter aims to review the literature and provide a background basis for the study of equine locomotion. Several unique cursorial musculoskeletal adaptations of the horse will be reviewed, the parameters of locomotor measurements defined, the currently available methods of measurement used in equine gait analysis described, and the basic gait characteristics in the athletic horse defined.

BIOMECHANICAL ADAPTATIONS IN EQUINE ANATOMY

The survival of the horse has depended on an effective protective flight response, optimizing the energetic cost of locomotion, enabling both travel over short distances at great speed to avoid predators and travel over long distances at a lesser speed to accommodate the environment (climate and food source changes). Because of the demand for faster, longer strides, the horse has evolved an unguligrade osseous formation—there is one retained weightbearing digit. Distally, three metacarpals (MCs) remain, but MCI and MCV have been lost. The three remaining MC have changed function—the elongated MCIII (or cannon) bone being the only weightbearing MC for the whole limb, with the vestigial MCII (medial) and MCIV (lateral) "splint" bones functioning only to support the carpal bones, providing maximal strength with minimal distal weight. The long bone density of MCIII has changed to increase strength, a small spongy medullary cavity leaving the slender shaft almost completely solid and more resistant to the compressive forces of locomotion.

Proximally in the forelimb, the forelimb–scapula complex and thorax are joined by an extensive composite of connective tissues (synsarcosis) rather than by a bony articulation (the clavicle is absent, and the coracoid process is fused to the scapula); the scapula has orientated to a lateral–vertical position because of a flattening of the thorax in a mediolateral direction; and the glenoid articulation is consequently redirected to face cranioventrally rather than laterally. This limits possible range of shoulder motion by its approximation with the trunk. The humerus, in turn, has rotated medially, thus drawing the forearm into pronation and leaving the radius dominant in forearm weightbearing. In sum, these bony changes have enabled greater weightbearing ability and efficient economy in locomotion in the forelimb by raising the center of gravity of the trunk and providing a more mechanically stable vertical limb alignment (Payne et al., 2004).

The structural arrangement of forelimb locomotive muscles has altered to accommodate the changed demands of altered body format, as has the ability of horses to move limbs in response to visual and vestibular sensory information in a changing environment. Proximally, postural muscles with interspersed elastic lamellae have developed, providing a secure muscular sling that suspends the trunk between the forelimbs, locomotive stability while allowing vertical and horizontal excursion of the thorax in relation to the limb, and absorption of vertical shock and horizontal propulsive forces

(Kainer, 2002). Distal musculature has moved more proximally, reducing the forelimb moment of inertia. The brachium has remained relatively short and muscular, providing proximal stability, while the antebrachium and distal arm have replaced muscle mass (weight) with tendons and ligaments to minimize locomotive demand. Similar muscle migration has occurred in the hind limb, resulting in a similar functional anatomy of the distal limbs fore to hind. The lack of muscular protection and the weightbearing demands on the distal limb leave a system of bone, tendon, and ligament with a huge burden of duty and a narrow safety margin for tissue failure. Musculoskeletal resilience and efficiency of responses in the dynamic mechanical environment of locomotion are variable according to component tissue type and age (Smith and Goodship, 2008). It is, therefore, not surprising that distal limb injuries account for the most common musculoskeletal injuries associated with the racing and training of Thoroughbreds worldwide (Ely et al., 2009); and the skeletal system is associated with a high incidence of other equestrian sporting injuries. These injuries include degenerative joint disease, tendon or ligament lesions, muscle lesions, and bony fracture.

LOCOMOTION ANALYSIS

Equine locomotion is a complex integration of linear and rotational movement patterns that are created by a combination of internal and external forces acting on the horse's body. Internal forces are attributable to the inherent qualities of the musculoskeletal system such as tendon extensibility, bone strain, and muscle strength, which are mostly only researchable by invasive measures (for more detail see Chapter 13). External forces are consequent to the environment, with the forces created by hoof strike and weightbearing (ground reaction force) allowing noninvasive measurement. External and internal forces are not independent of each other; therefore, many factors or combinations of factors can potentially predispose athletic horses to injury or poor performance. These factors include genetic makeup, biomechanical conformation, experience, age, sex, training regimes, activity-specific work demands, environmental working conditions such as hoof–ground interaction, saddle fitting, and shoeing.

Equine movement patterns can be examined as a whole body effect or by the individual contribution of specific body parts (Clayton, 2004). Biomechanical analysis provides an evidence-based platform to study these elements through the application of mechanical laws to living structures; therefore, describing and measuring motion, the forces causing movement, or both in live animals. This quantitative information can help to provide a better scientific understanding of equine locomotion, anatomy, and physiology and may also assist in validating or disproving previously subjective assessments. Prior to describing the measuring systems available today, a definition of terms, units, and measurable variables will be outlined in an attempt to standardize current nomenclature and methodology.

DEFINITION OF BIOMECHANICAL PARAMETERS

Gait can be analyzed scientifically through a single stride cycle. *Single stride* can be defined as the basic repeated unit of movement of an individual limb in a full cycle of limb motion and is measured from one footfall of a limb to the next footfall (Leleu et al., 2005). At any particular gait, this is a consistent, rhythmic, automatic repeating pattern for an individual horse. This linear measurement is representative of the distance moved by the center of mass during a single stride. The stride consists of the two following interactive phases: (1) the *stance phase*, which is measured from hoof landing to toe-off when the hoof is in contact with the ground; and (2) the *swing phase*, which is measured from toe-off to hoof landing when the hoof has no contact with the ground and is lifted and brought forward (protracted) in preparation for weightbearing (Figure 16-1). The stance phase has arbitrarily and simply been divided into a *restraint phase*, or *deceleration phase*, following heel strike, and a *propulsive phase*, with breakover and toe-off, mid-stance position bisecting these phases. Breakover begins at heel lift during the terminal part of the stance phase and ends at toe-lift. The mid-stance position in the forelimb is when the metacarpus is in a vertical position and in the hind limb when the hoof is directly under the hip. Measurement of these parameters in either geometric or force-related studies allow the terms in Table 16-1 to be calculated.

SYSTEMS FOR BIOMECHANICAL MEASUREMENT OF PARAMETERS

Current technology allows equine locomotion to be quantified in two main ways: (1) *kinematic*—the geometry of movement or description of the way body parts move in space without regard for the forces producing it; and (2) *kinetic*—examining the action of forces that produce movement without regard to the quality of movement. These are complementary ways of exploring the intricate combination of factors resulting in equine locomotion and may be used in isolation, in combination, or for comparison. Three primary orthogonal axes of coordinates are used for biomechanical analysis, and terminology differs among studies:

1. Transverse–horizontal, mediolateral, or yaw
2. Longitudinal–horizontal, craniocaudal, or roll
3. Vertical or pitch (Figure 16-2)

The axis designation as x, y, or z is arbitrary and must be defined in any data presentation or reporting.

Standardizing velocity is of utmost importance, as both kinematic and kinetic parameters have been demonstrated to be directly velocity dependent (Khumsap et al., 2001; Khumsap et al., 2002; Robilliard et al., 2007).When kinematic and kinetic data are synchronized with morphometric data (mass, center of mass, and mass moment of inertia of body segments) and mathematical formulas, more detailed gait analysis such as net joint moments, joint power profiles, and changes in center of mass position can be performed (see inverse dynamic analysis below). Statistical analysis of these data may or may not allow significant statistical conclusions to be drawn; however, trends toward or away from a biologically significant effect may be revealed. Biomechanical studies using these techniques can help in analysis of the characteristics of normal gait in multiple planes; detection of subtle gait asymmetries and compensatory changes, which has practical application for lameness evaluation; and assistance in performance assessment and quantification of the efficacy of intervention therapies and regimes.

Inverse Dynamic Analysis

Inverse dynamic analysis is a mathematical technique based on a biomechanical model of the limb as a linked series of rigid segments without friction or translation. Using this

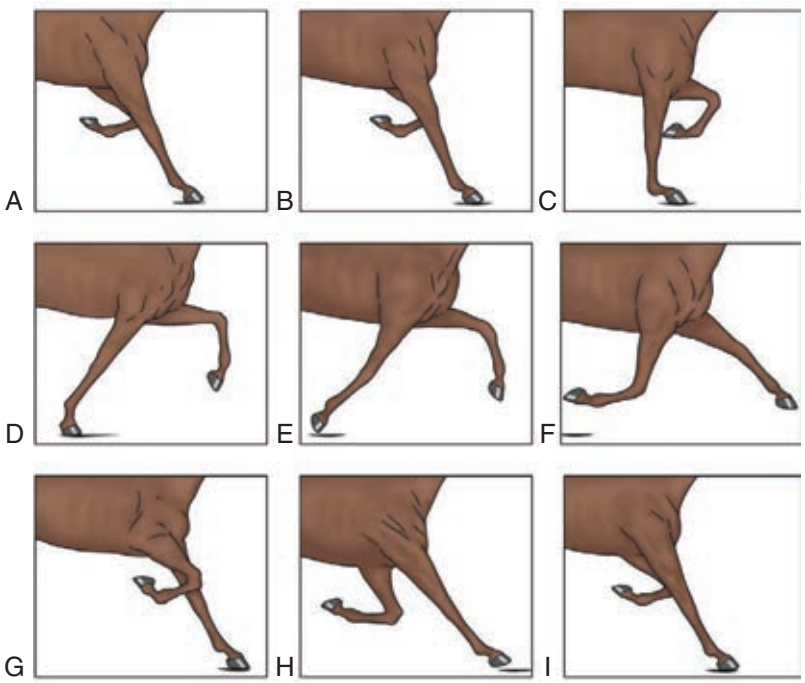

FIGURE 16-1 Sequence of right forelimb movement during one stride cycle of a trotting horse. The stance phase lasts from *A* to *E* and consists of the restraint phase from *A* to *C* and the propulsion phase from *C* to *E*. The swing phase lasts from *E* to *A*. **A,** Heel contact. **B,** Full contact. **C,** Mid-stance position. **D,** Beginning of heel-off. **E,** Toe-off. **F,** Beginning of hoof acceleration. **G,** Mid-swing. **H,** End of hoof deceleration. *(Adapted from Fredricson I, et al: A method of three-dimensional analysis of kinematics and co-ordination of equine extremity joints. A photogrammetric approach applying high-speed cinematography,* Acta Vet Scand *37(Suppl):1, 1972, with permission).*

TABLE 16–1

Definition of Terms and Units of Measure in Equine Gait Analysis

Descriptive Terminology	Definition and Units of Measurement of Stride Variables
Stride duration (SD)	SD is the time for one complete gait cycle or the time between any two identical events of a cycle (second [s]). SD decreases with velocity and is associated with a shortened stance phase.
Stride frequency (SF)	SF is the number of strides per unit of time (s). SF is equal to the inverse of stride duration (Leleu et al., 2005), is limited by protraction time and increases nonlinearly with velocity.
Stride length (SL)	SL is the linear distance a hoof travels (meter [m]) during a stride cycle and is deduced from the relationship of velocity and stride frequency (Leleu et al., 2005). SL increases linearly with velocity.
Duty factor	Fraction of the stride when the limb maintains contact with the ground (%), mostly reducing with velocity.
Protraction time/ swing phase	Fraction of the stride when the limb is moving forward and free from ground contact.
Advanced placement	Fraction of the stride that elapses between ground placement of specified limbs.
Overtracking	Distance between the hindlimb and forelimb hoof strike; it is positive if the hindlimb lands in front of the forelimb.

technique, interactions between the segments during the stance are summarized as net joint forces and net joint moments, which enables noninvasive in vivo evaluation of the net internal forces of peripheral joints and tendons at any time interval during this gait phase. A quasi-static equilibrium between internal and external forces and the moments acting on the joint is assumed, and inertial parameters are often disregarded as being negligible during the stance. Comparison of sequential time frame points enable the calculation of the work done (net torque) and power generated and absorbed about each joint. The application of this technique to the swing phase gives joint moments representing the net effect of muscular forces acting on segmental inertial properties to produce angular and linear acceleration of body segments against gravity (Lanovaz and Clayton, 2001). The design of the rigid segment model necessitates a simplification of musculoskeletal mechanics, thus limiting the extent of data interpretation. Friction and joint structure integrity are not considered; semirigid segments of the body cannot be accurately analyzed because of force attenuation ability (e.g., trunk); muscles crossing more than one joint (very common in

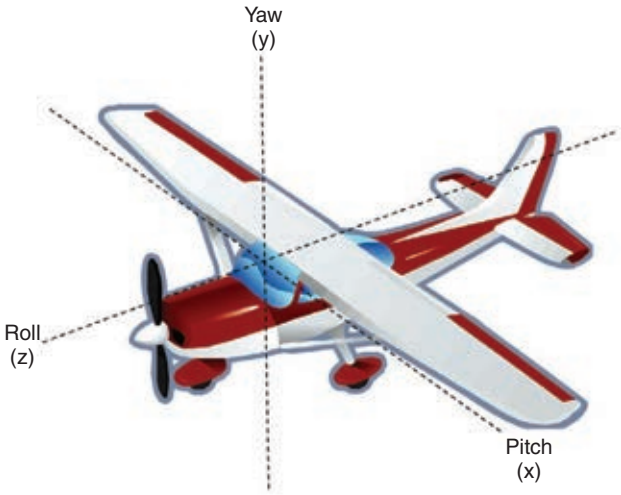

FIGURE 16-2 Orthogonal planes of movement. Axes denote the *axis of rotation* of the orthogonal plane movement. *Yaw*—"side to side" movement of wings. *Pitch*—downward movement of nose with upward movement of tail and vice versa. *Roll*—rotation around the longitudinal axis of the plane (*z*).

horses) are not well represented; joint moment data are a summary representation of mechanical effort; and movement in most models is restricted to a two-dimensional movement in the sagittal plane limiting force analysis to the flexor or extensor muscle groups acting on the joints. Isolation of individual muscle activity and characterization of the co-contraction of agonistic–antagonistic muscles cannot be determined as muscle action is represented as a net moment. Inverse dynamics analysis is also particularly sensitive to input data, so any inaccuracy will have important ramifications for resulting joint moment data.

Forward Dynamic Analysis

More recently, equine locomotion studies have employed *forward dynamic analysis*, which uses the input of internally applied joint forces and torques to simulate body section movement, thus predicting movement patterns. This analysis allows customization of modeling for individual subjects and prediction of movement patterns minimizing overall muscle effort. Computer modeling by indirect mathematical reconstruction of expression of movement based on easily obtainable input parameters may, to some extent, replace the often tedious and inconvenient current direct measuring techniques. The generalization inherent in such calculations will, however, limit the application and interpretation of the conclusions.

KINEMATIC ANALYSIS

Kinematic analysis of the horse in motion allows quantification of the components of equine gait which can be visually or subjectively assessed. Martens et al. (2008) confirmed that kinematic analysis is more detailed and reliable than visual appraisal, even with viewing of slow-motion cinematography. Videography and optoelectronic systems used for kinematic analysis can evaluate temporal (timing) variables such as stance times, swing times, duty factors; linear (distance) variables; and angular measurements, thus describing trajectories of body segments and joint angles in space over time. These data also allow the relationship between time and displacement (velocity and acceleration or deceleration) to be calculated. With appropriate software, this is a particularly valuable way to measure equine spinal mechanics, as vertebral access is difficult and joint movement amplitudes are small, which limits visual appraisal. Imaging can be either two-dimensional or three-dimensional, depending on the number of cameras and the orientation of camera angles. Data interpretation and comparison are dependent on the precise definition of the parameters measured and in the expression of statistical terms. Two-dimensional imaging is easily performed; however, data analysis is limited, and errors will be introduced if significant primary and coupled movements are not captured by the camera lens angle. While motion at the distal limb is primarily restricted to flexion or extension in the sagittal plane, the small movement components of rotation, circumduction, translation, or shearing, which may be relevant to locomotion or lameness studies, are not discernible in two dimensions. Furthermore, body parts may move temporarily out of camera view, and marker tracts may cross each other. Three-dimensional studies overcome these failings to a certain degree but are more complex in operation and analysis, and the setup expense limits its use to only a few specialty practices and academic institutions. Data presentation of kinematic gait analysis can be done by using spreadsheets, graphics, or stick figures (Clayton and Schamhardt, 2001). The most commonly employed plotting formats use trajectory versus time, which allows the comparison of different kinematic features occurring simultaneously or the comparison of simultaneous kinematic, kinetic, and biophysical data.

To allow serial videographic or optoelectric recording of skeletal range of motion through different planes of space and time, surface skin markers are applied to prominent, palpable anatomic (usually bony) landmarks, highlighting particular limb or trunk segments and joint angles. To standardize the position, markers should be located at the estimated center of joint rotation and the distal and proximal ends of each segment under investigation. Markers can be nonreflective material delineated and recognized in color by the kinematic system; retroreflective material, which reflects light back to the imaging source; or strobing light-emitting diode (LED) markers, which produce light the kinematic system can track. The respective *x*, *y*, and *z* positional values gained from kinematic recording allow mathematically based software programs to calculate linear and, more particularly, angular velocities and accelerations in some systems almost spontaneously.

A skin marker system will potentially introduce error to experimental design, as some landmarks are difficult to repeatedly and precisely identify because of the depth of overlying musculature or fat and the specificity of a small marker placement on a large bony landmark. Inadequate preparation of the marker site (e.g., not eliminating hair interference) may also introduce artefacts. More importantly, the task-dependent movement of overlying skin or soft tissue relative to the underlying bone, which occurs with positional change, will introduce error. Skin movement relative to bone is more apparent proximally than distally (Weller et al., 2006). Two-dimensional mathematical correction algorithms to adjust for skin displacement have been developed on Dutch Warmbloods at the walk and trot (van Weeren et al., 1992). The corrections are for a limited number of body segments and are only applicable to horses with comparable conformation, gait

style, and velocity. A preliminary three-dimensional method for skin displacement correction at the equine radius has been designed; however, further studies are needed to extend the application to the entire forelimb and hindlimb, which would allow more sensitive kinematic gait analysis (Sha et al., 2004).

Systems Used for Kinematic Analysis of Gait

The late 1800s saw the beginning of the quest to scientifically analyze equine locomotion. Muybridge (1899) is cited comprehensively in the literature as being the pioneer of kinematic movement analysis in horses, which involves the use of photographic stills to record relative stance durations during gait (Figure 16-3). The use of serial photography required the horse to move through the field of view of 24 cameras triggered in succession. Serial still photography only captures one instant of time per frame and records three-dimensional movement in two dimensions, so total movement analysis is not possible. Marey (1873) improved on this work by developing chronophotography, which captured several different images on one photographic plate; however, accurate kinematic measurements were difficult because of image overlap. High-speed cinematography, videography, and optoelectric systems evolved from these original concepts. Cinematography was used extensively in the past and employed a fine resolution (10 to 500 frames per second) and a wide range of shutter and camera speeds. The process was expensive and labor intensive in methodology and analysis (e.g., manual digitizing of coordinates, cumbersome mainframe computers). Currently, videography, combined with commercial software packages and optoelectric systems, is most often used for kinematic equine gait analysis. These systems all require calibration; automatic digitization of raw data, manual digitization of raw data, or both; integration of the calibration and digitized information (transformation) so that image coordinates can be correctly scaled to size; and smoothing to reduce the electric noise of digitalization caused by random fluctuation of electrical currents (usually performed using a low-pass filter, although piecewise quintic splines and Fourier series reconstruction may be used). This allows real-time data capture and analysis (Clayton and Schamhardt, 2001).

Videography

Videography can be in either analog or digital format. The hardware and software are comparatively inexpensive, easy to operate, and portable; and data collection is immediately available for analysis. Care must be exercised to prevent errors caused by skin marker placement and skin displacement as discussed previously. Additionally, the camera must be accurately orientated perpendicular to the segments of interest, and lighting conditions are critical for clarity of footage. Although videographic data collection can be performed outdoors in sunlight, some digital videographic systems allow manual identification of markers, which may be useful even in less-than-optimal lighting environments. The disadvantages of analog recordings are the likelihood of the VHS (video home system) recording medium degrading over time, unless loaded onto a computer, and the processing or digitizing of the video being time consuming. Options in digital processing include online or postprocessing autodigitizing of data collected which reduces time cost.

Optoelectric Systems

Optoelectric systems based on the emission or detection of infrared light are the most effective collection systems for kinematic data. Current infrared based systems, used in conjunction with reflective markers or LEDs, have high framing rates and high resolutions, enabling the capture of whole body movement even as precisely as measuring the deformation of the hoof wall during stance (Thomason and Peterson, 2008). Multicamera setups can localize markers

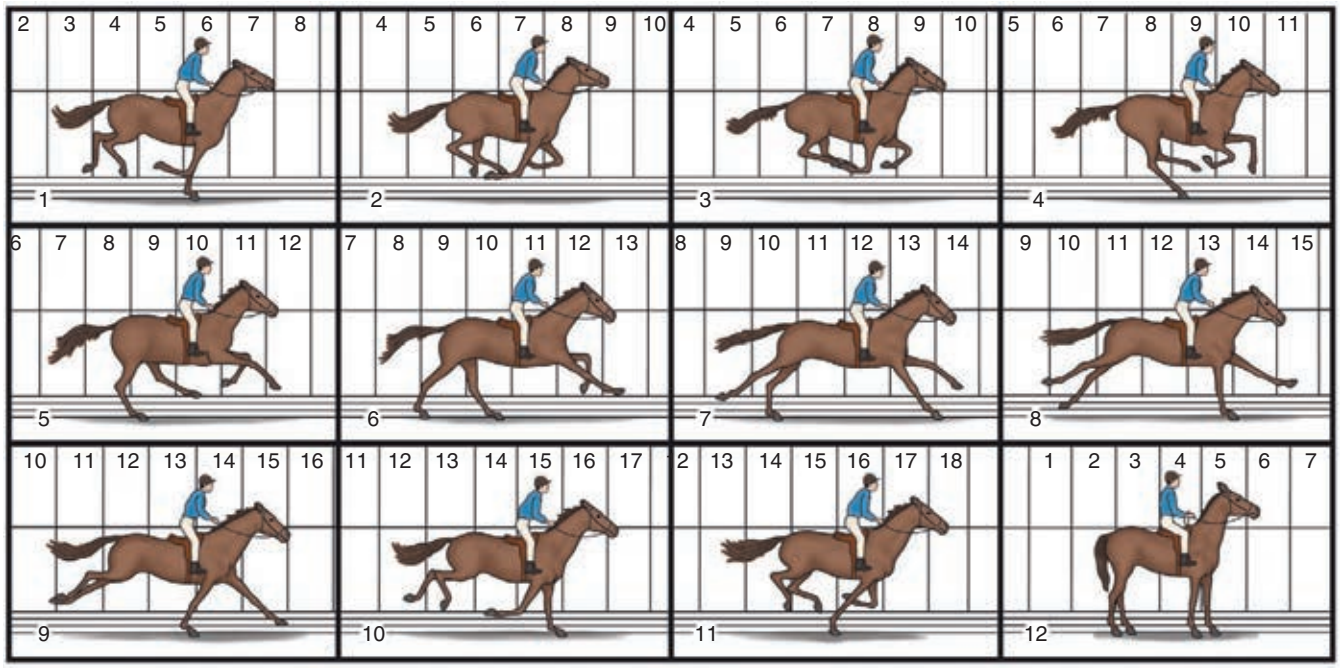

FIGURE 16-3 Photographs of the fast gallop after Muybridge. *(From Goubaux A, Barrier G:* The exterior of the horse *(Harger SJJ, editor), ed 2, 1892, Lippincott, Philadelphia p 487.)*

to within 0.6 mm in a predefined three-dimensional volume, allowing relative positions of markers to be determined using output coordinates (Pfau et al., 2005). The frame-by-frame representation of the changing marker position can be presented as computer-generated stick figures or graphs of joint position plotted against time. The major pitfall in using optoelectric systems is that aberrant markers may result from the sensitivity of the system to the infrared spectrum of daylight or any other reflective surface within the experimental area. Low-pass filtering to minimize marker trajectory noise may cause temporal shifts in key parameters of raw signals, thus distorting or dampening mean phase and magnitude values in the resulting data (Molloy et al., 2008).

KINETIC ANALYSIS

Kinetic analysis is the study of the mass distribution and dimensions of forces acting on the horse during the stance and motion, which are indiscernible to human sensory perception (Weishaupt, 2008). This movement analysis is concerned with the effect of forces on such factors as inertial properties, acceleration, energetics, and work. The main aims of analyzing force production during locomotion are to explain the influences of force on movement (sound or lame); maximal performance; the metabolic cost of locomotion; the possible triggers for gait transition; and the safety margins of musculoskeletal structures during high-speed motion indicating predisposition to injury.

Systems Used to Measure Kinetic Analysis of Gait

Marey (1873) is credited throughout the literature as being the pioneer of equine kinetic analysis. Using pneumatic principles, Marey developed various pressure sensor devices that were attached under the hoof and around the distal limb, measuring and recording pressure changes in cannon bone during hoof–ground contact at various gaits (Figure 16-4). Collaboration with other scientists produced a pneumatic force plate, which was used to measure force in the vertical axis. The same principle is used today, although modern technology allows much more detailed measurements of force and pressure at the hoof–ground interface to be recorded and analyzed. A variety of electronic force sensors have been used in walk, trot, canter, jumping, and lameness studies, and these will be summarized below.

Force Plates

The force plate provides a dynamic, noninvasive, quantitative assessment of the amplitude and orientation of ground contact forces transmitted through one limb during the stance phase. This assessment represents the sum of trunk and limb forces generated by and resulting from the stance and also reflects the acceleration of the body mass of the horse. The orientation of these forces is measured by deflection of sensing elements (strain gauges or piezoelectric quartz crystals) in the three orthogonal components of the ground reaction forces (GRF): (1) vertical, (2) longitudinal, and (3) transverse (Figure 16-5). Vertical GRF is of the greatest magnitude; it most directly measures limb specific weightbearing and sensitivity in grading lameness (Weishaupt, 2008) and so is the most commonly used measurement in force plate studies. Craniocaudal GRF quantitates forces affecting forward progression—braking (deceleration) and propulsion (acceleration). Mediolateral GRF has the smallest amplitude, so few studies have used this variable.

After recorded data are analyzed in all three parameters, peak forces, total impulses (total forces integrated over time), and average force or impulse can be determined, which helps describe the rate and pattern of limb loading and unloading. These data are often expressed in a *force-versus-time curve*, which can be mathematically broken-down into sine and cosine components, allowing convenient and reliable graphic curves to be studied. It is important to evaluate the shape of

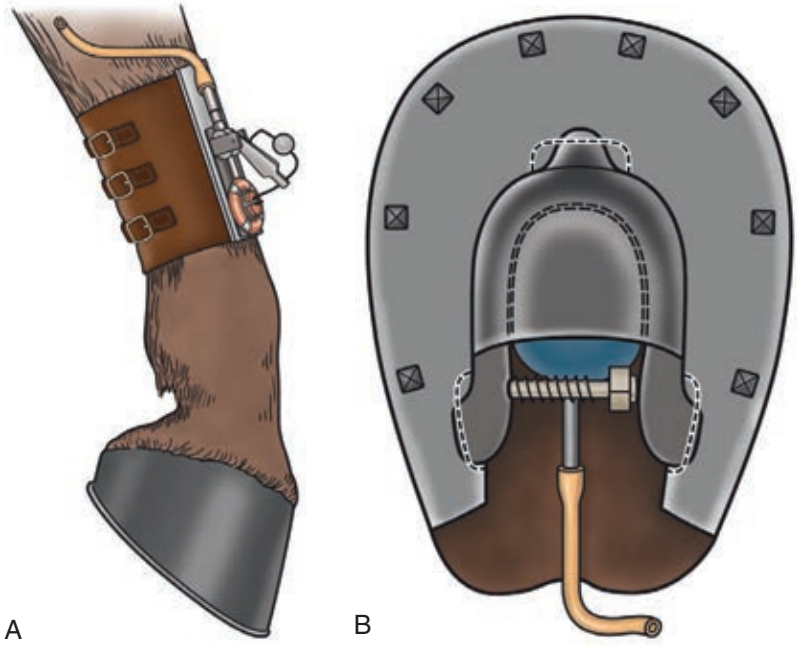

FIGURE 16-4 Pneumatic bracelet (*A*) and foot bulb (*B*) used by Marey (1873). *(From Goubaux A, and Barrier G:* The exterior of the horse *(Harger SJJ, editor), ed 2, 1892, Lippincott, Philadelphia p 175.)*

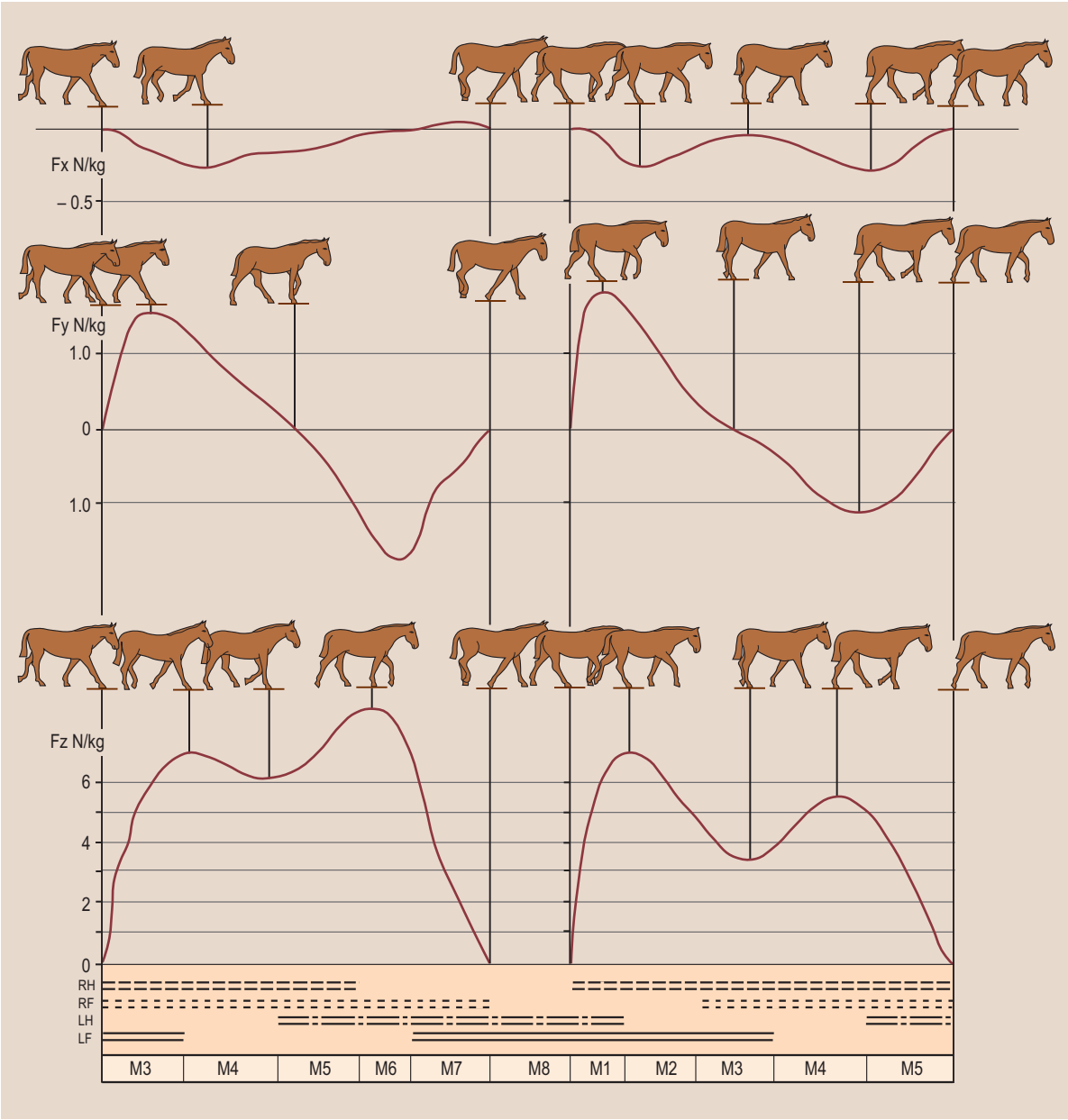

FIGURE 16-5 Limb positioning at the time of characteristic ground reaction force amplitudes of the right forelimbs and hindlimbs of a clinically sound Dutch Warmblood horse at normal walk. The phases of the concurrently loaded limbs are presented in a bar diagram. *(Reproduced from Merkens H: Quantitative evaluation of equine locomotion using force plate data [dissertation]. Utrecht, Netherlands, 1987, State University, with permission.)*

the force profiles in addition to peak values, as mild or transient differences that are meaningful may be identified. In addition, the force plate can identify the coordinates of the point of application of force, enabling measurement of the moment value, vertical torque, center of pressure, and coefficient of friction at this point (McLaughlin et al., 1996).

The sampling frequency and time of data recording can be altered, depending on force plate sensitivity. Although more minute changes in gait will be recorded with more sensitive settings, voluminous amounts of data will necessitate a large computer memory for storing and processing the information. Other possible restrictions of using force plates in equine studies are the initial expense of the force plate, the requisite mounting of the plate in a purpose-built runway in a laboratory situation, the difficulty in standardizing movement velocity, the limitations of high-speed data collection with a stride length greater than 5 meters (m) and the necessity for accurate foot placement on the plate (isolated foot strike centered on the plate) during gait (Witte et al., 2004). This requires repeated trials (five trials are recommended as a minimum), is time consuming, and also creates volumes of data requiring processing. Also, a single force plate can only record the forces through one limb at a time and load distribution generally sampled through at least two concurrent hoof–ground contacts. Increasing the size of the force plate or embedding a force plate in a high-speed treadmill will reduce these problems (see treadmill evaluation).

Pressure Plate

Pressure sensitive plates are an alternative noninvasive means of measuring and analyzing total vertical force and pressure during the stance, enabling calculation of variables such as regional force or pressure distribution (force or pressure per unit area) and total surface contact area (square unit) in a static or dynamic situation. The advantages of the pressure plate over force plate measurements are time efficiency; ease of use; portability for transportation; expense; and provision of immediate analysis of loading under the hoof in both clinical and research settings. The pressure plate cannot however measure horizontal or mediolateral forces, so data analysis is restricted to a basis of vertical force only. One example of a pressure plate is Footscan® scan (RSscan International, Olen, Belgium), which has previously been reported as a reliable tool to measure and quantify hoof balance characteristics (van Heel et al., 2004). The resolution of parallel sensors (four sensors per square centimeter [cm^2]) provided the ability to reliably quantify the different characteristics of force and surface area distribution.

Strain Gauges

Deformation of body tissues occurs with application of tensile or compressive forces, causing lengthening or shortening, respectively. When a bending force is applied, a continuous gradient of deformation occurs through the cross-section of the tissue under strain, with tensile forces generated on the convex side and compressive forces on the concave side. Manufactured from conductive materials such as metal foils or liquid metal, strain gauges are designed to change electrical resistance in response to unidirectional deformation on the axis of the small contact area between tissue and the gauge (1–2 square millimeters [mm^2]). The application of solitary strain gauges to a tissue subjected to a one-way force is an effective and reliable means of measuring tissue behavior under physiologic load, provided that the resting tissue length and the point of zero load can be established. The limitation of using solitary strain gauges in three-dimensional locomotive studies is readily apparent. This limitation is eliminated by combining three strain gauges assembled at 45 degrees to each other, forming a rosette gauge that enables measurement of amplitude and orientation of forces in three dimensions irrespective of the orientation of the gauge to the primary axis of strain. Resistance changes are converted into a voltage output proportional to the strain, which is then converted to digital data for computer processing and analysis. Strain gauges have been used independently to provide direct strain measurement of body tissues; to measure in vivo bone and tendon strains in relation to GRF; to measure muscle force as transmitted by tendons; and in force plates and horseshoes for research. Much of the body tissue research requires an invasive, surgical placement of the devices (which is beyond the scope of this section); however, gauges glued to the hoof wall can measure hoof wall deformation under a variety of loading conditions. In this instance, the shape of the hoof, the state of trimming or shoeing, and variations in surface conditions may influence the data collected (Keegan, 2007).

Instrumented Horseshoes

In an attempt to measure GRF during locomotion, several researchers have designed horseshoes or boots with one or more force sensors attached around the circumference of the hoof, commonly at the toe and quarters. Depending on shoe design, force sensors such as piezoelectric transducers and strain gauges can measure up to three components of the GRF at each point of application. The advantages of instrumented horseshoes are ease of direct application to the hoof; the ability for simultaneous data collection from multiple limbs over a large number of successive strides; and the ability for use on different track surfaces and at high-speed locomotion. The difficulties are producing a shoe stiff enough that its own deformation does not introduce error; maintaining the requirements of grip during locomotion; and preventing any influence on locomotion because of the mass and size of the force shoe (Witte et al., 2006).

Studies on the reliability of instrumented horseshoes or boots are encouraging. Significant correlation between instrumented horseshoe and force plate data was shown when all force sensors were in contact with the ground; however, breakover introduced a variance between these comparative measurements (Roepstorff and Drevemo, 1993). Rollet et al. (2004) successfully and objectively compared the three components of GRF over the hoof on soft ground and hard ground by sandwiching four triaxial force sensors between a support shoe and a GRF recording shoe. Recently, more lightweight (490–860 grams [g]) three-dimensional dynamometric horseshoes have been developed and have been demonstrated to be equally reliable at slow speed (Chateau et al., 2009b), at high speed (Robin et al., 2009), and on the treadmill (Roland et al., 2005). This shows promising results for furthering research outside the laboratory environment.

Accelerometers

Accelerometers detect and measure the magnitude of acceleration and deceleration (gravity induced reaction forces) of the surface to which they are attached and are being increasingly employed in the analysis of equine gait. Single (unidirectional) and triaxial (three orthogonal directions) models are commercially available, and after appropriate processing, these express voltage outputs as linear vector quantities. From these data, the essentially kinematic characteristics of foot-on or foot-off can be determined, stride timing variables calculated, and peak vertical GRF determined. The benefits of accelerometers are multifold. The device is small and lightweight; is easy to apply and use; is cost effective; can capture data from many gait cycles; and, because of its high portability, may be applied in the field, during racing conditions at high speed, and on different track surfaces. The most significant potential source of error in accelerometry is an inconsistent orientation of the sensitive axis of the device. Without accurate information and repeatability of device orientation, the ability to distinguish between the inertial and gravitational components of the signals becomes complex and test–retest studies become noncomparable. Anatomic location should also be carefully considered, as contamination of accelerometric signals from body-mounted devices may also be caused by skin displacement and muscle activation artefacts (Kavanagh et al., 2006).

Most equine studies have applied accelerometers to the hoof wall, thereby eliminating skin displacement and muscle activation artifacts. The resulting data have been used to determine foot landing patterns, impact force characteristics, and associated accelerations. These data allow comparison between various shoeing and trimming techniques and evaluation of different track properties (Chateau et al., 2009a; Ratzlaff et al., 2005). The versatility of accelerometry is evident

in the widely divergent number of equine studies using this methodology. As examples, accelerometers have been applied to the sternum via an elastic girth to measure basic longitudinal and dorsoventral accelerations of the trunk during the walk, the trot, and induced lameness (Barrey et al., 1994); the sternum to measure biomechanical variables between French Standardbred trotters (Leleu et al., 2005); the sternum and sacrum to measure the kinetic characteristics of the forelimbs and hindlimbs at the walk and the trot (Biau et al., 2002); the metacarpal and phalanges via bone screws for in vivo and in vitro quantification of the attenuation of impact vibrations transmitted through the forelimbs with and without shoeing (Willemen et al., 1999); to the head and dorsal pelvis to evaluate lameness (Keegan et al., 2004); and on limbs to measure stride parameters in racehorses at the gallop (Witte et al., 2006). Combining accelerometers with other measuring tools (gyroscope, magnetometers, and thermometers) allows more complex experimentation such as estimation of internal work related to the motion of the center of mass of a horse ridden at high speeds (Pfau et al., 2005).

Recent attempts to determine the reliability of accelerometer-based systems in equine locomotion analysis are positive for future research. Data quantifying forelimb and hindlimb lameness in horses trotting on a treadmill was synchronously collected by a motion sensor system and an accelerometer–gyroscope combination with high linear correlation (Keegan et al., 2004). Direct comparison between force plate and accelerometer data also showed a high correlation (Witte et al., 2004). A preliminary study on three horses found accelerometers to be reliable in measuring the biomechanical differences between two track surfaces at high speed, thus indicating a sensitivity to detect rapid movements despite corresponding small displacements (Chateau et al., 2009a). As in all gait studies, velocity needs monitoring when accelerometers are used. To this end, some recent studies have utilized an electromagnetic tachometer (Leleu et al., 2005), a radar gun calibrated for use at long distances to a sensitivity of 0.16 kilometer per hour (km/h) (Dallap Schaer, 2006), and a global positioning system (GPS) (Pfau et al., 2006; Witte et al., 2006).

The last decade has seen the development of wireless data acquisition systems (WDAS) to be used in conjunction with accelerometers. One such system designed by Ryan et al. (2006) weighs only 148 g and comprises three triaxial piezoresistive accelerometers, a completely wireless system (radio frequency transceiver, data logger, lithium battery), and a remote trigger to sample data from a distance of approximately 25 m. This experimental design has shown promising preliminary results in the measurement of hoof acceleration over several surfaces and exercise or shoeing conditions (Dallap Schaer et al., 2006; Ryan et al., 2006). Although currently limited to individual assessment of load deformation characteristics, further research in larger populations may describe generalizations for a specific population as a whole, allow tailored testing programs designed for individual elite athletic horses, assess biologic risk with specific accelerations in the impact profile, or all of these (Dallap Schaer et al., 2006). Wireless data acquisition eliminates methodologic difficulties previously encountered when foot-mounted accelerometers were hardwired to trunk-mounted computers or data loggers. The placement of wires that cross multiple limb segments is time consuming in application, is difficult to secure, and may be irritating to the horse, thus affecting normal locomotion.

Some technical difficulties may arise when mounting multiple telemetry units in proximity. These include possible radio modulation and signal interference in data transmission; repeat radiation, shielding effects of the horse, or both, which may reduce the transmitting range of the units; and the use of automatic gain control in FM (frequency modulation) analog radio systems, which prevents determination of actual acceleration values, the output voltage being proportional to the acceleration through the stride. MP3 recorders have been tested for logging data from multilimb hoof-mounted accelerometers (Parsons and Wilson, 2006). Mounted distally in custom-made exercise boots at the third metacarpals or metatarsals, the MP3 enabled accurate data collection on timing variables of foot-on and foot-off, but information on signal amplitude was not available.

Electromyography

Normal locomotion is enabled by a combination of stabilizing and dynamic muscle contractions, the coordination and execution of each function determined by an organized recruitment of motor units. A motor unit can be defined as a motor neuron and its accompanying innervated muscle fibers that have a resting membrane potential between –70 millivolts (mV) and –90 mV (depending on slow-twitch or fast-twitch fibers) and an activated membrane potential of +40 mV with respect to the surrounding extracellular medium. Muscle fiber recruitment is preceded by an electrical activation of resting motor units, and the sum of change in the electrical activity across the multiple muscle fiber membranes in contact with the electrodes can be detected, measured, and evaluated using electromyography (EMG). The measurement of electrical activity can be made at rest, during reflex contraction (local reflex arc), and during voluntary contraction as modulated by the central nervous system.

The timing and magnitude of electrical activity associated with muscular contraction can be used to determine the initiation and cessation of muscular activity. This describes muscular activation patterns during various work demands, the number of active motor units, and the frequency at which they fire—*muscle specific recruitment*. After analysis, these data can help elucidate the motor and neural controls of movement, as shown by the coordination of muscles in a given activity, and help describe the functional role of particular muscles in generating movement (Herrel et al., 2008).

Methods of electrode placement for EMG studies can be performed by using either surface electrodes for superficial muscles or fine-wire intramuscular electrodes for deeper muscles. Neither of these methods appears to cause discomfort to the horse, and both appear to be well tolerated during locomotion. A few studies of EMG used in conjunction with cinematography in equine locomotion have been published (Tokuriki and Aoki, 1995), but recent studies using EMG have been scant (Penham and Schobesberger, 2006; Schuurman et al, 2003). This may be attributed to challenging methodologic issues: reduction of interference by proper preparation of the electrode sites; possible influence of adjacent muscles; and the ability to effectively secure electrode placement and surface contact.

To obtain repeatable results, standardization of electrodes and signal treatment is necessary. Konow and Gerry (2008) reported that variant repeatability during intramuscular EMG studies can be attributed to repeated implants; a shift in position of existing implants; fine-wire implanting into different muscle

fiber types, with consequent different activity patterns; electrode morphology; resistance or impedance in electrical equipment; sampling rate; signal conditioning; and the behavior of the subject. For more detailed information on the reliability of EMG, refer to more specialized reviews (e.g., Herrel et al., 2008).

EMG is potentially a powerful tool for research; however, used alone, it has limited utility, as it cannot determine the integrated study of movement or the forces exerted during movement (Loeb and Gans, 1986). To be more effective, this method of measurement is best used in conjunction with other kinesiologic techniques.

TREADMILL EVALUATION OF LOCOMOTION

The treadmill, combined with both kinematic and kinetic techniques, has been used extensively in equine locomotion studies. The major advantages of treadmill study are the ability to control velocity over repeated strides; the ability to collect variables from multiple strides in a single session; the ability to change experimental conditions (e.g., gait analysis with or without induced lameness; with or without altered surface types; with or without surface inclinations; with or without unmounted, ridden, or weight-loaded subjects); and the objectivity of treadmill gait analysis eliminates subjective bias. The disadvantages of treadmill gait studies are as follows: the method is dependent on a laboratory environment; an energy transfer may occur between the treadmill and the subject; the treadmill belt may change speed during stance phases moderating acceleration and deceleration; gait speed is typically limited up to 15 ms^{-1}, which may constrain maximal performance of an elite athletic horse (gallop speed of a Thoroughbred reaches up to 20 ms^{-1}); and the horse requires habituation for steady-state treadmill locomotion prior to meaningful studies. Three sessions of habituation at the trot was shown to be sufficient to obtain constancy in stride parameters; however, after 10 sessions at the walk, some horses still displayed significant variations in stride duration and limb movement angle (Buchner et al., 1994b). These findings are possibly related to an increased need for a more efficient gait at speeds higher than 6 ms^{-1}; the horse may concentrate more at higher speeds (van Weeren et al., 1993), or the complex combinations of multiple and varying overlapping stance times at the walk may affect reproducibility of gait parameters.

In addition, some authors claim treadmill gait creates artefacts and is not perfectly equitable to unrestrained natural locomotion in linear, temporal, or energetic parameters (Clayton and Schamhardt, 2001; Jones et al., 2006; Pfau et al., 2006; Witte et al., 2006). Buchner et al. (1994a) found increased vertical trunk oscillations and a 7% longer stance duration of the forelimb during treadmill gait. Conversely, a recent study by Gomez Alvarez et al. (2009) found no difference in stride parameters. Two possible explanations are advanced by these authors: (1) a closer approximation of surface compliance between treadmill and overground locomotion studies (gravel versus tarmac) and (2) a higher level of habituation of their subjects. These authors found minor differences in vertebral kinematics in this study. Despite possible artefacts in stride parameters, the treadmill is a useful tool for comparing and reassessing *treadmill* locomotion under clinical or research conditions.

Instrumented Treadmills

Treadmills fitted with an inbuilt dynamometric platform designed to record kinetic forces allow simultaneous force data collection from any weightbearing limbs during multiple and consecutive motion cycles at a controlled speed during different gaits. Though this system allows diverse studies during locomotion, this is not a commonly used tool in research because of the prohibitive setup cost and expertise required for operation (Weishaupt, 2008; Weishaupt et al., 2009).

NORMAL LOCOMOTION: GAIT CLASSIFICATION IN HORSES

Gait is defined as a characteristic, repeating sequence of footfalls recognized by a consistent limb coordination pattern. Horses display many different innate gait patterns, and training can enhance these and even develop other unnatural gaits. This section will deal primarily with the major natural symmetrical and asymmetrical gait patterns shown by athletic horses. *Symmetrical gaits* are the gaits in which each forelimb or hindlimb is considered to be employed equivalently with very similar kinematics (movement) and kinetics (forces). Left and right footfalls occur with a regular rhythm or at approximately equal time intervals. The common symmetrical equine gaits are the walk, the trot, and the pace, which are distinguished from each other by speed, interlimb coordination of thoracic and pelvic limbs, relative durations of the stance and swing phases, and the presence or absence of the suspension (aerial) phases.

Asymmetrical gaits are the gaits in which individual limb function is considered to be different both in kinematics and kinetics. An uneven stance phase causes alternating short and long intervals in locomotive rhythm with the footfalls occurring as couplets (Clayton, 2004), interlimb coordination is far more complex than with symmetrical gaits, and a suspension stage is always present. The last forelimb to leave the ground prior to the suspension or aerial or flight phase is known as the *lead limb*, the other forelimb being known as the *nonlead* or *trailing forelimb*. Common asymmetrical equine gaits in athletic horses are the gallop and the canter (collected gallop) and are differentiated similarly to the symmetrical gaits. All the mentioned gait styles are described in detail below using the following abbreviations: forelimb (FL), hindlimb (HL), left hind (LH), left fore (LF), right hind (RH), right fore (RF). The beat described is the number of foot strikes distinguishable per single stride, whether these are individual or simultaneous landings.

SYMMETRICAL GAITS

Walk

The walk is a slow-speed, regular, four-beat gait with independent and distinct ground contact phases for all limbs. This provides an alternating tripedal (2 HL/1 FL or 2 FL/1 HL) and bipedal (lateral or diagonal pair) support sequence so facilitating balance the majority of the time. Head and neck flexion and extension also help maintain balance between braking and acceleration during this gait (Khumsap et al., 2002). The limbs move a quarter period (or 25%) out of phase in a "figure-of-8" wave (Collins and Richmond, 1994), resulting in the following footfall sequence: LH, LF, RH, RF recurring. These footfalls are theoretically equally spaced in time; however, Clayton (1995) showed frequently that either an early or delayed placement of the forelimb can affect the relationship of footfalls between forelimbs and hindlimbs without affecting the left–right symmetry. There is no suspension phase in this gait, so an increase in velocity is dependent on moving the

center of mass of the horse further forward during the stance period. As the walk speed increases, the stance phase of individual limbs shortens; thus, the large overlap of multilimb support decreases, bipedal support increases, and there is an increasing dependence on forward momentum to maintain balance (Clayton, 1995).

Trot

The trot is a medium-speed, regular, two-beat leaping gait with a primarily *diagonal* bipedal support base, facilitating balance in addition to forward momentum. With increasing speed, the trot footfall pattern tends to adduct (move toward the median plane) toward the center of mass of the horse, thus increasing stability. The equilibrium between energy expenditure and forward movement at the trot make it the horse's natural choice of gait.

Biphasic vertical excursions of the head and neck into flexion and extension are seen during a single stride at a normal trot but are far less compared with the walk because of the diagonal stability of this gait. Indeed, excessive or asymmetrical head-and-neck movement at the trot is a clear clinical indication of some degree of lameness. Diagonal limb pairs move mostly synchronously in half periods (or 50%) out of phase with one another with a short suspension phase between each diagonal ground contact creating the footfall pattern: LH/RF, suspension, RH/LF, suspension.

As the trotting speed increases, the synergy between forelimbs and hindlimbs tends to become slightly out of time, which is referred to as *advanced placement* or *diagonal disassociation*. This is a desirable movement quality that is dependent on the intended task of the horse. A *positive diagonal disassociation*, where the hindlimb is grounded before the forelimb, is preferred in dressage horses. It appears to be related to high trot scores, independent of the degree of collection, and may be indicative of the horse's natural balance. Conversely, at race speeds, Standardbred trotters tend to ground and take off with the forelimb in advance of the diagonal hindlimb—*a negative diagonal disassociation*. The support base in this gaitpattern requires interlimb coordination and central balance, as a brief period of single forelimb support is followed by a bipedal support phase and a brief hindlimb support phase. From a biomechanical aspect, this, in theory, would allow the forelimb to absorb large amounts of locomotive forces via a nonbony thoracic attachment and also allow the articulated hindlimb to exert higher propulsive forces with less resistance from forelimb ground contact.

Pace

The pace appears to be genetically determined and is found primarily in Standardbred racehorses (Cothran et al., 1987). It is similar to the trot in that it is a medium-speed, regular, two-beat leaping gait, the difference being the *unilateral* synchronous forelimb and hindlimb movements, which, together, form a bipedal support base with strides left to right moving 180 degrees out of phase with each other: RF/LF, suspension, LF/RF suspension. Increasing speed causes a tendency for significant asymmetry between subsequent stride lengths, interlimb coordination, and flight phases in the pace (Drevemo et al., 1980; Robilliard et al., 2007).

ASYMMETRICAL GAITS

Gallop

The gallop is a rapid, four-beat, asymmetrical leaping gait, and horses typically use a transverse gallop, where the limb placement crosses the body axis giving a footfall sequence of LH, RH, LF, RF, suspension on a right lead (Figure 16-6). Occasionally, the horse will use a rotary gallop, the footfalls following a circular pattern: LH, RH, LF, RF on a right lead. Fatigue may induce

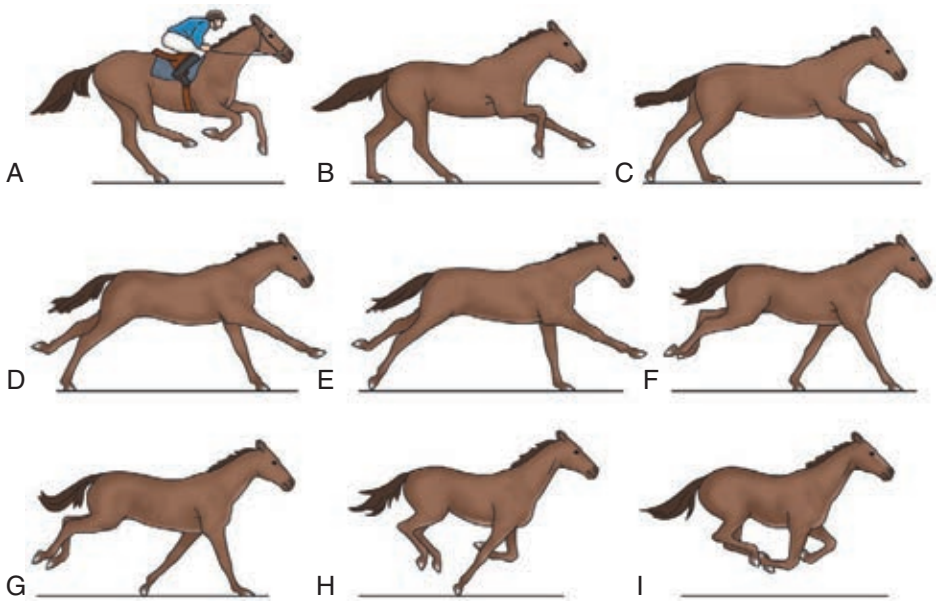

FIGURE 16-6 Right lead transverse gallop. The gait characteristics during one complete stride cycle at 16 meters per second (m/s) (960 m/min). **A,** Nonlead limb landing. **B,** Lead hindlimb landing. **C,** Nonlead hindlimb takeoff. **D,** Nonlead forelimb landing. **E,** Lead hindlimb takeoff. **F,** Lead forelimb landing. **G,** Nonlead fore limb takeoff. **H,** Lead fore limb takeoff. **I**, Suspension phase and stride stance phase (A-H). *(From Hellander J, et al:* Basic gait variables of the canter and gallop in relation to horse velocity, vol 35, p 75–82 Svensk Vet Tidn *(Suppl 3):75, 1983, and artist Bo Furugen, with permission.)*

a change in the lead or style of the gallop. In the gallop, the forelimbs and hindlimbs work in two skipping pairs, with velocity-dependent overlap in the stance phases of each limb. Increasing speed results in less stance duration for each individual limb, a higher stride frequency, and a higher stride length with consequent decreased overlap of multilimb ground contact, with overlap time approaching zero at maximum speed (Witte et al., 2006). There is generally only one suspension phase, the duration of which is independent of speed (Witte et al., 2006). The vertical excursion of the center of mass appears to fall in a curvilinear manner with increasing speed at the gallop; however, craniocaudal and mediolateral displacement of the center of mass have been shown to increase (Pfau et al., 2006). Balance is, therefore, possibly maintained primarily through momentum and is reliant on the horse's natural stability.

Canter

The canter is a controlled, three-beat, leaping gait that is best considered by foot placement and support base. The beat is created by the grounding of the trailing hindlimb, followed by a mostly synchronous landing of the diagonal trailing forelimb and leading hindlimb and then the leading forelimb. The support base alternates between one, two, and three overlapping phases: LH, RH/LF +/– LH, LF/RF +/–RH, RF, suspension in a right lead canter (Figure 16-7). This is a similar sequence of footfalls as the gallop, but the second hindlimb and the first forelimb leave the ground at the same time. In this gait, it is speculated that one hindlimb provides propulsion, while the other three limbs are moving forward. A tripedal stance follows, with both hindlimbs and the diagonal forelimb bearing weight, followed by one propulsive forelimb initiating forward momentum and the other three limbs being airborne and moving forward.

GAIT TRANSITION

After the descriptions of the various traditional classifications of gait styles, it should be mentioned that there has long been a suggestion that equine gait may be a continuum of variations in locomotive patterns rather than the discrete gait entities as described. Analyzing footfall events, Robilliard et al. (2007) found clusters of force patterns within the continuum of the running gaits (trot, pace, canter, and gallop), which supports this hypothesis. The walk was found to be a separate entity. This is in keeping with the inverted pendulum versus elastic recoil energetics of gait and the presence or absence of the suspension phases. If this is the case, it is important to understand what prompts a horse to move through the continuum during free locomotion.

The primary aim of equine locomotion is to generate forces to counteract the effects of perturbations within the movement and the environment while maintaining the desired speed and direction of movement with minimum metabolic expenditure. The horse's "preferred speed" during any particular gait tends to hover within a narrow range of velocities central to the broader spectrum of speeds available, which suggests an energy economic strategy. Proprioceptive input from muscle resulting from metabolic activity (conversion of metabolic energy to mechanical power) has been suggested as the mechanism controlling preferred speed and, thus, maintaining metabolic economy (Wickler et al., 2001). Velocity can be increased by an energy-expensive extension of the kinematic characteristics of stride or by changing the gait style. Gait transition occurs at a characteristic speed; however, the triggers governing this locomotor paradigm are still unknown. Metabolic economy and mechanical cueing as determinants of gait selection have been the primary focus of equine studies; however, no definitive consensus has emerged. This may be attributed to varying methodologies within the research, such as different breeds and sizes of horses, varying gait speed increments, and the different gait transitions studied (walk to trot, trot to gallop, and vice versa).

Minimization of energetic cost has been proposed as the trigger for gait transition because of the variation of preferred speed and speed at transition (Griffin et al., 2004; Wickler

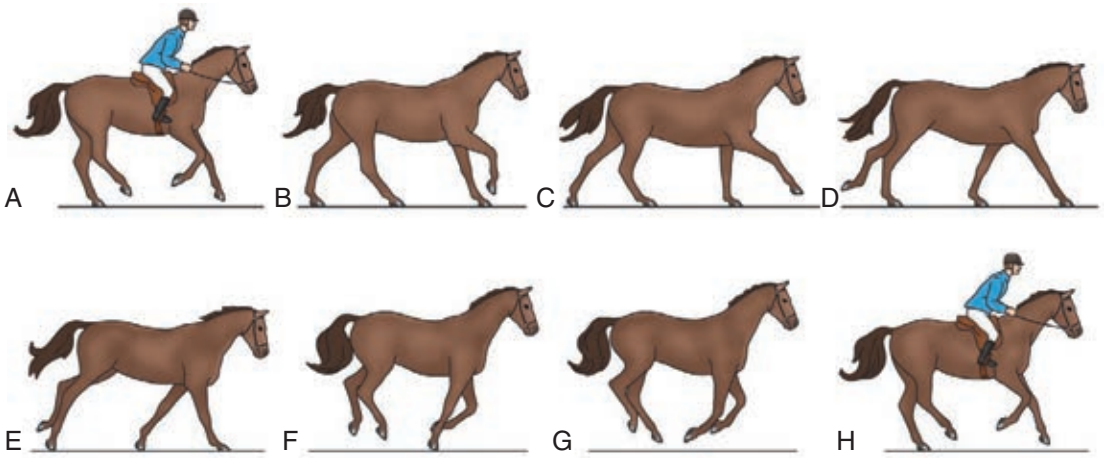

FIGURE 16-7 Right lead transverse canter. The gait characteristics during one complete stride cycle at 6 meters per second (m/s) (360 m/min). **A,** Nonlead hindlimb landing. **B,** Lead hindlimb and nonlead forelimb landing simultaneously. **C,** Nonlead hindlimb takeoff. **D,** Lead forelimb landing. **E,** Lead hindlimb and nonlead forelimb takeoff. **F,** Lead forelimb takeoff. **G,** Suspension phase; **H,** Nonlead hindlimb landing and stride stance phase (A-F). *(From Hellander J, et al:* Svensk Vet Tidn Basic gait variables of the canter and gallop in relation to horse velocity, vol 35, p 75–82 *(Suppl 3):75, 1983, and artist Bo Furugen, with permission.)*

et al., 2003). This theory has mixed support in the literature because of the difficulty in determining a definitive link between metabolism and gait selection. As horses naturally change to a faster gait at speeds slower than predicted by metabolic cost, which actually increases energetic cost, it has been postulated that gait selection is reliant on mechanical cueing when peak GRF causes musculoskeletal stress to reach a critical level (Farley and Taylor, 1991). This does not explain gait transition from the gallop to the trot, as forces and strains will increase during this transition (Wickler et al., 2003). Reliant on the intrinsic properties and integrity of musculoskeletal structures, sensory input from muscles, joints, ligaments, tendons, and bone, in combination with motor outflow responses (as quantified by muscle activation), may cause a sense of effort of locomotion and trigger a gait transition. Similar description of gait transition in human studies has been found where higher joint moments at a fast walk require increased muscle activation to swing the leg, thus increasing the sense of effort and triggering a run (Prilutsky and Gregor, 2001). The run–walk transition in this study was correlated with increased activity of core stabilizing musculature. This is perhaps an explanation for the gallop–trot transition, as a decrease in stride frequency causes a longer swing phase, reducing stabilizing demands. The source of control attempting to balance gait requirements of forward stable locomotion may, therefore, be a neural response either at a supraspinal level (Pribe et al., 1997) or from a local decentralized autonomous reflex network (Geyer et al., 2003). A supraspinal control center may also support the previously mentioned concept that each gait style is not entirely independent of the next but is a transitionary movement pattern in response to neural activation. Some studies could hypothetically add credence to one or more of these theories. Drevemo et al. (1980), for example, demonstrated a horse trotting at speeds close to its normal transition to the canter or the gallop shows the most variable asymmetry of stride length and contralateral interlimb coordination. This may indicate an energetic trigger, a peak GRF trigger, a neural balance trigger or a combination of triggers. It is clear from this brief synopsis that more research is necessary to elucidate the definitive mechanism for preferred speed of locomotion and gait transition in horses. Drawing from human gait studies to conclude this section, it is proposed that possible broad parameters for future equine research in this area include kinematic discontinuities; mechanical loading; muscle activation and prevention or overuse; central motor control; decentralized autonomous responses; mechanical restrictions such as maximal joint angles; and critical velocity of joint motion.

INTERLIMB COORDINATION AND THE PRINCIPLES OF NEUROMOTOR CONTROL

It is widely accepted that limb coordination, rhythm, and speed through all gaits is generated and controlled, in part, by central pattern generators (CPGs). These specialized interneuron circuits located within the spinal cord have an ability to produce rhythmic patterned locomotion independent of supraspinal input (Clayton, 2004; Collins and Richmond, 1994; Pribe et al., 1997). There are separate control centers for thoracic and pelvic limbs, although they are interconnected by long neurons to allow phasic coordination between the front and the rear of the horse. Normal locomotion also depends on the ability of the central nervous system (CNS) to receive and process all sensory information (such as proprioceptive, cutaneous, visual, and vestibular inputs) to initiate and complete movement in an effective, coordinated, energy conserving fashion. CNS feedback in response to afferent input can be either through a monosynaptic reflex arc or through the action of the CPGs. As the CPGs can sequence appropriate activation of motor neurons in addition to determining the threshold of other interneurons involved in afferent and efferent transmission, the final motor output and CPG competency during locomotion is modulated by an intact afferent and supraspinal system. The complex relationship between neural and muscular (motor) control of locomotion is referred to as *neuromotor control*.

NEUROMOTOR CONTROL

Neuromotor control not only modulates the dynamic limb patterns of locomotion, it is also highly important in providing a stabilizing "core" to support the skeleton during locomotion. Unwarranted and unnecessary joint motion or muscle activation places the musculoskeletal system at risk for injury. This has been shown to have important implications in humans, especially with regard to the spine and pelvis and also the peripheral joints.

Spinal stability and control of movement is highly dependent on the contribution and coordination (the pattern of when and how the muscles activate) of the muscular system. In the past, attention has focused primarily on muscle strength. It is really about real-time neural feedback, compensatory mechanisms, and motor adaption to changing environments. It is now realized that the CNS control of muscle coordination is probably of greater importance to the musculoskeletal ability to satisfy reactions when perturbations and forced environments challenge the dynamics of movement (for a review, see Hodges, 2004). The CNS must plan suitable strategies of muscle recruitment and levels of activity to finetune the demands of internal and external forces, thus initiating appropriate motor responses to unexpected disturbances of movement and function.

In humans, all of the many muscles of the back and trunk contribute to movement production, spinal control, and support. Nevertheless, research has shown that the deep, local muscles of the region (e.g., the transverses abdominis and the segmental lumbar multifidii) have key roles in modulating the stiffness of the lumbar spinal segments and pelvic joints during limb and lumbopelvic movements (Hodges et al., 2005; Indahl et al., 1997; Richardson et al., 2002). It has also been shown that the CNS preprograms activity in certain trunk muscles in preparation for limb movement, but activity in the transverses abdominis always occurs prior to limb movement. This appears to be regardless of movement direction, purportedly to use the attendant rise in intra-abdominal pressure and tensioning on the thoracolumbar fascia to increase segmental stiffness for spinal segmental support prior to loading (Hodges and Richardson, 1997; Hodges and Richardson, 1999). Ironically, several key studies have shown that in the presence of low back pain, the strategies used by the CNS to control trunk muscles may be altered, and less efficient muscle recruitment strategies result (Hodges, 2001). Patients with back pain, for example, display delayed activation of the transverses abdominis, depriving the painful and injured spinal segments of timely support.

Knowledge gained from this research related to the changes in neuromotor control that occur with back pain have

translated to the development of new rehabilitation strategies for the lumbopelvic muscles in patients with back pain. MacDonald and colleagues (2006) have recently reviewed the application of this evidence into clinical practice. This rehabilitation places emphasis on motor relearning to optimize motor control for spinal dynamic stability. The rehabilitation first uses the end organs of the neuromotor system—the muscles—with the aim that cognitive, repeated contractions of the muscles and correct movement patterns will result in a transition to automated use such as in skill training (O'Sullivan et al., 1998). Initially, deep muscles such as transverses abdominis and lumbar multifidus are repeatedly activated in the relearning process during rehabilitation. Movement patterns and strategies for all trunk muscles are then re-educated to perform painless and controlled functional activities. The stability system is then functionally challenged with load (static and dynamic exercises) as control is improved. Most important, there is growing evidence that this exercise approach can reduce low back pain and possibly reduce its recurrence rate (O'Sullivan et al., 1997). Specific physiotherapeutic intervention in people with multifidus dysfunction following an episode of acute back pain reduced the rate of recurrence of injury to 30% in the physiotherapy intervention group compared with 84% in the control group (Hides et al., 2001).

SENSORIMOTOR SYSTEM AND PERIPHERAL JOINT FUNCTION

The sensorimotor system is another related area of human physiotherapy research that has had a large impact on the treatment and rehabilitation for peripheral joint injuries, including the ankle, knee (Lephart et al., 1998), and shoulder (Myers et al., 2006). Prevention of these injuries and maximizing performance in athletes has been a subject for research on the sensorimotor system and proprioception of joints (Riemann and Lephart, 2002a; Riemann and Lephart, 2002b). Research in this area involves using evidence-based techniques to determine the normal controlled movement of any particular joint and restoration of that control when it is lost because of injury or dysfunction. Stability in a joint is controlled by a combination of its mechanical restraints (such as soft tissue and bone geometry) and dynamic restraints, which are mediated by the sensorimotor system, including proprioceptive feedback and neuromuscular control (Myers et al., 2006).

Taping techniques are often used to enhance the sensorimotor system in humans at any level of sport. The aim of taping techniques is to stimulate mechanoreceptive and proprioceptive activity in skin, fascia, ligaments, and joints. This affects the sensory afferent activity from that region, modulating or altering the neuromotor control of locomotion. Patellar taping was shown to improve control of the knee joint in pain-free people with poor joint proprioception (Callaghan et al., 2002) as well as in people with femoropatellar pain (Herrington, 2006; Worrell et al., 1998). Similarly, the combined mechanical and proprioceptive benefits of taping for preventing ankle sprains as well as recurrence of sprains is well documented (Arnold and Docherty, 2004).

APPLICATIONS IN HORSES

Taping has been used clinically in horses for similar purposes as in humans (Figure 16-8). One study investigated the biomechanical effects of taping the fetlock in the forelimbs of horses (Ramon et al., 2004). In this model, mechanical effects, rather than sensorimotor effects, were being investigated, and the results suggested that the observed changes may be a reflection of proprioceptive adaptations. Effects on ground reaction forces and joint ranges of motion were observed, but further research is warranted. More recent physiotherapist-led research has clearly shown the effects of tactile stimulation of the distal hindlimb in horses, causing increased hoof flight arc, increased peak flexion angles in the hindlimb, and increased concentric activity of the tarsal musculature (Clayton et al., 2010). This is clearly an appropriate area for equine research, yet research, to date, has been very limited.

Research in equine physiotherapy using both functional biomechanics and the human physiotherapy model has been used to investigate various equine problems, including sacroiliac joint biomechanics (Degueurce et al., 2004; Goff et al., 2006) and equine back pain (Denoix, 1999; Stubbs et al., 2006). Stubbs and colleagues (2006) applied the human research model of the multifidus muscle to the horse and found striking similarities in structure and function. The multifidus in the horse has been shown to be morphologically orientated in a similar manner and thus functions in a comparable way to that in humans.

Current epaxial muscle research in the horse is underway. Ultrasonography has been used to detect changes in multifidus muscles associated with back pain, as has been seen in humans (Stubbs et al., 2010). This may influence the way we diagnose and treat equine back pain, as in the case of humans.

Research into the biomechanics and neuromotor control of the human sacroiliac joint (SIJ) has contributed to the ability of clinicians to diagnose sacroiliac dysfunction in humans. It has been shown that noninvasive, manual SIJ provocation tests are as predictive for SIJ being the source of pain as are diagnostic joint blocks (van der Wurff et al., 2006). These tests are described as pain provocation tests for the SIJ and are designed to compress the SIJ articular surfaces, stress the extra-articular structures of the joint, or both. Manual tests based on the amount (hypomobility or hypermobility) and quality of motion at the SIJ are also used clinically to gather diagnostic information regarding the functional status of the SIJ. This includes the degree of movement of the SIJ during the application of manual force and, specifically, in analyzing the movement of the sacrum relative to the pelvis in weight-bearing through the pelvis (Lee, 2004).

Past research on the diagnosis of equine sacroiliac disease using manual tests, similar to those used in humans, has been alluded to in the literature (Haussler, 2003; Varcoe-Cocks et al., 2006). Recent research documenting motion between the sacrum and ilium during the application of manual force has been reported (Goff et al., 2006). Manual forces applied by a physiotherapist were used to demonstrate the direction and amount of movement of the ilium relative to a fixed sacrum in an in vitro model. The application of this research to horses in vivo and in horses affected by sacroiliac disease would support the use of manual tests in horses as in humans.

The net result of neuromuscular control allows the complex musculoskeletal design of the horse to counter changes in the physical environment and execute appropriate stabilizing and voluntary movements for the intended task. It must also be remembered that normal locomotion requires a synergy between musculoskeletal, cardiovascular, respiratory, metabolic, and neurologic systems, so any performance is dependent on a myriad of factors. Aspects of these interrelated systems are addressed in other chapters of this text.

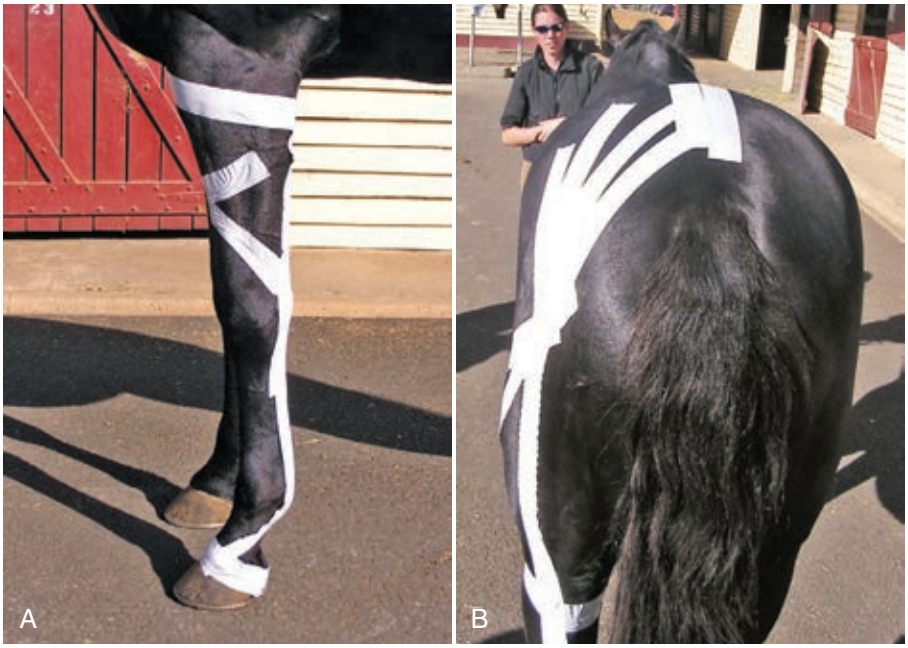

FIGURE 16-8 Examples of proprioceptive taping. The tape is worn during the rehabilitative exercises including all forms of locomotion including work in hand and under saddle. Tape used should be elastic as this is not a mechanical restraint. Kinesio® (www.kinesiotaping.com) is one product suggestion as the tape is porous and allows for sweating without loss of adhesiveness. The tape is best applied to a short coat or shaved body region. In these figures the tape used is purely for demonstration purposes for these images. **A,** Distal forelimb taping technique to stimulate proprioceptive feedback for the superficial digital flexor tendon from origin to insertion in the forelimb. **B,** Hind quarter taping technique to stimulate proprioceptive feedback for pelvis and pelvic limb position sense, and Biceps Femoris and superficial gluteal muscle activation.

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CHAPTER

17 Kinematics of the Equine Back and Pelvis

RENE VAN WEEREN

The axial skeleton consists of the vertebral column from the first cervical vertebra down to the last coccygeal vertebra, and the pelvic bones. Together with the skeletal structures in the limbs, called the *appendicular skeleton*, it forms the bony basis of the equine musculoskeletal system that gives the horse its speed and agility. The species was originally domesticated approximately 5000 years ago (Dunlop and Williams, 1996). The role of the horse in society has changed significantly over the past 50 years from an economically, militarily working animal to a popular sports and leisure animal. The musculoskeletal system of the horse is considered the horse's main asset. It may be not surprising, therefore, that disorders of the musculoskeletal system are the main reason for wastage of racehorses (Rossdale et al., 1985; Williams et al., 2001). Epidemiologic studies of equestrian disciplines other than racing are virtually nonexistent, but there is general consensus among equine veterinarians that the same applies to almost all other equine athletic activities.

Historically, equine orthopedics has focused on limbs rather than the back. This is partly because not much overt lameness has its origin in the back but also because of the limited accessibility of this vast, complex structure. However, in the modern elite sport horse, it is often not the obvious, overt lameness that presents the biggest challenge to the equine practitioner but the marginally worse-than-normal performing horse that may suffer from very subtle pathologies or, more often, a combination of very minor to subclinical pathologies, adverse environmental conditions such as suboptimal riding or training techniques, ill-fitting tack, and so on. In this subtle interplay of physical factors and environmental conditions, as well as the mental states of both the horse and the rider, any impairment of function of the back or the pelvis may be hard to define, as is the case in humans, in whom lower back pain is one of the most important, but equally intangible, causes of occupational disability (Waddell and Main, 1998). The difficult accessibility of the equine back for diagnostic purposes, the complex interaction of back (dys) function with other physical and mental factors, and the lack of objective quantitative methods to evaluate therapies have provided an ample opportunity for the myriad of "equine therapists," with highly variable degrees of skill and trustworthiness, who claim to be able to influence the function of the equine back. Installed therapies range from treatments that at least theoretically could have some physiological basis, for example, chiropractic treatment, to completely irrational actions such as imposition of hands or sending a hair from the affected animal to somebody with a black box, who will provide the diagnosis by mail (Jeffcott, 1998).

Thanks to the significant and rapid advancements in both diagnostic imaging techniques and kinematic analysis in the past two decades, our understanding of the function and dysfunction of the equine back has improved considerably. These technological advances have greatly improved our diagnostic capacity and are a great help in the rationalization of the therapeutic approach to the equine back. In fact, they may help in the demystification of many therapies that claim effectiveness on irrational grounds. In the following sections of this chapter, which discusses the kinematics of the equine back and pelvis, a short introduction is given into the current biomechanical concept of the quadrupedal back. After a quick overall review of the fundamental research that has led to the currently used techniques to quantify kinematics of back and pelvis (for a more in-depth review, see van Weeren, 2009), the discussion will move on to the applications of back-related kinematical research to the modern performance horse and the significance of research outcome for both equine health care and the equestrian sports. The chapter concludes on a more speculative note, discussing some likely future developments and their possible impact.

BIOMECHANICAL CONCEPT OF THE QUADRUPEDAL BACK

The Roman physician Galen (129–200 A.D.) is the first scientist known to have written about the concept of the mammalian back, describing it as a vaulted roof sustained by four pillars, that is, the limbs (cited by Slijper, 1946). The next concept dates from the middle of the nineteenth century. It depicts the back as a bridge, with the limbs representing the land abutments of the bridge, the gap between these being spanned by the bridge itself (Bergmann, 1847;Krüger, 1939; Zschokke, 1892). The bridge consists of an upper ledge, representing the supraspinous ligament; a lower ledge, representing the vertebral bodies; and a number of smaller girders between them, pointing in either the craniodorsal or the caudodorsal direction, representing the spinous processes and the interspinous ligaments (Figure 17-1). This model was generally accepted until the time of World War II and is even used by some today when the biomechanics behind equine locomotion and certain equestrian activities are discussed. However, it contains an important conceptual error. The upper ledge representing the supraspinous ligament and the lower ledge representing the string of vertebral bodies imply that the upper ledge is loaded under tension and that the lower ledge is under compression because ligamentous structures (such as the supraspinous ligament) inherently cannot withstand compressive loads. In reality, however,

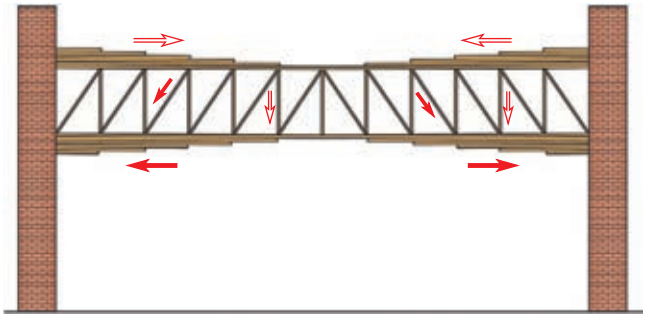

FIGURE 17-1 The bridge concept of the vertebral column as depicted by Krüger. Open arrows represent tensile forces and closed arrows compressive forces. *(From Krüger W: Über die Schwingungen der Wirbelsäule–insbesondere der Wirbelbrücke- des Pferdes während der Bewegung,* Berl Münchn Tierärtzl Wschr *13:129, 1939.)*

the always-downward-facing gravitational forces that act on bridges and mammalian trunks alike cause compression in the upper ledge and tension in the lower one.

The bridge model has been replaced by the so-called "bow-and-string" concept, in which the bow is the thoracolumbar vertebral column and the string is the "underline" of the trunk, consisting of the *linea alba*, the *rectus abdominis* muscle, and related structures. The concept was already described in the late eighteenth century by Barthez (1798), but apparently he was too ahead of his time, and the model did not become generally accepted until its rediscovery by Slijper (1946) (Figure 17-2). The bow-and-string model is the first concept that takes a holistic view of the entire trunk and is not limited to only the thoracolumbar vertebral column with adnexa. As in an old-fashioned hand-bow, the entire system is under intrinsic tension due to a dynamic balance, and changes in one component will inevitably influence the biomechanical dynamics of the other. There are many factors that influence this dynamic balance (Figure 17-3). Gravitational forces will always act along a vertical line in a downward direction. They will, thus, tend to straighten the bow, that is, extend the back or make the back hollower. Of course, every horse is subjected to gravitational forces, but all factors that increase the mass on which the gravitational forces act will make the effect more pronounced. These factors include pregnancy (the typical hollow-backed broodmare), tack, and, of course, the rider, whose weight is of considerable importance, as elegantly demonstrated by DeCocq et al. (2004). Muscular action is another factor that influences the dynamic equilibrium between the bow and the string. Contraction of the ventral musculature will tense the bow, that is, flex the back or make it more arched. Contraction of the massive epaxial musculature will have the opposite effect because the work line of these muscles runs dorsal to the axis through the centers of the vertebral bodies. The only dorsally located muscles that have a flexing effect on the back are the *psoas* muscles. Because these are located along the pelvis, the ventral aspect of the lumbar, and the last three thoracic vertebrae (Koch, 1970), they will principally affect lumbosacral flexion. Further toward the craniad direction, there is no musculature ventral to the vertebral column, hence no flexing action on the spine occurs. Protraction and retraction of the limbs are important events that strongly affect the balance in the bow-and-string system. Protraction of the hindlimbs results in a more forward position of the point of support. Given the anatomic connection between the *gluteus medius* muscle and the lumbar and sacral spinous processes through the gluteal and lumbodorsal fascia (Koch, 1970), this will have a flexing effect on the back (tensing of the bow). Retraction of the forelimb will have a similar effect through increased forward traction on the spinous processes of the first few thoracic vertebrae that form the basis of the withers. The opposite movement of the limbs, that is, protraction of the

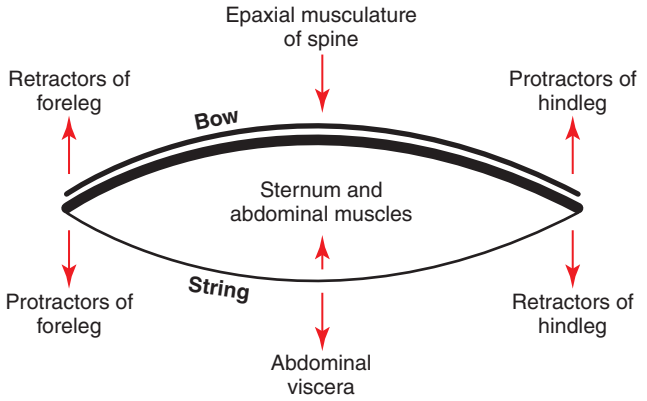

FIGURE 17-3 Factors that determine the motion of the back according to the "bow-and-string" concept. Upward-pointing arrows indicate a flexing effect on the back and downward-pointing arrows represent an extending effect.

FIGURE 17-2 "Bow-and-string" concept of the back, according to Slijper. The vertebral column is the bow, the ventral musculature and the sternum are the string. The ribs, lateral abdominal musculature, spinous processes, and ligamentous connections are additional elements. *(From Slijper EJ: Comparative biologic-anatomical investigations on the vertebral column and spinal musculature of mammals,* Proc K Ned Acad Wetensch *42:1, 1946.)*

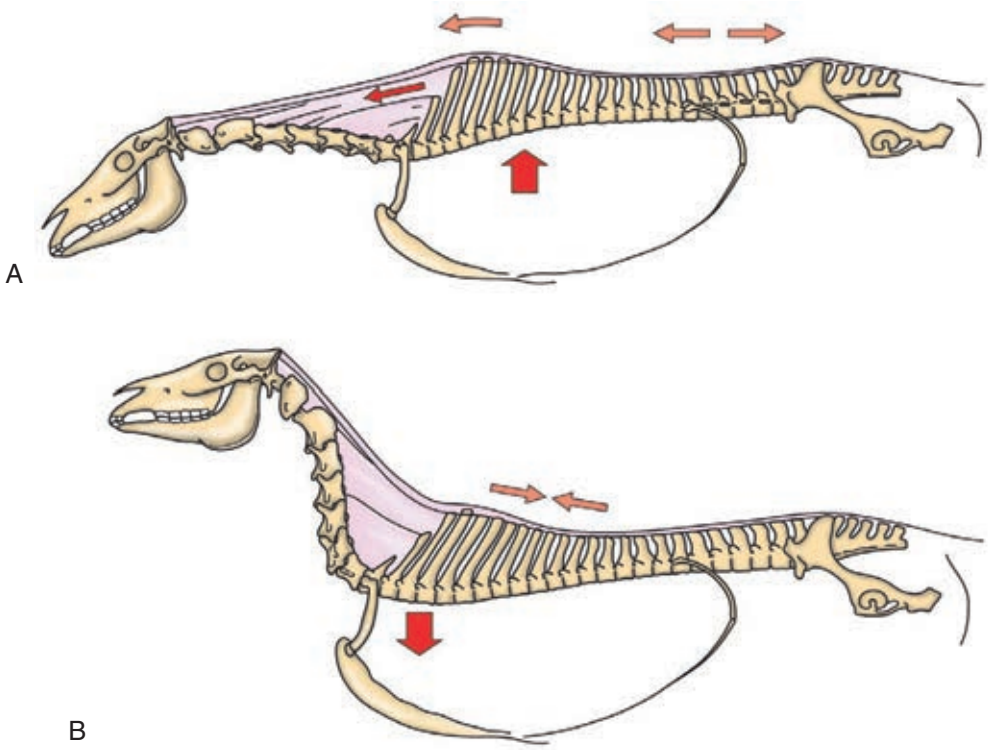

FIGURE 17-4 The effect of head and neck motion on thoracolumbar flexion and extension. **A,** Downward movement of the head and neck will flex the back (tense the bow of the bow-and-string model). This leads to compression of the vertebral bodies and tension on the supraspinous ligament. **B,** Upward motion of the head and neck has the opposite effect: extension of the back with the spinous processes moving closer to each other. *(From Denoix J-M, Pailloux J-P:* Approche de la Kinésithérapie du cheval, *ed 2, Paris, France, 2001, Maloine.)*

forelimbs and retraction of the hindlimbs, will have an opposite effect on back motion and thus produce a hollow (extended) back. The last factor, which has been central to the recent discussions on training techniques for dressage horses, is the effect of head and neck position. Lowering of the neck will tense the nuchal ligament and exert a forward rotating moment on the spinous processes of T2–T6. These long spinous processes provide a big lever arm, and traction on them in the forward direction will provoke flexion of the back (tensing of the bow). Elevation of the head will produce the opposite effect (Figure 17-4).

The bow-and-string model is at present considered by the scientific community as the best representation of the biomechanical concept of the equine back. It should be realized, however, that the model is an oversimplification of the real, highly complex situation. For instance, it does not account for the function of the pelvis and does not describe the interaction among hindlimbs, the pelvis, and the sacrolumbar spine (Haussler et al., 2007).

RESEARCH LEADING TO CURRENT ANALYTICAL TECHNIQUES FOR EQUINE THORACOLUMBAR KINEMATICS

Eadweard Muybridge and Etienne Marey (both 1830–1904) were, through the combination of their pioneering work on serial photography and their interest in horses, the founding fathers of what has been called the "first golden age of equine locomotion research" (van Weeren, 2001). In their wake, much research into many aspects of equine locomotion was initiated, using the novel techniques of photography and cine film, especially by the flourishing German veterinary anatomists in the era between the beginning of the twentieth century and World War II. Most of the work focused on limb motion (Schmaltz, 1922; Walter, 1925), but Wilhelm Krüger from Berlin dedicated part of his research effort to studying equine back motion (Krüger, 1939) and was also interested in the relative position of the center of gravity (Krüger, 1941). Krüger still held the traditionalist view of the back being a bridge spanning the gap between the limbs but was probably the first to perform in vivo kinematic research explicitly aiming at studying equine back motion, filming his subjects from above with help of a camera mounted on a tree.

There was significant decline in equine kinematic research following World War II, until the start of what has been named the "second golden age of equine locomotion research" (van Weeren, 2001) in the early 1970s. Around that time, in Sweden, a novel technology from the aeronautics industry was introduced to study equine gait (Fredricson and Drevemo, 1971), but it took a while before interest in equine back motion resurged. First, a number of ex vivo studies using cadaver specimens were conducted in Canada (Townsend and Leach 1984; Townsend et al., 1983) and in France (Denoix, 1987; Denoix, 1992). These studies provided interesting and much-needed data on bony and ligamentous constraints on equine back motion (Figure 17-5), but these studies were not representative of the in vivo situation, as the specimens were isolated and the studies did not include muscular action.

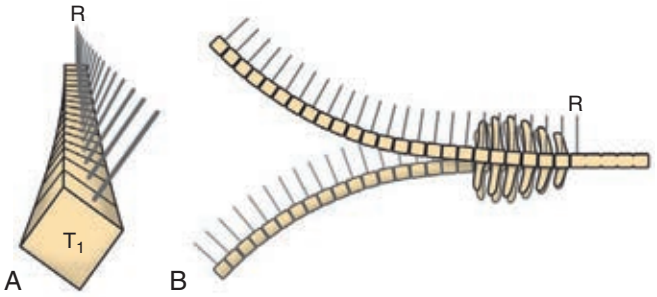

FIGURE 17-5 Schematic representation of the method used to assess axial rotation (*A*) and lateral bending (*B*) ex vivo with a reference Steinmann pin in the sacrum and other Steinmann pins in the vertebral bodies further craniad. *(From Townsend HG, Leach D, Fretz PB: Kinematics of the equine thoracolumbar spine,* Equine Vet J *15:117, 1983.)*

BASIC KINEMATICS

The motion of any rigid body in space can be described as the resultant of three translations and three rotations, respectively, around the three axes of a Cartesian orthogonal coordinate system that defines the space. The translations describe the progression of that rigid body within the space; the rotations describe positional changes of the body. For the description of back motion in relation to the rest of the animal, knowledge of the three rotations is sufficient, as rotation around the vertical axis (Z-axis) represents lateromotion or lateral bending (LB), rotation around the axis along the line of progression (described as Y-axis, or sometimes as X-axis) represents axial rotation (AR), and rotation around the remaining axis perpendicular to the sagittal plane (X-axis, sometimes designated as Y-axis) represents flexion–extension (FE) (Figure 17-6). In some publications, (aero)nautical terminology—*pitch*, *yaw*,

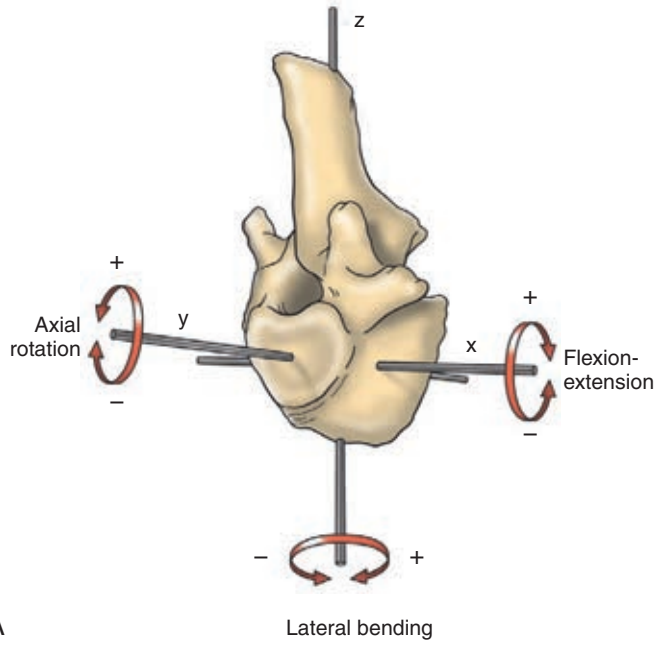

FIGURE 17-6a The basic movements of the back depicted as rotations of an individual vertebra around the three axes of an orthogonal coordinate system. *(From Townsend HG, Leach D, Fretz PB: Kinematics of the equine thoracolumbar spine,* Equine Vet J *15:117, 1983.)*

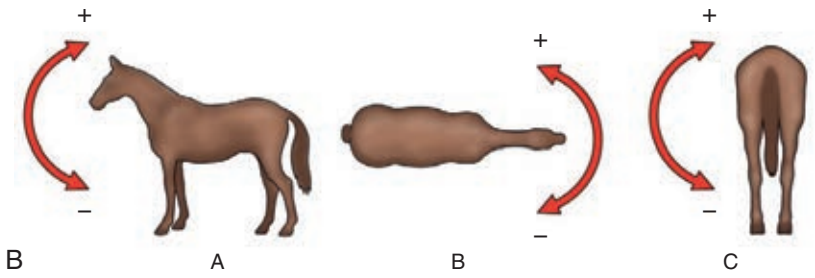

FIGURE 17-6b The three basic movements of the equine back: flexion–extension/FE (*A*), lateroflexion or lateral bending/LB (*B*), and axial rotation/AR (*C*).

and *roll*—is used for FE, LB, and AR, respectively. It should be realized that the motion of thoracolumbar vertebrae relative to each other is severely limited because of the anatomic constraints posed by the numerous ligaments connecting the vertebrae and the geometrical shape of the intervertebral joints (Denoix, 1999). For this reason, lateral bending cannot occur without a certain degree of axial rotation (Figure 17-7). The mutual dependency with respect to motion between the constituting elements of the thoracolumbar vertebral column is much larger than that between individual limb segments.

Most of the currently used techniques for kinematic analysis rely on the use of skin markers for motion capture of segments of the body. Although convenient, the use of skin markers introduces an important source of error, which is the sliding of skin over skeletal structures, which are the real objects of study (Fick, 1910). When using skin markers for in vivo capture of spinal motion, this artefact is even more important than when studying limb motion because of the much smaller range of motion of the vertebral segments in comparison with limb segments and because of the intrinsic coupling of motion patterns as indicated above. The latter factor makes it impossible to quantify the degree of axial rotation and lateral bending underlying the displacement from the midline of a marker positioned on top of any of the thoracolumbar vertebrae. The skin displacement artefact in the limbs has been investigated by using invasive techniques to detect the position of the skin marker and underlying bone simultaneously (van Weeren and Barneveld, 1986; van den Bogert et al., 1990; van Weeren et al., 1990). For the accurate measurement of spinal motion in all three planes, a similar approach was used by Faber et al. (2000), who implanted Steinmann pins mounted with markers into the spinous processes of a number of thoracic and lumbar vertebrae. In this way, rigid bodies composed of the vertebra, the Steinmann pin, and the marker were created, which permitted drawing conclusions about vertebral motion based on motion capture of the markers. For data analysis, the method used allowed for the determination of three-dimensional spinal kinematics without defining a local coordinate system (Faber et al., 1999). Because the equine subject had been measured before surgery with skin markers fixed to the insertion sites of the pins, a program could be developed that could translate noninvasively collected skin marker data into motion patterns of the underlying vertebrae with reasonable accuracy and repeatability (Faber et al., 2002). This so-called Backin®[1] program has been used in many more applied studies focusing on equine thoracolumbar kinematics, which will be discussed later in this chapter.

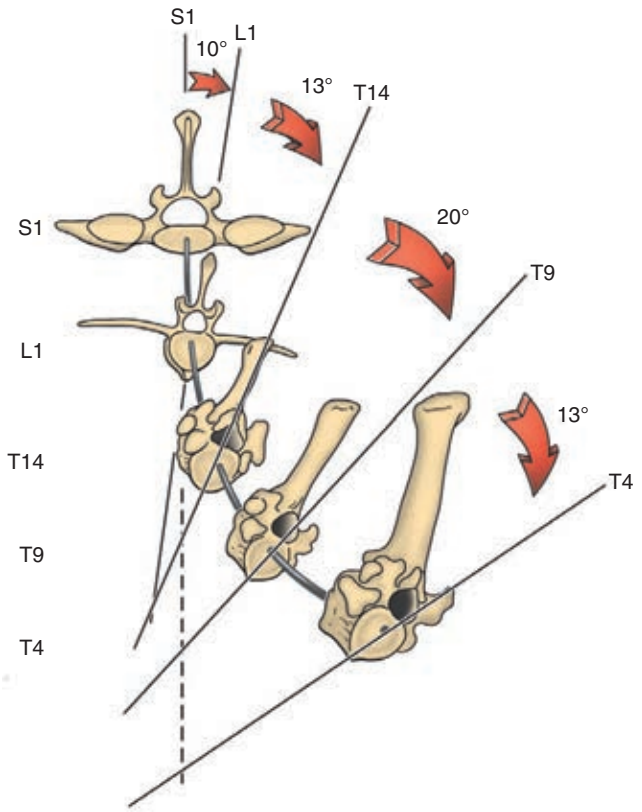

FIGURE 17-7 Schematic drawing of the coupling of lateral bending and axial rotation.

THORACOLUMBAR MOTION IN THE HORSE

Using the invasively obtained data, Faber calculated the three rotations describing spinal motion for the thoracic vertebrae T6, T10, T13, and T17 and the lumbar vertebrae L1, L3, L5, and S3 at the walk, trot, and canter (Faber et al., 2000; Faber et al., 2001a; Faber et al., 2001b). Motion patterns of all three basic rotations had a sinusoidal shape with FE showing two peaks, and LB and AR only one (Figure 17-8). This is caused by the fact that FE is induced by hindlimb motion and is identical (in symmetrically moving horses) for the left and right hindlimbs, whereas left and right hindlimb motion induces opposite patterns (clockwise and anti-clockwise, respectively) with respect to LB and AR, resulting in a single sinusoidal shape. In the method used by Faber et al. (2001c), the angle of a given vertebra is defined as the angle with a horizontal line through the marker of this vertebra that is parallel to the line connecting the markers of the two adjacent vertebrae, that is, the angle of L3 is defined by the line connecting L1 and L5. Outcome parameters of kinematic measurements of back motion are, thus, angular motion patterns (AMPs), which describe the thoracolumbar position, and also range of motion (ROM), which describes the extent of thoracolumbar motion in terms of FE, LB, and AR.

As expected, thoracolumbar motion is limited compared with the motion patterns of most limb segments. Maximal ROM of FE is about 8 degrees at the walk, does not exceed 6 degrees at the trot, and is greatest at the canter with approximately 16 degrees (Faber et al., 2000; Faber et al., 2001a; Faber et al., 2001b). At the canter, most of the FE motion is found at the lumbosacral junction, although in vivo recorded motion is considerably less than the maximal ROM found in ex vivo experiments (Townsend et al., 1983). Figures that were a little lower but of the same order of magnitude were found by Haussler et al., who used a different invasive technique based on liquid metal strain gauges connecting the Steinmann pins inserted into the spinous processes of the vertebrae (Haussler et al., 2000; Haussler et al., 2001). In a more recent study, Johnson and Moore-Colyer (2009) determined the relationship of FE ROM at the lumbosacral junction with speed at the canter. They found a linear relationship, with FE

[1]Qualisys AB, Gothenburg, Sweden

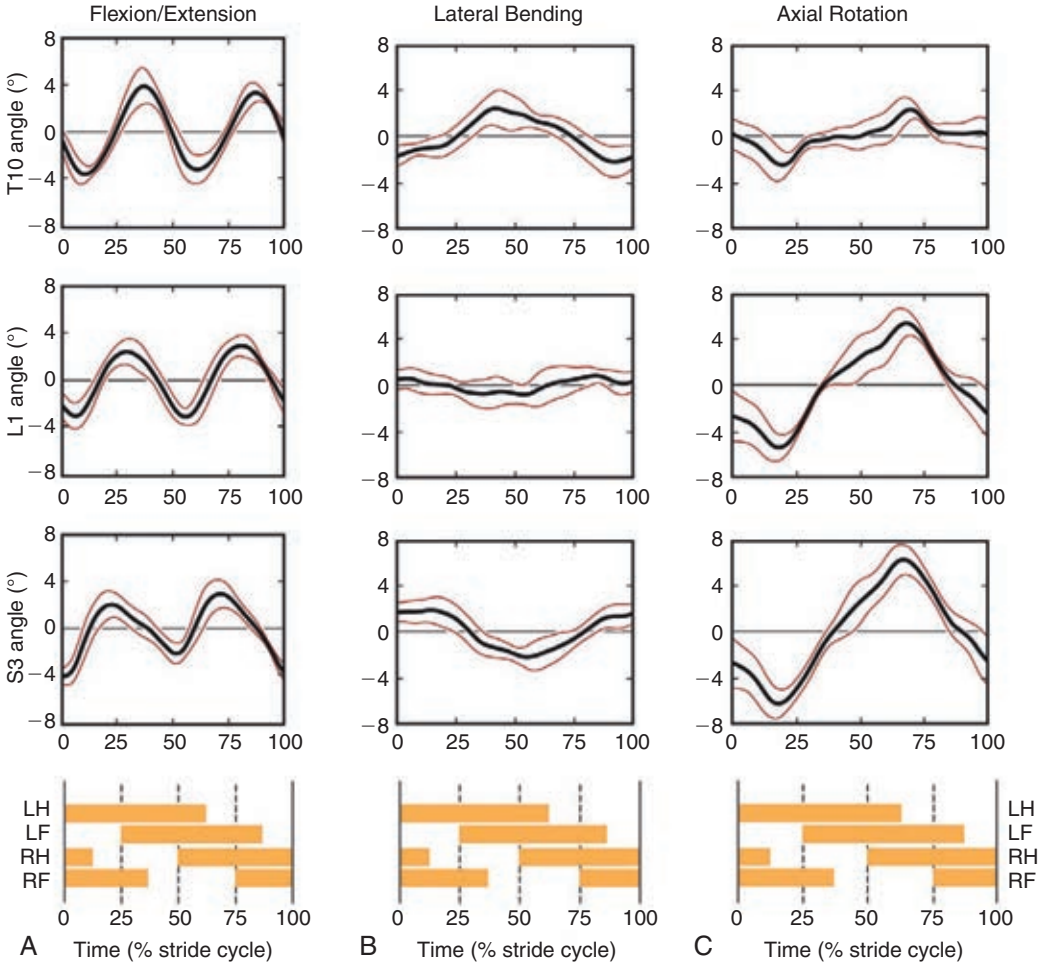

FIGURE 17-8 Mean (*thick line*) ± standard deviation (*thin lines*) motion patterns of three vertebrae (T10, L1, S3) of five horses walking on a treadmill at a speed of 1.6 meters per second (m/s). The stride cycle is represented by the bars below (*LH*, left hind; *RH*, right hind; *LF*, left fore; *RF*, right fore). The bar is closed when the limb is in contact with the ground (stance phase). **A,** Flexion and extension. **B,** Lateral bending. **C,** Axial rotation. *(From Faber MJ, Schamhardt HC, van Weeren PR, et al: Basic three-dimensional kinematics of the vertebral column of horses walking on a treadmill,* Am J Vet Res *61: 399, 2000; with permission from the American Veterinary Medical Association.)*

ROM increasing from 6.1 ± 1.9 degrees at 6 ms^{-1} to 7.2 ± 1.9° at 8 ms^{-1}. The ROM for LB is generally less than for FE. At the walk, maximal values of approximately 5 degrees were found that reduced to 1.9 to 3.6 degrees at the trot and were 5.2 ± 0.7 degrees at the canter (Faber et al., 2000; Faber et al., 2001a; Faber et al., 2001b). Axial rotation, for the calculation of which a reference line through the markers on both tubera coxae was used, varied at the walk between 4 degrees (T6) to 12 degrees (S3) and was 3.1 to 5.8 degrees at the trot and 7.8 ± 1.2 degrees at the canter (Faber et al., 2000; Faber et al., 2001a; Faber et al., 2001b). From these figures, it is clear that the trot is the gait at which the back is held most rigid in all possible directions of motion.

Variability in back motion is considerable, with within-horse variability ranging from 6% for AR at the walk to 7.8% to 18.2% for LB and also at the walk. Between-horse variability was highest for LB as well, and between-horse variability was four to five times higher compared with within-horse variability; for FE and AR, this was two to three times higher (Faber et al., 2001a; Faber et al., 2001b). The high variability in back motion should be taken into account when assessing the effects of interventions on individual horses and also when judging back motion in horses that are presented for the first time with suspected back dysfunction.

SACROILIAC MOTION

The sacroiliac junction is often incriminated as the seat of performance-limiting pathology, but relatively little fundamental research has been conducted investigating the basic kinematics of sacropelvic motion due to the very small ROM and the inaccessibility of the area. In an ex vivo study, Degueurce et al. (2004) showed that nutation and counternutation motion (the rotation of the sacrum with respect to the pelvic bones in the sagittal plane) was restricted to 1 degree only. In another ex vivo study by Goff et al. (2006), who used three-dimensional orientation sensors on cadaveric sacroiliacal joints, forces were applied from directions other than from the sagittal plane, and these authors showed a range of

motion for yaw (i.e., rotation respective to the Z-axis) of up to 2.5 degrees when applying the load from the lateral aspect. Applying these forces alters the tension in the ligament that runs from the tuber sacrale of the ilium to the abaxial surface of the spinous processes of the sacral vertebrae, which translates into changes in the cross-sectional area when evaluated ultrasonographically. By measuring these changes in living horses that were subjected to manual manipulation of the pelvis, sacroiliac motion could be demonstrated indirectly in the living animal (Goff et al., 2006). In later in vivo studies using horses equipped with motion sensors rigidly attached to the sacrum and pelvic bones by implanted Steinmann pins, the same research group succeeded in recording sacroiliac motion at walk in the trot in vivo (Goff et al., 2010). In a detailed ex vivo study, Haussler et al. (2009) induced nutation–counternutation, left–right lateral bending, and clockwise–anticlockwise axial rotation to sacropelvic specimens equipped with marker triads for stereophotogrammetric analysis. Displacements in all planes were in the order of 3 to 6 mm for most recorded motions, with none of them larger than 10 mm. The ROM of axial rotation was 7.9 ± 2.5 degrees, which is considerably more than the value reported by Goff et al. (2006). This was thought to be caused by the direct application of mechanical force versus unquantified manual force. From the relative motion of various sites of the pelvis, it was concluded that pelvic deformation occurs; this makes the usual assumption that the pelvis can be seen as a rigid structure invalid for studies aiming at the detailed investigation of pelvic motion. Haussler et al. (2009) also found considerable left–right asymmetry in pelvic motion. Pelvic asymmetry is a common feature of horses suspected of having chronic sacroiliac injury (Jeffcott et al., 1985) but is also often seen in horses without any known history of pelvic lameness (Haussler et al., 1999). Chronic hindlimb lameness is likely to induce asymmetric muscular and ligamentous loading of the pelvis, which might result in visible pelvic asymmetry through gradual bone remodeling and ligamentous adaptation (Goff et al., 2008).

APPLIED RESEARCH INTO THE KINEMATICS OF THE EQUINE BACK AND PELVIS

Although research on the kinematics of the equine back has lagged behind with respect to the developments in equine gait analysis in general, the past 10 to 15 years have certainly seen an attempt to close this gap. A large variety of studies focusing on the kinematics of the equine back and pelvis have been performed. Many of those have used the Backin® program, which had been developed on the basis of the large invasive in vivo study described before, but there have been others using entirely different technology as well. The subjects of these studies can be divided grossly into three areas: (1) studies aiming at gaining more knowledge of basic aspects of equine thoracolumbar motion and the interaction with limb motion under normal and pathologic conditions, (2) investigations into the effect of therapeutic intervention, especially chiropractic or other forms of manual treatment, and (3) studies in which back kinematics are used as a possible predictor for performance or as an outcome parameter to evaluate the effect of factors related to the use of the horse, such as saddles, other tack, and the effect of specific training techniques. The last category will be dealt with only briefly, as horse–rider interaction and the biomechanical effects of other use–related items are covered by the following chapter.

FUNDAMENTAL ASPECTS OF SPINAL MOTION AND INTERACTION WITH THE APPENDICULAR SKELETON

Basic Kinematics

As might be expected, conformation affects back motion. A long thoracic back results in significantly more LB in the lumbar area (Johnston et al., 2002). This may be the background of the empirically well-known increased vulnerability of an elongated back that was already signaled by Xenophon, who saw a long back as a sign of weakness (Xenophon, 430–354 BC, cited by Schauder, 1923). Further, horses with a large stride length have more FE ROM in the caudal saddle region (Johnston et al., 2002), which is also a confirmation of empirical knowledge, as large horses are known to be rather uncomfortable for short riders, especially when ridden in the sitting trot. In a study attempting to create a database for normal thoracolumbar kinematics, the same group of researchers found a decrease of FE ROM with increasing age, demonstrating increased stiffness in the elderly horse, also larger ROMs for LB at T10 and T13 in dressage horses than in show jumpers (Johnston et al., 2004). The latter finding may reflect the higher degree of gymnastic training required for a dressage horse.

Audigié et al. (1999) used a different kinematic analysis system, developed by Pourcelot et al. (1998), to measure back kinematics in sound trotting horses. The system is based on four skin markers and is, therefore, suitable to measure flexion–extension but not lateral bending or axial rotation. The same system was used by Robert et al. (2001) to analyze the effect of treadmill speed on back kinematics. Interestingly, they measured trunk muscle activity (*longissimus dorsi,* ventral abdominal muscles) simultaneously in an attempt to relate muscle activity to kinematics. Increasing treadmill speed decreased the maximal flexion angles, whereas extension angles remained the same. Electromyography (EMG) measurements showed that the large trunk muscles at the trot basically act to restrict (swinging) movement and do not induce it. A similar finding came from another EMG study by Licka et al. (2004). A combination of EMG and kinematic measurements was also carried out by Peham et al. (2001), who used yet another kinematic analysis system (Licka and Peham, 1998; Licka et al., 2001), again based on skin markers. They concluded that EMG recordings could best be performed at the height of T12, as amplitude was highest there. The more fundamental question to what extent back motion on a treadmill is similar to overground locomotion was not addressed until years later by Gómez Álvarez et al. (2009), who concluded that vertebral kinematics during treadmill locomotion was not identical to overground locomotion but that the differences were minor. The flexion–extension ROM was similar in both conditions, but LB ROM of the lumbar angles T17-L1-L3 and L1-L3-L5 was less during treadmill locomotion. During overground locomotion, the symmetry pattern of the lumbar vertebral angles was diminished, indicating increased irregularity of the movement.

Back Pain

The clinical diagnosis of back pain is often subjective, sometimes contentious, mainly because few disorders of the back

can be localized unambiguously by local analgesia, which is the gold standard in the orthopedic workup of lameness. The introduction of the algometer as an aid in the quantification of back pain by Haussler and Erb (2006) is a step forward but has not yet brought the relatively high degree of objectivity as in lameness diagnosis. Wennerstrand et al. (2004) compared sound horses with horses with back pain (on palpation) and found reduced ROM of AR and FE, with a concomitant decrease in stride length. Lateral bending was increased in the mid-thoracic region (T13), which might have been a compensatory effect. A number of these horses suffered from "kissing spines" (detected on radiographs, but not confirmed by local analgesia), others showed muscle soreness without radiographic signs. Interestingly, the same group investigated the effects of local analgesic blocks in the interspinous spaces of T6-L2 in back pain–free, asymptomatic horses. The blocks resulted in significantly larger ROMs for FE and LB at the walk, with much less effect on the trot. A lesser degree of ROM increase was seen when saline was injected instead of a local anaesthetic (Roethlisberger-Holm et al., 2006). The mechanism of both effects is thought to be an influence on the proprioception of back muscles. It is known that the deep musculature of the thoracolumbar vertebral column, especially the *multifidus* muscle, plays an important role in the internal stabilization of the back in humans. Poor dynamic control of the *multifidus* muscle has been reported as a predictor for lower back pain in humans (Cholewicki et al., 2005; San Juan et al., 2005); it is not unlikely that the muscle has a similar function in the horse. However, little is known about this, and more advanced studies into the deeper musculature of the equine back and its neuronal control have been undertaken only recently (Stubbs et al., 2006).

Back Pain and Lameness

Another notoriously contentious area related to back function is the mutual relationship between back pain and lameness. Both the axial and appendicular skeleton of the horse pertain to the same biologic system, so mutual influencing is possible. The questions are to what extent and which factor can be assumed to be the primary one in most cases. Field studies in the area are few and extremely difficult to carry out because of the subjectivity of the diagnosis and the fact that when simultaneous lameness and back problems are diagnosed, it is hardly ever possible to determine with certainty which of the two came first. Landman et al. (2004) found both lameness and back pain in 26% of horses presented with orthopedic problems. They used horses presumed to be asymptomatic and presented for prepurchase examination as controls; they found lameness with concurrent back problems in only 5%. However, not all animals underwent exactly the same examination. Dyson reported concurrent forelimb or hindlimb lameness due to an unrelated cause in 46% of horses with thoracolumbar or sacroiliacal pain but failed to prove that there was, indeed, no relationship (Dyson, 2005).

Many of the practical problems related to the use of horses with naturally occurring back pain were overcome in an experimental study in trotters by Jeffcott et al. (1982), who induced reversible back pain by injecting a strong solution of lactic acid into *longissimus dorsi* muscles. They noticed no effect on temporal or linear stride characteristics (stride length and stride frequency) but observed a notable increase in rigidity of the back. Detailed analysis of thoracolumbar kinematics was not performed at the time, but this was done in a recent repetition of this experiment in Warmblood horses. Back pain was induced in a similar way and horses were kinematically analyzed before and, at several occasions (up to 1 week), after induction of back pain. In this study, there were, again, no effects on spatial and temporal gait characteristics. However, back kinematics showed significant (though minimal, often not exceeding 0.5 degree) changes. A two-stage response was attributed to direct reaction to the painful injection, followed by muscle stiffness in the following days (Wennerstrand et al., 2009). In the same study, a detailed analysis of limb kinematics was also performed. Here, too, changes were limited in number and extent, not resulting in visible lameness (Gómez Álvarez et al., 2007a).

The effect of overt lameness on back motion has been documented (Buchner et al., 1996; Pourcelot et al., 1998), but Gómez Álvarez et al. (2007b, 2008a) were the first to investigate the effect of very subtle (maximally 2 of 5; Stashak, 2002) lameness, in either the forelimbs or the hindlimbs, on back motion (Gómez Álvarez et al., 2007b; Gómez Álvarez et al., 2008a). Forelimb lameness increased vertebral ROM and changed thoracolumbar back movement in both the sagittal and the horizontal planes, and this was interpreted as an attempt to move the center of gravity away from the affected limb. Hindlimb lameness resulted in hyperextension, increased ROM of the thoracolumbar back, a stiffer lumbosacral segment, and rotational motion changes of the pelvis. Recognizing that the model used in the study (solar pressure; Merkens and Schamhardt, 1988) resulted in acute lameness, whereas in clinical practice chronic lameness is more important, the authors felt that their conclusion was justified and that the study provided support for the widely assumed role of subclinical lameness in back dysfunction. When drawing conclusions from the induced back pain study and the induced lameness study together, both of which were conducted by the same international group of researchers under identical conditions, it was stated that already existing subtle lameness could result in significant changes in back motion, whereas even considerable back pain did not lead to obvious lameness. Therefore, it might be advisable to check thoroughly for the presence of lameness before embarking on advanced thoracolumbar diagnostics in any horse presented for back dysfunction (Wennerstrand et al., 2009).

Assessment of the Effects of Therapeutic Interventions on Back Motion

Manual manipulation is a common, although not yet universally accepted, form of treatment for back pain in humans (Gaumer, 2006; Hurwitz et al., 2006). There are many types of manual treatment, including the application of high-intensity, short-lever thrusts and the use of more long-lever techniques by chiropractors and osteopaths, respectively, but the common factor in all of them is that they try to correct spinal disorders through mechanical intervention. This treatment form has been used in horses for a long time (Herrod-Taylor, 1967) but has always met with widespread scepticism in the veterinary profession, partly because of the use of such terms as "subluxation" for functional diagnoses, which did not correspond to the commonly used pathologic terms. This scepticism was reinforced by the lack of objective evaluation of the treatments, often dismissed as being illusory or short-lived at best. The advent of reliable, repeatable measurement techniques for equine spinal kinematics has changed this.

Faber et al. (2003) applied their recently developed kinematic analysis technique to show the changes in thoracolumbar kinematics after manual manipulation in a single horse diagnosed with back pain. The results of their study were somewhat ambiguous, as they succeeded in showing clear improvement of symmetry in back motion after treatment and also the longevity (up to 7 months) of this effect; however, the eventual positive outcome of the study was associated with an unrelated change of trainer, not with the treatment-related improvement in symmetry. The same analytical technique was used by Gómez Álvarez et al. (2008b) in a group of 10 horses diagnosed with back pain. They showed a less extended thoracic back, reduced inclination of the pelvis, and improvement of the symmetry of the pelvic motion pattern as overall beneficial effects of treatment. However, the changes were subtle and not all of them lasted until the second measurement session 3 weeks after initial treatment. Different analytical methods have been used in other studies. Haussler et al. (2007) demonstrated a beneficial effect of manual manipulation of the spine in horses with back pain induced by the implantation of Steinmann pins. Sullivan et al. (2008) were able to show that chiropractic treatment had a superior effect compared with massage or treatment with phenylbutazone in asymptomatic horses (suspected of having low-grade back pain).

It can be concluded that the measurement of spinal kinematics has certainly contributed to the wider acceptance of manual treatment of the equine vertebral column as an auxiliary treatment, by showing a limited, but undeniable, effect on the thoracolumbar kinematics of such treatment.

USE OF EQUINE SPINAL KINEMATICS TO ASSESS PERFORMANCE OR TRAINING TECHNIQUES

Performance Evaluation

The four-marker system developed by Pourcelot et al. (1998) has been used to evaluate the possible usefulness of kinematic analysis to distinguish good and bad show jumpers. When evaluating two groups of show jumpers of strongly different levels, there were, indeed, significant differences between these groups, such as less lumbosacral extension in bad jumpers during the airborne phase and increased thoracolumbar and lumbosacral flexion before take-off in the badly performing horses, possibly indicating a less efficient strutting action when converting forward movement into upward motion (Cassiat et al., 2004).

In a 5-year longitudinal study on the effects of early exercise on jumping performance (see also Chapter 25), Santamaría et al. used the technique developed by Faber to capture the kinematics of the limbs and spine of show jumpers from foal age until age 5 years. They showed that the jumping technique was very consistent over time (Santamaría et al., 2004); when jumping performance was quantified at age 5 years as the ability to successfully complete a puissance competition, some kinematic parameters that were predictive at foal age for future performance could be identified (Bobbert et al., 2005). No parameter directly related to back kinematics was predictive, but the degree of hindlimb retroflexion (i.e., the degree to which hindlimbs are extended backward in relation to the position of the back) when clearing the jump was among the predictive parameters.

The discussion about the pros and cons of training horses with the head in an extremely low, "rolled up" position that has been called "Rollkur" (Meyer, 1992), "hyperflexion" (Jeffcott et al., 2006), or "low, deep, round" (Janssen, 2003) has had a significant influence on top-level dressage in the past few years. The technique, which was already known by the "grands écuyers" (great riders) from the baroque period, had been reintroduced in show jumping in the early 1970s. It had also been adopted by a few well-known and very successful top-level dressage riders. However, it met with fierce resistance from proponents of the more classic approach, who thought it to be an important welfare issue. The ensuing debate was intense but was based on empiricism and private opinions rather than on scientific data, as research was lacking (Balkenhol et al., 2003; Janssen, 2003; Schrijer and van Weeren, 2003). This is a complex issue and no definitive answer can be given for the question whether the use of the technique is justified or not based on research on any single aspect of the problem. However, it was research on equine spinal kinematics that produced the first scientific arguments in the discussion. An international Swiss–Dutch–Swedish research group performed a large-scale study on the effects of various head and neck positions on the biomechanical variables of the horse and the rider, starting from preliminary data generated by the Swedish group (Rhodin et al., 2005). In one of the publications emanating from this project, Gómez Álvarez et al. (2006) reported that in unridden horses, in agreement with the theoretical bow-and-string concept, differences in head-and-neck position had clear effects on vertebral angular motion patterns in the sagittal plane (FE). An extended head-and-neck position extended the back and reduced the ROM, whereas the very low "Rollkur" position flexed the back and increased the ROM for FE. This overall pattern was confirmed in a parallel study under the same conditions in ridden horses (Rhodin et al., 2009). The general conclusion was that a high position of the head and the neck impeded normal motion more compared with the natural position and the low position. In fact, the very low position (Figure 17-9) could be said to have positive effects for the gymnastic training of the horse through the increased FE ROM, confirming the claims of some trainers (Janssen, 2003).

FUTURE DEVELOPMENTS

The equine back is not an unknown entity or "black box" anymore. Research from the past few decades has yielded important fundamental information, and the basic motion patterns of the equine thoracolumbar vertebral column have now been described for the principal gaits of the horse. Also, there is some, though still limited, knowledge on pelvic motion. This does not mean that all has been said, as we are still far from "properly understanding the pathogenesis of the various thoracolumbar disorders" (Jeffcott, 1979). Although we can describe equine back motion to a certain extent, we still know very little about how the equine back actually works. Investigations on the steering mechanism have just begun (Stubbs et al., 2006), and knowledge about the motion chains in the equine body that generate back motion is still insufficient, although some of it is used on an empirical basis (Janssen, 2003). We know that there is a mutual influence of limb motion and back behavior (Gómez Álvarez et al., 2007b; Gómez Álvarez et al., 2008a; Wennerstrand et al., 2009), but the interaction is complex and certainly not yet fully understood. There is much fundamental research ahead before we will

FIGURE 17-9 The "low, deep, round," "hyperflexed" or "Rollkur" position (*A*) as performed by the threefold Olympic dressage champion Anky van Grunsven. This position enhances flexion–extension range of motion in the thoracic and lumbar back. As the position is extreme from an anatomic and physiologic viewpoint, its use is not without danger and should be undertaken with caution. Using it for short periods only and frequent transitions to more relaxed head-and-neck positions (*B*) are important.

fully understand equine back function and the working mechanisms of the influences on it.

It is likely that in the future, modeling algorithms will play a much larger role in equine back research than has been the case so far. A research group in Vienna has already done some work in this field (Peham and Schobesberger, 2004; Schlacher et al., 2004), but much more refinement and, above all, validation are needed before these models will really start to play an important role in the study of equine spinal kinematics.

Fundamental research will always be carried out in a laboratory environment, as almost all research related to equine spinal kinematics, until now, has almost exclusively been performed in an academic setting. This picture is already changing, with more nonacademic and traditionally more practical institutions embarking on equine gait analysis, especially in the United Kingdom (Johnson et al., 2009). The situation is likely to change further in the (near) future with respect to applied research. Even though the equestrian community is notoriously conservative and resistant to novel techniques or ideas (van Weeren, 2008), technology is slowly entering many parts of the equine world. Technological advances in the areas of micronization of electronic devices and wireless data transfer have given rise to the development of easily applicable, ready-to-use measurement systems that can be used under field conditions. It is probable that tiny, wireless devices consisting of combined accelerometers and gyrosensors will, in the near future, replace the more cumbersome, costly optoelectronic gait analysis systems, at least for more applied research. Saddle pressure mats (von Rechenberg, 2006) that give indirect information about back kinematics are already widely used, rightly or wrongly, by commercial saddlers in the field. A fascinating development that may account for the relatively large interindividual variations in back movement in the horse is the introduction of pattern recognition technology to equestrian sports (Schöllhorn et al., 2006). Together with measuring of individual peculiarities of gait and back motion through ready-to-use equipment based on novel technologies adapted for use in equestrian sports, such an approach might prove very suitable for the individual tailoring of training techniques, tack, shoeing, or any other external influence to specific horse–rider combinations.

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CHAPTER

Functional Biomechanics: Effect of the Rider and Tack

18

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For every role the horse has served since its domestication—from warfare, agriculture, and transport to modern-day use as a sports and leisure animal—people have had to communicate with the horse to control speed and direction of movement. This influence of man on the horse is mediated through *tack*, which functions as an interface between the horse and the human being(s) using it. This chapter focuses on these influences on the ridden horse. The first section concerns the influence of tack such as the saddle, the bit, and reins. The second section describes the direct (biomechanical) effect of load carriage and the effects of signals given by the rider to the horse, including factors that influence this communication (e.g., riding style and riding level). In both areas, much progress in research has been made in the last two decades, largely due to important technical developments in instrumentation techniques of various forms of tack, an area that is still characterized by rapid progress and innovation.

EFFECT OF TACK ON THE HORSE

Several interfaces between horse and rider exist. Ridden horses are normally equipped with a bridle and a saddle, which serve as the main transmitters of signals from the rider. Other tools such as spurs and whips are also used to communicate with the horse. Tack often is connected to both horse and rider and, therefore, is suitable to incorporate measuring devices. The most commonly used tools in this respect are strain gauges. The electric resistance of strain gauges changes with the force exerted on them. Strain gauges can be used to assess forces on bits, reins, and stirrups. Furthermore, flat pressure (or "normal") force sensors incorporated in pads can be placed underneath the saddle, underneath blankets or even simply between the horse and the rider's legs. Horse–rider combinations with instrumented tack are shown in Figure 18-1. The instrumented tack can be used not only to study the effect of tack itself but also to study the interaction between rider and horse (see below).

EFFECT OF SADDLE AND SADDLE PADS

The use of a saddle, or saddle-like structure, goes back to a long time before the Christian era, but the stirrup is a more recent addition that was invented by the Chinese in the third century AD (Dunlop and Williams, 1996). Although the concept of the saddle has not essentially changed since then, research into the effect of the saddle on the horse is much more recent. Harman (1994) was the first researcher who used a computerized pressure measuring device (SaddleTech) to evaluate the effect of a saddle and saddle pads on the horse.[1] This first saddle pressure system was equipped with 256 sensors, which used pressure-sensitive ink printed on a polyester film that changed resistance when subjected to pressure. Harman (1994) studied several saddle pads: cotton-quilted pads, open-cell foam pads, gel pads, and a balancing shim. The most common saddle fitting problem she identified was "bridging." In "bridging," the front and the rear panels were contacting the horse, but there was no or reduced pressure in the central area in between. Only 35% of the tested saddle pads improved the saddle fit or at least did not change it in a negative way. The remainder, in fact, increased pressure, which was compared by Harman (1994) to the effect of a sock in a shoe that is too tight.

The study of Harman encouraged other researchers to take up the topic. Pullin et al. (1996) used a newer technique, force-sensing array (FSA), for the evaluation of an equine athletic saddle pad and saddle liners.[2] They identified several potential sources of error within the system that could affect the objectivity of data collection and interpretation. They stressed the importance of numerical scoring based on specific measurements rather than relying on subjective impressions, the importance of the calibration procedure, the position of the sensor pad, and the position of both rider and horse. They further stated that measuring without a rider is not useful, as the weight of the rider is required to evaluate the weight distribution underneath the saddle.

Jeffcott et al. (1999) investigated the validity of the FSA technology. In principle, according to Newtonian laws, the force on the horse's back should be the sum of the weight of the saddle, the force exerted by a tightened girth, and the weight of a rider. There should, therefore, be a linear relationship between total weight and the pressure measured underneath the saddle. They tested this hypothesis on both a wooden horse and a live horse placed in the standing position. The correlation between weight and measured pressure appeared to be high, indeed. They also presented preliminary data on characteristic changes of the center of pressure at the walk, sitting trot, rising trot, and canter. De Cocq et al. (2006) focused on the validity and repeatability of the FSA technology. They not only confirmed the overall results of Jeffcott et al. (1999) but also demonstrated that there may be considerable drift during a single

[1]SaddleTech, EquiTech, Woodside, CA.
[2]FSA, Vistamedical, Winnipeg, Manitoba, Canada

FIGURE 18-1 Two horse-rider combinations with instrumented tack. **A,** Horse–rider combination with normal force sensors incorporated in a blanket underneath the saddle and between the legs of the rider and the horse. On both rider and horse infrared light reflecting markers are placed to measure kinematics. **B,** Horse–rider combination with strain gauges incorporated between bit and reins and a saddle force device underneath the saddle. *(Courtesy of* Bit Horse Magazine, *Lonneke Ruesink.)*

measurement day, urging frequent (re)calibration. Furthermore, repeatability of the measurement was low when a rider dismounted and remounted a horse. The FSA device was also not able to distinguish satisfactorily between two saddle-fitting procedures, although the filling of the panels (special air-filled panels were used) was significantly different. The newest saddle force measurement technique uses capacitometric sensors (Pliance).[3] De Cocq et al. (2009a) tested the Pliance system with respect to validity and repeatability. The reliability of the calibration of the Pliance system decreased during one measurement day but remained within acceptable values. Repeatability of measurements was acceptable as long as the saddle device and the saddle remained on the horse in-between measurements. This stresses the importance of the position of the device on the horse's back. The Pliance system was able to detect differences when weight was added to the saddle or the rider and between rider positions.

The Pliance system has been used in several studies on the effect of saddles and saddle pads. Werner et al. (2002) compared standard and custom-made saddles. They combined the saddle measurements with a clinical examination of horses, which included back palpation. A correlation was found between high pressure values (>3.5 newtons [N]/cm^2) and both pain reactions and the occurrence of muscle atrophy affecting the area of the back under the saddle. The following criteria for good saddle fit were identified: a wide, uniform contact area between saddle and horse, maximal pressure values not exceeding 3.0 N/cm^2, and no pressure peaks or "bridging." Meschan et al. (2007) studied the effect of the width of the tree on the forces and force distribution underneath the wooden tree saddle. They demonstrated that the load under poorly fitting saddles was distributed over a smaller area than under properly fitting saddles, with the potential to lead to harmful pressure peaks. Mönkemöller et al. (2005) demonstrated that an adjustment of saddle fit can enlarge the contact surface underneath the saddle and hence, by reducing pressure in the region of the caudal thoracic spine, reduce the number of horses demonstrating back pain. Nyikos et al. (2005) subdivided the contact area of the saddle into six regions. They found that the lumbar area was more sensitive to pressure than the area of the withers. They concurred with Harman (1994) that "bridging" was the worst problem related to saddle fit. Harman (1994) had stated that saddle pads under poorly fitting saddles may often be detrimental, and Kotschwar et al. (2010) investigated the use of a pad under an overly wide saddle, theorizing a beneficial effect. Although significant intrahorse effects were demonstrated, there was no significant interhorse effect. The choice of a saddle pad to improve the fit of an excessively wide saddle (if such a saddle is to be used anyway) should, therefore, be based on highly individual criteria for each horse.

Latif and colleagues (2010) used the Pliance system to measure saddle pressures generated underneath three different racing saddle types: wooden tree, treeless, and flexible tree. With a rider taking the jockey seat, canter and gallop exercise peak pressures occurred underneath the front of the saddle, with these shifting caudally during the trot exercise. They showed that horses were at greatest risk of high peak forces during trotting exercise, especially in the mid-saddle

[3]Pliance, Novel, Munich, Germany.

region in a treeless saddle, and in the hind region of the wooden tree saddle. The resultant effect of high saddle pressures is saddle soreness, so owners should be encouraged to check their horse for dry spots underneath the saddle which may be an indicator of high saddle pressures and resultant soft tissue damage (von Peinen et al., 2010).

Although the use of saddle measuring devices has proven its value for scientific research, the use of these devices for the evaluation of saddle fit in individual cases is fraught with problems. Apart from saddle fit, there are several other factors influencing the measurements. First, the user should be aware that the systems should be recalibrated frequently, as reliability decreases considerably with use and unreliable measurements may easily lead to false conclusions. Second, saddle measurements are only useful when both rider and horse are measured, as discussed above. The position of the rider influences weight distribution and should, therefore, be standardized. Further, the exact positioning of the system with respect to horse and saddle is of importance. Saddle pressure measuring may improve the procedure of fitting a saddle only when all these items are dealt with satisfactorily. The most reliable criterion to judge saddle fit is the contact surface. A large contact surface will distribute the forces better and, thus, prevent pressure peaks.

EFFECT OF REINS AND BRIDLE

Research on the effect of the bridle has, thus far, been focused on the bit. The bit is probably the oldest piece of tack that has been introduced in horse riding. Evidence of the use of bits has been found in two different archeological sites of the Botai, a culture located in the Aqmola province of Kazakhstan, famous for its very early use of the horse dating from about 3500 to 3000 BC (Anthony and Brown, 2000). The bit is in direct contact with the horse via the mouth, and aids given by the hands of the rider are transmitted through the reins and the bit directly to the horse's mouth.

As a large part of the bit is hidden from view, fluoroscopic techniques have been used to evaluate the position and action of several bits (Clayton, 1985; Clayton and Lee, 1984). The first bit that was studied was a joined snaffle bit. In the resting position, the mouthpiece was interposed between the tongue and the hard palate, indenting the dorsum of the tongue. When applying an equal force to both reins simultaneously, the bit was moved caudally, deepening the indentation in the horse's tongue. When applying asymmetrical force, the net effect depended on the relative forces applied to the active and opposing rein. It was not possible to produce an independent effect on one side of the mouth. The jointed mouthpiece was suspended in a more horizontal position when keepers were used to fix the position of the bit rings relative to the cheek pieces of the bit. The keepers also reduced the mobility of the bit within the oral cavity. Less intraoral mobility was also observed in bits with a single mouthpiece. A bit that had two joints connected to an angled plate could be positioned in ways that the plate lay either parallel or perpendicular to tongue and palate, which makes a marked difference with respect to the severity of the impact of the bit.

The fluoroscopic studies demonstrated that the force exerted on the bit greatly influences the position and movement of the bit in the mouth of a horse. A next logical step was to measure the force that riders apply to the reins and which is transmitted to the bit. In several studies, force sensors have been attached in between the bit and reins to measure rein forces (Clayton et al. 2005; Preuschoft et al., 1999; Warren-Smith et al., 2005). Preuschoft et al. (1999) analyzed the functions of a number of bridles ("head gears") biomechanically. Most bridles are designed to transmit tensile forces applied through the reins or a lunge to the sensitive parts of the horse's mouth. The direction, duration, and magnitude of these forces are essential factors in controlling the horse. Several bridles and two major types of bits (with or without levers) were analyzed using a device that could roughly quantify these forces. Rein forces were found to show regular patterns and to be dependent on the horse's gait. During competition, forces between 20 and 147 N were measured; in recreational use the range was 20 to 49 N. Clayton et al. (2005) used a more precise load cell and found similar rein force patterns consisting of a series of spikes with frequencies corresponding to two per stride in the walk and trot and one in the canter. They established a maximum force of 104 N in the canter. Warren-Smith et al. (2005) focused on developing a low-cost and practical sensor and recording system that could be used in everyday training. They tested two sensors on horses that were being led, lunged, and ridden and measured forces in the range of 0 to 30 N for light rein contact. As other studies had reported considerably higher forces, they claimed that horses might be subject to unnecessarily high forces and that the education of horses and riders could be improved. In a follow-up study, the same authors (Warren-Smith et al. 2007) focused on specific equitation exercises: left turn, right turn, going straight and halt, in both long-reining and riding. The rein force required for going straight was lower, and the force required to elicit the halt response was greater than for any other activity. The mean force exerted during long-reining was 10.7 ± 1 N and for riding was 7.4 ± 0.7 N. These, again, were lower values than those recorded by others in earlier studies.

Heleski et al. (2009) used a combination of behavioral observations and rein force measurements to study the effect of martingales and rein inserts. There is controversy about the use of these tools. Some claim that they can reduce discomfort caused by inexperienced and unsteady hands. Others consider them inappropriate "crutches." No differences in conflict behavior were observed in horses with or without martingales or rein inserts. Mean rein forces were higher for martingales than for controls or rein inserts. The head of the horse was lower for horses ridden with martingales. It can be concluded that carefully fitted martingales might have a place in riding schools that teach novice riders.

EFFECT OF RIDER ON THE HORSE

Riders have direct and indirect biomechanical effects on the horse through their sheer mass and through the aids aiming at the horse's sensory system with which they actively try to influence locomotion (Meyer, 1999; Preuschoft et al., 1995), apart from visual and acoustic signals (Figure 18-2). Besides the gravitational force elicited by the mass of the rider, the distribution of this weight on the horse is also an important factor. Studies on rein force have already been described previously. Leg force is limited by the long lever arms of the reaction force, although this may be altered, for example, by spurs used to create a relatively high local pressure. The effect of the rider on the horse is further modulated by the

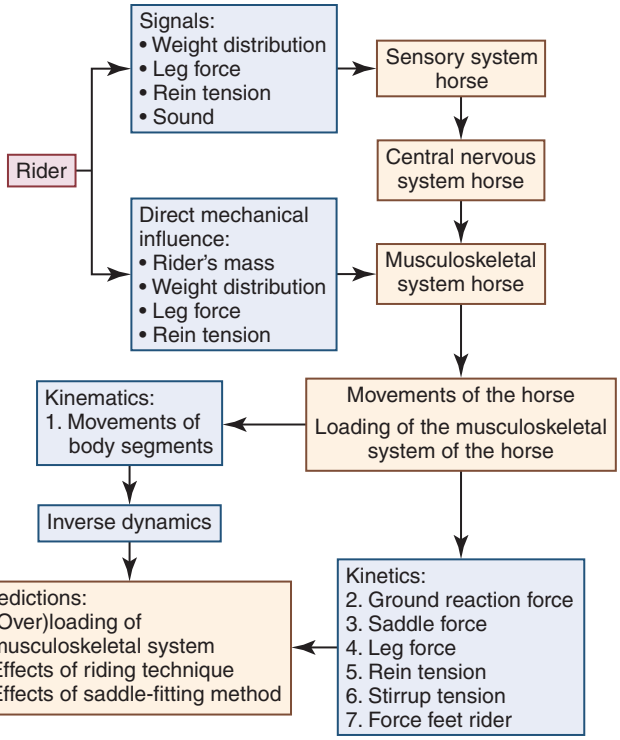

FIGURE 18-2 Diagram of the influence of the rider on the biomechanics of the horse and the biomechanical approaches to study these influences.

riding technique of the rider and, associated with this, the level of riding. This section focuses on the direct effect of the rider's mass, the influence of riding technique and riding level.

EFFECT OF BODY MASS OF THE RIDER

During horseback riding, apart from the extra energy required due to the increase in total mass, the weight of the rider has a direct biomechanical effect on the musculoskeletal system of the horse. According to Slijper's bow-and-string model (Slijper, 1946; see Chapter 17), a weight exerted on the horse's back will lead to an extension of the thoracolumbar vertebral column. This theory has been supported by research demonstrating that horses have a more extended posture of their vertebral column when saddled with 75 kilograms (kg) of extra weight (De Cocq et al., 2004). With just the saddle, changes in back extension occur only at the canter, and application and tightening of a girth strap without the saddle does not lead to any change in posture. Similar alterations in back kinematics were also observed between an empty saddle and a rider in the sitting (but not rising) trot (De Cocq et al., 2009b). In each study, changes in back movements were accompanied by changes in limb movements with increased retraction of the forelimbs at the walk and trot being the most salient feature. As these changes in limb retraction have a flexing action on the back (see Part 3, Chapter 17), it appears that the horse is trying to compensate for the altered back posture.

In certain equestrian disciplines, there are minimum requirements for the weight that the horse has to carry. When the rider is too light, weight is added to ensure a fair competition. The question is how this added weight (or an increase of body weight of the rider) affects the horse. During jumping, several kinematical differences can be observed in horses carrying only a rider compared with a rider with added weight. Two of these differences involve increases in maximal extension of the fetlock and carpal joints (Clayton, 1997). When comparing trotting horses with or without a rider, only small changes in fetlock kinematics were observed (Clayton et al., 1999). When the weight is added asymmetrically to a rider on a standing horse, this will lead to an asymmetrical force distribution underneath the saddle (de Cocq et al., 2009a). This asymmetrical force distribution is likely to influence the musculoskeletal system of the horse. Rider asymmetry has anecdotally been associated with poor performance and injuries. This may be an important issue, although it has been poorly researched to date. Symes et al. (2009) demonstrated that asymmetries in the movements of the shoulders of riders are very common. The method for describing asymmetries presented in their paper could possibly also be used to evaluate the effect of an asymmetrical position of the rider on the loading and performance of the horse.

This gives rise to the question whether the effect of the mass of a rider can be imitated by the use of dead weight. Schamhardt et al. (1991) compared the effect of a rider and a sandbag of the same mass on ground reaction forces. Compared with the sand bags, the riders were able to shift part of the weight toward the hind limbs. Clayton et al. (1999) compared ground reaction forces between ridden and unridden horses. Although the absolute peak vertical ground reaction forces were higher with a rider, the mass-normalized peak vertical ground reaction forces were lower. There also was a change in timing of the peak ground reaction forces. A ridden horse, therefore, did not seem to be equivalent to a proportionally larger horse with the same total mass. In contrast, Sloet van Oldruitenborgh-Oosterbaan et al. (1995; 1997) compared the effect of a rider and a weighted saddle on

limb kinematics and did not find differences between these situations, although this discrepancy may have occurred because of differences in riding technique between studies (see below).

The average vertical force on the horse's back must be equal to the weight of the rider, but fluctuations around this average value can be expected because the rider accelerates and decelerates during locomotion. This force on the horse's back can be measured with the saddle force devices described earlier. Frühwirth et al. (2004) evaluated the force patterns at the walk, trot, and canter and demonstrated a fluctuating pattern. Von Peinen et al. (2009) related the saddle force pattern of a walking horse to the movements of both rider and horse. However, there are some limitations in using saddle force measurements. The piezoelectric and capacitometric techniques actually measure normal force, not pressure. The difference between these newer techniques and the pressure-sensitive ink, as used by Harman (1994), is that the newer systems are only sensitive to forces that are applied perpendicularly to the surface, that is, normal force. However, shear forces play a role, too, and for correct measurements, these forces should be taken into account. Unfortunately, it is not possible to measure these nonperpendicular forces when the system is placed underneath the saddle. For the measurement of forces on the horse's back, the system is, therefore, limited to the summation of the magnitudes of normal forces, thus carrying an inherent error. An alternative approach to evaluating the effect of the force on the horse's back is to use rider kinematics to calculate the force of the horse on the rider, which has the same magnitude but is opposite to the force exerted by the rider on the horse's back. This approach has been used to study the effect of riding technique (de Cocq et al., 2010).

EFFECT OF RIDING TECHNIQUE

Taylor et al. (1980) hypothesized that it is the energetic cost of generating force to support body mass that determines the energetic cost of running, more than the mechanical work that has to be done. Several studies indicate that an elastic coupling between carrier and load reduces peak forces, thus reducing energetic cost. In horse riding, the riding technique has a strong influence on the coupling between carrier (horse) and load (rider) (de Cocq et al., 2010).

In the trot, riders can choose between three different riding techniques: sitting trot, rising trot, and two-point seat. De Cocq et al. (2009b) studied the effect of the sitting trot and rising trot on back movements and head and neck position of the horse. They concluded that the back movements during the rising trot showed characteristics of both the sitting trot and the trot without a rider. The rising trot is similar to the sitting trot when the rider is sitting in the saddle and similar to the unloaded trot when the rider stands in the stirrups. Roepstorff et al. (2009) compared vertical ground reaction forces and the kinematics of horses during the sitting and standing phases of the rising trot. They found an increased ground reaction force and several changes in the kinematics of the horse during the sitting phase compared with the standing phase, concluding that the rider's movement in rising trot induces an uneven biphasic load that affects the back, pelvis, and limbs of the horse. This biphasic load was confirmed by studies evaluating the loading of the horse's back with saddle force equipment (Peham et al., 2008; Peham et al., 2010) and also in a study in which loading was calculated from the rider's kinematics (de Cocq et al., 2010). All these studies supported the idea that the rising trot is less challenging to the horse's back, making the technique useful for the training of young horses that have to be accustomed to the rider's weight and for the rehabilitation of horses with back problems.

The fact that the standing phase of the rising trot is the phase where loading is least raises the question whether standing in the stirrups during the total stride cycle would even be less challenging to the back of the horse. Peham et al. (2010) looked at the two-point seat and found that peak forces were, indeed, lowest in this riding technique. The two-point seat can be seen as a precursor of the rider position that is used during horse racing (Pfau et al., 2009). The current riding technique of jockeys was developed in the late nineteenth century. During the same time, racing performance improved tremendously. It appears that the jockey uncouples himself from the horse by moving relative to his mount. The jockey's body moves little with respect to the world inertial frame, and therefore, the horse supports the jockey's weight but does not have to accelerate and decelerate him during each stride cycle. This, again, leads to lower peak forces on the horse's back and might be the reason that the horses are able to gallop faster.

EFFECT OF RIDER EXPERIENCE

Another factor that is likely to influence the performance of the horse is the experience of the rider. An experienced rider is able to maintain an upright body position, whereas a beginner rider sits more forward (Schils et al., 1993). This change in body position affects the force distribution underneath the saddle, as shown by de Cocq et al. (2009a). Upright, forward, backward, and sideward positions of the rider were compared using saddle force equipment. In the forward position, riders shift about 90 N of their weight to the front part of the saddle, a big change in weight distribution. Further, a rider is more stable in the upright position. Terada (2000) found instability of the upper body of a novice rider in the sitting trot, which, as suggested by electromyographic (EMG) data, provoked imbalance between the erector spinae and the rectus abdominis muscles. This effect was not observed in advanced riders. The stability of the rider might also affect the stability of the equine gait. On this same topic, Lagarde et al. (2005) found an increase in the regularity of the oscillations of the trunk of the horse when comparing an expert rider to a novice rider. The expert rider was able to move in phase with the horse, whereas the novice rider was not.

Even if it is clear that rider experience influences the quality of movement of the horse in a positive way, this does not mean that in all cases an experienced rider is required to obtain good performance. Ridden horses have more constant motion and more repeatability of jumping efforts while being ridden. Peham et al. (2004) developed a method to evaluate the variability of horse motion pattern and demonstrated that the movement of the horse is more constant when ridden. Lewczuk et al. (2006) studied the repeatability of horses' jumping parameters with or without a rider. The jumping parameters were more repeatable with a rider while jumping a 1.20-meter (m) fence, but there were no differences when jumping lower fences. Powers and Kavanagh (2005) studied the effects of novice and experienced riders on the jumping kinematics of experienced jumping horses. Their results

suggested that the rider's body position and body movement have no effect on the horse's jumping kinematics. However, it can be questioned whether the combinations were challenged enough by a 1.05-m fence. In an earlier work, Powers and Harrison (2002) demonstrated that a rider can influence the jumping kinematics of a young horse when jumping a 1-m high fence. It, therefore, seems that the individual jumping technique of a horse is less susceptible to rider influence in experienced horses. Rider experience, horse experience, and fence height all seem to influence the jumping performance of a horse–rider combination.

CONCLUSION

Horse riding started probably more than 5000 years ago (Anthony and Brown, 2000), and the horse has played a crucial role in many human societies for millennia, but scientific studies into the effects of the interaction between horse and rider have only recently begun. Greatly helped by technological developments in computing and engineering, devices have been developed that permit the study of various aspects of horse–rider interaction. The studies performed thus far have yielded interesting results, partly confirming and partly negating, long-held beliefs regarding horses that were based on empiricism and subjective perception. Taking into account the global interest in and popularity of equestrian sports and the ever-accelerating technological developments, it can be anticipated that this line of research will increase in the foreseeable future. This will undoubtedly increase our knowledge of the complex, close interaction of two individuals belonging to different species, which is a characteristic of all major equestrian activities. The outcome of this type of research may also further our understanding of the impact of a variety of equestrian practices on equine welfare and thus help in the discussions on ethics and associated regulatory affairs.

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CHAPTER 19

Training Regimens: Physiologic Adaptations to Training

DAVID R. HODGSON

The major objectives of training are to prepare a horse for the rigors of athletic competition, to decrease the risk of injury, and to increase the work capacity. By nature, horses are gifted athletes that are also capable of undergoing substantial adaptations in response to training. Investigations examining the adaptability of the musculoskeletal, cardiorespiratory, hemolymphatic, and thermoregulatory systems have been undertaken over several centuries. Some of the findings will be summarized here. Most of the measurements discussed have been defined and used only in experimental laboratories. However, more recently many more "in-field" measurements have been procured.

MUSCULAR RESPONSES TO EXERCISE

ENZYMES IN MUSCLE

Aerobic Enzymes

Muscle is a remarkably plastic tissue that is remodeled when exposed to the stresses of training. In general, although by no means universally, training results in an increase in mitochondrial density, with resulting increases in the activities of enzymes in the tricarboxylic and lipid metabolic pathways, both of which contribute to increased oxidative capacity of skeletal muscle. Endurance training produces the greatest increase in the activities of aerobic enzymes, and in the first few months of training, overall increases of more than 100% over pretraining values occur. These changes also may be associated with an increase in the number of oxidative muscle fibers within the working muscle. Additionally, an increase occurs in the density of capillaries surrounding muscle fibers.

Although the metabolic advantages of increases in oxidative capacity in skeletal muscle will be greatest in animals required to undertake more prolonged exercise, for example, steeple-chasing, eventing, or endurance racing, positive effects occur in animals participating in more intense activities, with a prolongation of work capacity.

The mechanism by which the increase in oxidative capacity exerts its effects is by more efficient utilization of substrates by the metabolic pathways within the skeletal muscle. This occurs as a result of a more rapid translocation into the mitochondria of the adenosine diphosphate produced during muscular contraction. Since an increased ratio of adenosine diphosphate–adenosine triphosphate (ADP:ATP) within the cytosol of working muscle is one of the stimuli for an elevation in glycolytic (anaerobic) energy production, the increase in oxidative capacity serves to keep this ratio low via the rapid mitochondrial uptake of ADP. A decrease in the ADP:ATP ratio reduces the stimulus for glycolysis and increases the contribution of fat to total energy production. The capacity for greater utilization of fatty acids by muscle during submaximal exercise results in a sparing of glycogen within the working muscle. This glycogen-sparing effect assists in delaying the onset of fatigue during endurance events because of a direct relationship between exhaustion of the intramuscular glycogen store and the onset of fatigue.

The lower intracellular ADP:ATP ratio is also likely to have important effects during exercise at higher intensities. A beneficial effect would be provided by the augmented aerobic capacity, since this allows a greater proportion of energy to be produced by the aerobic pathways early in the exercise. Thus, the production of lactate and hydrogen ions will be delayed, reducing the potential of these byproducts to adversely affect the contractile apparatus, a factor that contributes to fatigue. This increase in oxidative capacity is reflected by increases in values for the metabolic variables such as in response to standardized exercise tests, or more practically improved racing times.

Glycolytic Enzymes

Equine skeletal muscle possesses an intrinsically high glycolytic capacity, which is reflected by the high activities of the glycolytic enzymes. Such is the magnitude of the activities of the glycolytic enzymes that when compared on the basis of protein concentration, the glycolytic activity is more than tenfold greater than for the aerobic enzymes. However, some changes occur in the activities of glycolytic enzymes in response to most routine training programs. In some cases, modest decreases in the activity of lactate dehydrogenase occur in response to training. These findings of few, if any, changes in the activity of the glycolytic enzymes are similar to those reported for several other mammalian species.

In contrast, when training involving short-term, intense bursts of exercise is undertaken, it results in an increase in the activities of several glycolytic enzymes. This also parallels the findings in human and murine studies, in which intense training has been shown to result in an increase in the glycolytic potential.

Enzymes Associated with Purine Nucleotide Metabolism

The effects of training on the activities of enzymes directly associated with purine nucleotide metabolism have been

reported. Analogous to the situation for glycolytic activity, the intrinsic activities of creatine kinase (CK) and adenosine monophosphate (AMP) deaminase are high in equine skeletal muscle. The greater the capacity for speed of an animal, the higher are the enzyme activities. Training has been reported to result in limited increases in the activities of CK and AMP deaminase. The purpose of these increases has not been determined, but it has been suggested by some that the increase in AMP deaminase activity may be responsible for ensuring a rapid stimulation of glycolysis during the most intense forms of exercise.

CAPILLARITY

The number of capillaries surrounding muscle fibers has been shown to increase in response to training. The purpose of this increased capillarity appears not to be related to an increase in the supply of blood to the working muscle per se but to prolongation of the transit time for blood through the capillary bed of the muscle. This increased transit time improves the potential for exchange of substrates to and metabolic byproducts from muscle fibers. During prolonged exercise, these effects allow greater uptake by muscles of glucose and free fatty acids, which are ideal fuels for the metabolic pathways, whereas during intense exercise, the capability for the offloading of oxygen and glucose and the removal of carbon dioxide, lactate, and hydrogen ions from the contracting muscles is increased.

ALTERATIONS IN FIBER TYPES

The proportion of type I fibers in the skeletal muscles of several breeds of horse has been shown to increase in response to advancing age (from juvenile to adult) and training. In contrast, a growing body of evidence indicates that conventional race training of adult horses induces few transformations in fiber type proportions, although fast-twitch fibers have been demonstrated to undergo substantial increases in oxidative capacity. The most consistent change in fiber type proportions in equine training studies is a shift resulting in a higher type IIA (fast twitch, high oxidative) to type IIB (fast twitch, high glycolytic) (2a:2x) fiber ratio.

GLYCOGEN CONCENTRATION

The concentration of glycogen in equine skeletal muscle, usually in the range of 500 to 650 millimoles (mmol) glycosyl units per kilogram (dry weight), is about 50% higher than that in humans. In general, training results in a moderate increase in the intramuscular glycogen store, particularly if an adequate plane of nutrition is maintained.

Repletion of glycogen following prolonged exercise is relatively slow in the horse, taking, at times, up to 48 hours for complete replenishment. Assuming that the diet contains sufficient calories, nutrition apparently has little effect on the rate of glycogen repletion. Similarly, supplementation with glucose, either orally or intravenously, does not affect the rate of replenishment. In humans, a combination of prior training, a bout of exercise to induce glycogen depletion, and subsequent consumption of a high-carbohydrate diet result in what has been referred to as a "supercompensation" in the muscle glycogen store. To date, this effect has not been readily reproduced in horses. However, feeding of diets high in fat has been suggested to result in both a glycogen sparing effect during exercise and an increase in the resting concentration of muscle glycogen. The mechanism for this increase in muscle glycogen reserve has not been established.

BUFFERING CAPACITY

Horses possess a large intramuscular buffering capacity that assists in offsetting the deleterious effects of proton accumulation during intense exercise. These systems are so important that the capacity to perform high-intensity exercise is thought to be linked to the concentration of buffers within muscle. Hydrolysis of phosphocreatine provides buffering in response to energy production in the myofibrils and is referred to as *dynamic buffering*. *Physiochemical buffering* refers to the static buffering systems within skeletal muscle provided by proteins, dipeptides, and inorganic phosphate in the myofibers. Of the physicochemical buffers, carnosine provides a large proportion of the buffering capacity of equine skeletal muscle.

Training appears to exert a variable but beneficial response on muscle buffering capacity. The advantage of a training-induced increase in buffering capacity is to allow the horse to tolerate a higher proton (H^+) load in working skeletal muscle during exercise, thereby postponing the onset of fatigue.

HEMATOLOGIC RESPONSES TO TRAINING

HEMOGRAM

Erythrocytes are a vital conduit for the transport of oxygen from the lungs to the working muscle. Increases in the total red cell pool have been reported following training in racehorses. This would provide its greatest advantage, in terms of improved oxygen transport, during intense exercise, when maximal splenic emptying has occurred. Training has been shown to result in moderate increases in the resting hematocrit, hemoglobin concentration, and red cell count. The significance of these increases is not clear, since they may only reflect the variations in these variables encountered in response to repeated sampling. Changes in the horse's temperament in response to training also may influence these responses. In general, as horses progress through training, they become a little more excitable when handled, particularly when procedures such as venipuncture are being performed. This apparent short-lived anxiety may result in increases in the variables described above, making their physiologic significance open to question. The circulating white blood cell pool, however, undergoes few, if any, changes in response to routine race training.

When compared with other types of racehorses, Endurance horses frequently have lower values for resting hematocrit and other red cell indices. This may be the result of an increase in the plasma volume in response to training, although this is by no means a routine effect of training.

PLASMA BIOCHEMISTRY

Plasma biochemical values are often integral to the diagnosis of disease syndromes in horses. For example, elevations in the plasma activities of the enzymes CK and aspartate aminotransferase are common in association with myopathies, whereas increased activity of gamma glutamyl transferase has been linked to hepatic dysfunction or other nondefined changes occurring in association with training. Despite the utility of these markers for certain diseases, minimal changes are reported when many commonly measured plasma biochemical variables are measured repeatedly throughout a training program.

BLOOD AND PLASMA VOLUME

Training has been reported to result in an increase in the plasma volume. This increase in plasma volume coupled with fixed hemoglobin concentration in blood indicates that an increase in the oxygen-carrying capacity of the blood also occurred. This is likely to contribute to the increase in maximum oxygen uptake $\dot{V}O_{2max}$ that occurs early in response to training. An expansion of the plasma volume is also likely to contribute to improved capacity for thermoregulation, since it would allow increased blood flow to the skin while maintaining blood flow to working muscle during exercise.

CHANGES IN CARDIORESPIRATORY VARIABLES IN RESPONSE TO TRAINING

HEART RATE

A reduction in heart rate during submaximal exercise in response to conventional and treadmill training has been reported in horses. However, this finding is not common to all studies, with a number of reports describing no change in the heart rate response to submaximal exercise following training. Maximal heart rate does not appear to be affected by the state of training.

Recovery of heart rate following exercise has been suggested to be correlated with the level of fitness in racehorses. The recovery of heart rate in the first 5 minutes following galloping in Thoroughbreds was reported to be a useful indicator of fitness. Heart rate recovery became more rapid as fitness improved. In contrast, several other studies have failed to show a similar response to training.

Heart rate recovery following endurance exercise is likely to be a valuable tool in the assessment of training effects. From studies conducted over the decades at competitive endurance rides, it is apparent the fittest horses suffering the fewest metabolic derangements (e.g., dehydration) at the compulsory rest stops (vet gates) have the most consistent and rapid drop in heart rate during the rest period. This is supported by other observations where more poorly performing Endurance horses have slowest heart rate recoveries and, as such, are at risk of being eliminated from the race.

STROKE VOLUME AND CARDIAC OUTPUT

Training has variable effects on stroke volume during exercise. When trained at low speeds, some suggest that a small but significant increase occurs in stroke volume in response to submaximal exercise following the training. Also, there appears to be an increase in stroke volume and cardiac output during maximal exercise following training. These findings are not always repeatable, however, as some authors report no changes in stroke volume in response to training.

MAXIMAL OXYGEN UPTAKE ($\dot{V}_{O2MAX}$)

Increases in $\dot{V}O_{2max}$ have been reported following training, with the most substantial increases occurring in the first few weeks of training. Up to 25% increases in $\dot{V}O_{2max}$ have been reported following training in Thoroughbred horses. This increase is associated with an elevated plasma volume, arteriovenous oxygen difference, and stroke volume.

METABOLIC RESPONSES TO TRAINING

LACTATE PRODUCTION AND METABOLISM

Lactate is a product of muscular metabolism and accumulates in muscle and blood at higher intensities of exercise. One of the effects of training is to increase the exercise intensity at which lactate begins to accumulate within the circulation. This is referred to as the *onset of blood lactate accumulation* (OBLA), and the derived variable V_{LA4} is the speed at which a blood lactate concentration of 4 millimoles per liter (mmol/L) is achieved. Generally, this is the blood lactate concentration above which lactate is known to increase rapidly in the blood. An intensive 5-week training period resulted in an increase in the V_{LA4} from 7 to almost 8 meters per second (m/s). The advantage of this effect would be an increase in the speed at which the deleterious effects of proton accumulation occurs during high-intensity exercise.

APPLICATION OF INDICES OF FITNESS TO HORSES IN TRAINING

RESTING MEASUREMENTS

A number of measurements that may give some insight into a horse's athletic potential have been described. These include conformational indices, estimates of heart size and blood volume, and determinations of the proportions of fibers within the muscles of locomotion. In contrast, few measurements, when performed in the resting horse, provide information regarding the animal's fitness despite attempts to link the two.

CONCLUSION

The horse is a naturally gifted athlete that also possesses great capacity to respond to the repeated stresses imposed by training. Although a substantial body of information about the adaptations occurring with training is now available, the need for more data relating to specific competitive events persists. One of the major problems in training horses is the objective assessment of fitness. The more widespread use of sophisticated field testing may permit changes in fitness to be assessed more critically in the future.

CHAPTER

20 Training the Thoroughbred Racehorse

DAVID R. HODGSON

For decades, many well-credentialed trainers of Thoroughbred racehorses have been successful without any formal knowledge of the science of exercise or training. However, over recent decades, modification of traditional techniques and application of new scientific findings have, in some cases, assisted in obtaining better results. Quite a few trainers are now using the science of equine exercise as an aid to training. Therefore, this chapter presents some of the more research-based principles that are relevant to the training of Thoroughbred racehorses. Although training Thoroughbred racehorses may, on the surface, appear relatively easy, this endeavor is quite complex. The metabolic demands on Thoroughbred horses in racing over the common racing distances of 1000 to 3200 m (5 to 16 furlongs) are quite different. In addition, there have been no published studies that apportion the contribution of anaerobic and aerobic adenosine triphosphate (ATP) supply in such events. Application of portable mask systems indicates that in the Thoroughbred, racing uses more aerobic than anaerobic metabolic contributions to exercise. This contribution may be up to 90% of the energy being contributed by aerobic pathways in a 2-mile race (see Chapter 3).

Thoroughbred trainers must, therefore, train horses in such a way that demands are placed on both aerobic and anaerobic energy pathways to increase endurance and speed. A comparison of training methods for Thoroughbreds in different countries illustrates that these objectives can be achieved in many ways. Most Thoroughbred training regimens can be divided into a variety of phases, as discussed below.

PHASES OF TRAINING

Generally, three phases of training exist:

- Phase 1: Endurance training at speeds less than 600 meters per minute (m/min)
- Phase 2: Combined aerobic and anaerobic training at 70% to 80% of maximal speed (750 to 850 m/min)
- Phase 3: Anaerobic training for development of speed and acceleration

PHASE 1: ENDURANCE TRAINING

Endurance exercise at the start of any Thoroughbred training program is vital for the racing future of the horse. It involves exercise at slow speeds (trot and canter at speeds up to 600 m/min) over long distances. The distances used vary greatly among trainers.

Such exercise rapidly increases maximal oxygen consumption ($\dot{V}O_{2max}$). Within 2 to 6 weeks, 10% to 23% increases in $\dot{V}O_{2max}$ occur. However, it is likely that prolonged endurance training over many months will result in gradual improvement in the maximal aerobic capacity of the horse, as in humans.

The other important role of the initial weeks of endurance training is the development of strength in bone and soft tissue in the limbs, that is, the response to Wolf's law. This will result in remodeling of all of the major bones, ligaments, and tendons used in locomotion and support of the animal during racing. However, little is known about the durations or intensities of exercise that promote optimal adaptive responses in bone or soft tissue (see Chapter 13). A 14-week period of treadmill training has demonstrated the effects of training on third metacarpus bone quality in 13- to 14-month-old Thoroughbred horses. The training did not produce any clinical signs of bucked shins but did result in several important adaptive responses in the cortices of the metacarpal bones of the young horses compared with unexercised controls. These included increased subcortical bone, bone mineral content, and bone stiffness.

It is likely that the greatest adaptation will occur if the "overload" principle is followed. This principle is relevant to the adaptation of many body systems in horses in training. It refers to the necessity for a gradual increase in the training stimulus every 2 to 3 weeks. This period gives time for adaptation to the current training demands before increasing the stress of the training to induce further adaptations. Many of the problems found in Thoroughbred training, such as bucked shins and periodic inappetence, are probably related to rapid increases in the intensity of training such that time for adaptation has been insufficient.

Endurance exercise training of Thoroughbreds can be conducted in many ways besides the traditional use of a jockey. Treadmill exercise at the trot and canter, jogging horses behind trucks or beside horses in carts, and swimming have been used successfully. It is important not to rely exclusively on exercise without using a jockey, especially in young horses, since this period of training is as important for development of the horse's behavior as it is for the physical adaptations to training.

Swimming should be used sparingly in endurance training of Thoroughbreds. It does promote cardiovascular fitness but probably does not develop limb strength or gait coordination. Frequent use of swimming also breaks one of the major rules of training, that is, specificity. *Specificity* refers to the need for training to mimic the gait that is employed in competition so that structural changes in the limb are appropriate to the stresses of competitive events. Although a disadvantage in training, the reduced limb load bearing in swimming is an advantage for rehabilitation programs.

Typical slow-speed training in Australia involves only 4 to 5 weeks of training at the trot and canter over 3 to 5 km (2 to 3 miles) per day before moving on to faster exercise at greater than 20 seconds per furlong. Thoroughbred trainers in England tend to employ greater durations of slow exercise over a period of 3 months or more, especially in 2-year-old horses. Subjective assessments indicate that English 2-year-olds have a lower incidence of bucked shins compared with their Australian or North American counterparts. Rapid preparation of 2-year-olds in Australia has been identified as an important contributing factor in the incidence of bucked shins and other joint ailments.

It is not possible to make specific recommendations about the duration of the trotting and cantering endurance training of Thoroughbreds. Generally, most Thoroughbreds can canter about 10,000 meters at about 500 m/min in one bout 6 days per week after a suitable 3- to 5-month prior training and adaptation period. More prolonged endurance training also has been recommended. An obvious balance has to be struck between the likely adaptive advantages and the financial cost of prolonged training periods. If conditions are hot and humid, it is appropriate to break up the endurance training into 10- to 15-minute sessions if relatively high-speed cantering is being used. This will obviate the risk of exhaustion caused by hyperthermia or heat stroke.

Slow-speed training has been subdivided into a slow, long-distance training phase and a phase at slightly faster speeds termed *cardiovascular fitness work* to improve the oxygen transport system. This division is entirely arbitrary and varies according to trainer, horse, track, and climate. This seems not be of great consequence, as no differences have been demonstrated in the degree or rate of change in cardiovascular fitness during training at different speeds of submaximal exercise. However, the recommendation that a prolonged period of exercise at speeds up to about half pace (800 m/min) with gradual increases in the stress of training every few weeks is likely to be physiologically appropriate.

PHASE 2: COMBINED AEROBIC AND ANAEROBIC TRAINING

The overload principle dictates that the training speed should gradually approach racing speed. Training at about 70% to 80% of racing speed results in accumulation of lactate in the muscle and blood of the horse. This indicates that anaerobic glycolysis has been employed by some muscle cells to support the need for ATP supply. Exercise at speeds that produce high blood lactate concentrations, for example, 15 to 20 millimoles per liter (mmol/L), which approximate those found after racing, cannot be maintained for prolonged periods or be repeated daily. The inevitable consequence of attempts to do so will be lameness, a state of overtraining, or both, reflected in weight loss, inappetence, loss of interest in racing, and poor performance.

However, training at 70% to 85% of racing speed (about 14 to 16 seconds per furlong) is an important component of Thoroughbred training, since it stimulates muscle adaptations that reflect improved capacity for anaerobic ATP resynthesis. Such adaptations include increased percentage of type II fibers, buffering capacity, and concentrations of enzymes involved in anaerobic metabolism. Large variations seem to exist among trainers and countries in the techniques and relative importance of this type of training.

After a period of 4 to 5 weeks of training at the trot and canter over 1600 to 2400 m per day ("pretraining"), Thoroughbred trainers in Australia usually alternate between fast and slow mornings. On slow days, horses are exercised on average 5500 m at speeds between 4 and 7 meters per second (m/s) (trot and slow canter). On fast days, horses exercise at speeds between 12 and 16 m/s over 1000 to 2000 m after a warmup of 1000 m.

Conventional training in North America reportedly employs "breeze" work at about 75% of maximal speed every 7 to 10 days interspersed with walking, jogging, and swimming. A conventional racetrack program in the United States also has been described as gallops at near-maximal speed for 600, 800, or 1000 m every fifth day. On intervening days, horses were walked, trotted, and cantered over a total distance of about 5 km.

A detailed description of a typical 9-week conventional Thoroughbred training program in the United States included the following: trotting 2400 m each day in week 1 and 4000 m each day in week 2. After 2 weeks of training at slow speed, horses were introduced to additional 1200-m gallops at about 400 to 500 m/min. The distance of the gallops was increased to 3200 m in the sixth week. Fast gallops, or breezes, at 900 to 950 m/min were then employed every 5 days over distances of 600 to 1000 m. These gallops were followed by rest days and then 3 days of the trot and gallop exercise in a 5-day rotation. The percentages of maximal heart rate achieved in the trotting, gallops, and breezes were approximately 50% to 60%; 70% to 90%; and 90% to 100%, respectively.

In England, training of the aerobic and anaerobic systems includes regular multiple exercise bouts on hills, combined with exercise on level ground over 800 to 1600 m at about 14 to 15 seconds per 200 m. For example, 6 days of training could include 10 canters up an 800-m incline at about 20 seconds per 200 m and two workouts over 1200 m at the higher speeds. Horses canter up hills one to three times a day at speeds that usually generate postexercise blood lactate concentrations of 3 to 15 mmol/L. These concentrations are similar to those found after single bouts of 1200-m exercise at 14 to 15 seconds per 200 m on level ground.

Two main intensities of exercise on flat sand tracks have been described for training Thoroughbred horses in Germany. In addition to slow work at speeds less than 11 m/s for less than 3 minutes, horses also work at faster speeds that generate high blood lactate concentrations. At a mean speed of 13 m/s for 100 seconds, average maximal postexercise blood lactate concentration was 16.3 mmol/L.

Strategies for increasing the volume of training which stimulate anaerobic glycolysis without causing fatigue and overtraining include increased training distance or frequency and use of exercise on hills or treadmills inclined at 5% to 10%.

Many horses in Australia are exercised at 70% to 80% of top speed over distances much less than race distance, although some trainers frequently use exercise at about 15 seconds per 200 m over distances anticipated during racing. It appears that Thoroughbreds can successfully perform exercise at that speed over 1000 m at least once daily 5 days per week in addition to daily treadmill exercise. Treadmill exercise can consist of 1600 m of trotting and slow cantering, 3-minute rest periods, and then fast canter exercise over 1200 to 1600 m. Such fast canters usually result in postexercise blood lactate concentrations of about 4 mmol/L.

Increased training frequency enables use of training at appropriate speeds on more days per week compared with usual or interval training. Interval training refers to the use of multiple workouts on the same day separated by short rest periods. Interval training has been advocated as a way of increasing fitness, but few investigations of the possible advantages of interval training compared with continuous training have been conducted.

Interval training on a treadmill does result in increased fitness, as measured by the heart rate response to exercise. Recent evidence also suggests that interval training may increase the anaerobic capacity of Thoroughbreds. This conclusion was based on a finding of higher rates of lactate production and increased plasma lactate clearance rates in the interval-trained horses. However, differences in the responses to training in interval-trained and conventionally trained horses in this study may have been caused by differences in the total amount of training performed rather than being a reflection of the use of interval training. The relationships between anaerobic capacity and blood lactate responses to exercise in racehorses have not been fully described.

An interval training program in Thoroughbreds was used to investigate skeletal muscle adaptations to training. After a warmup trot and canter over 1200 m, horses performed increasing numbers of 600-m gallops at speeds of 820 to 860 m/min. One-minute rest periods were given between gallops. In weeks 1 to 2, 3 to 4, 5 to 6, and 7 to 8, horses completed 1, 2, 3, and 4 × 600-m gallops, respectively. Horses galloped 3 days per week in week 1 and 5 days per week in other weeks, with 2 rest days 3 days apart. This training program significantly increased the mean VLA4 (the exercise speed that results in a blood lactate concentration of 4 mmol/L). Values for VLA4 were derived from a racetrack exercise test requiring horses to gallop at 540 to 670 m/min.

The advantage of hill or treadmill exercise for strenuous submaximal training is the reduced speed of the exercise and reduced likelihood of injury. When treadmills are used for such training, the work speed can be carefully controlled, and blood lactate concentrations, heart rates, or both are easy to monitor to regulate the intensity of exercise.

Swimming is not appropriate for improving the anaerobic endurance of horses, since heart rates during swimming are generally less than 180 beats per minute (beats/min). Moreover, swimming is not specific to competitive exercise and, therefore, may not stimulate adaptation in muscles recruited during running.

PHASE 3: ANAEROBIC TRAINING

Thoroughbred horse racing over any distance employs some exercise at top speed and usually necessitates rapid acceleration at some stage of the race. It is, therefore, important that this aspect of training not be ignored. Traditionally, very little training of Thoroughbreds has been used specifically for speed and acceleration. Most top-speed exercise is given at the completion of work at submaximal speeds. For example, horses often exercise over 600 to 1600 m at 14 to 16 seconds per 200 m and then gallop over another 200 to 600 m at 95% to 100% of top speed.

Specific speed and acceleration training could employ frequent exercise at top speeds over 200 to 400 m (1 to 2 furlongs). Interval-training techniques would be appropriate. Sprints shorter than 40 to 45 seconds duration are probably necessary to improve anaerobic capacity. The number of brief-duration, high-speed intervals should be increased gradually every 2 to 3 weeks, with intensive training days probably limited to twice weekly. Other training days through the week can be of the slow or moderate training intensities described above. Some race-fit and nearly-fit Thoroughbreds in England are maintained on a 3-day rotation twice weekly. Horses are given phase 1, 2, and 3 exercises on successive days.

However, frequent use of high-speed interval training over 600 m or more is probably inappropriate. In eight Thoroughbreds that performed four 600-m exercise bouts at near-maximal speeds with 5-minute rest periods between exercise sessions, muscle glycogen concentrations were reduced by approximately 50%. Therefore, it may be unwise to employ frequent interval training at 95% to 100% of top speed over distances of 800 to 1200 m (4 to 6 furlongs). Such training may lead to overtraining and the possible loss of 3 to 5 months of adaptation to prior training.

Maintenance of fitness does not require intensive and frequent training. The frequency of training can be reduced when horses are racing every 2 weeks as is often the case in Australia and the United States.

CONCLUSION

The difficult task for the trainer of the race fit horse is to balance the demands of racing, postrace recovery, maintenance of fitness, and mental attitude of the horse. It is unlikely that scientific formulas will ever be devised to give trainers exact recipes for management of the fit racehorse from week to week and race to race. The experience of the individual trainer and recognition of the individual attitudes, attributes, and needs of each horse in the stable then become even more important.

CHAPTER

Training Standardbred Trotters and Pacers

21

KARYN MALINOWSKI AND RYAN AVENATTI

In the 15 years since David Lovell authored the chapter on training the harness horse for the first edition of the *Athletic Horse*, the Standardbred horse has consistently improved its ability to perform. Race times continue to be lowered, and one can only wonder when the Standardbred will approach speeds equivalent to that of the Thoroughbred running horse. One might ask: Can this be attributed to an improvement in the horse? Are training methods the reason behind this? Is it an improvement in equipment and racetrack surfaces? Is it advances in the science of what makes a horse race faster and the application of new technology to its care and management?

The answer to the last question is that while equine exercise physiologists know more about "what makes horses tick," equine scientists have not gone so far as to transfer the science to practical application for use in the field for training and conditioning horses. The real challenge for equine scientists, if a difference is to be made in the racing community, is to increase the scientific knowledge of trainers to the extent that they find the science understandable and useable. Conditioning programs will not change unless suggested changes are practical in nature and laid out with a clear understanding of how they promote positive adaptations in exercise performance.

The Standardbred horse, genetically, is a more superior athlete today than it was 15 to 20 years ago. It is more naturally gaited, possesses innate speed and intelligence, and has more human contact and handling from birth onward, making it easier to break and train. Standardbred horses in previous decades were more heavily boned and not as refined in their conformation. Trainers had to teach them to perform. Horses were more difficult to break and gait, and speed had to be developed. The stallion Meadow Skipper genetically changed the conformation of the horses he sired. Horses became more streamlined and taller, with higher withers, finer bones, and longer legs, thereby creating more speed. Speedy Crown and Speedy Somolli changed the trotting breed, making trotters more naturally athletic and better gaited. Contemporary Standardbreds have natural speed and, according to Kelvin Harrison, "fall out of their mothers pacing." Today's Standardbred trainer primarily develops and maintains fitness; all of the horses can "get up and go." Modern trainers are conditioners, who attempt to ensure cardiovascular fitness and soundness. In the words of the veteran trainer Harry Harvey, "Horses don't require as much savvy today to train. Our title has changed from trainers to conditioners."

During the preparation of this chapter, trainers were asked, "What makes a horse great?" Top trainers today are looking for class and speed. Horses may be able to pace a 1:49 mile and still not be able to win the race. A $100,000 claimer may be able to race just as fast as an Open horse, for instance, but still cannot beat that horse. Great horses are genetically made to have the physical aptitude and the mental aptitude to be champions. Great horses are extremely durable and tolerate stress well. The reality of training horses is that a perfect horse without weaknesses does not exist. A winning trainer has the ability to identify a horse's weaknesses and then devise a plan to overcome them. A great horse must have innate courage to win races in spite of everyday aches and pains that would bother other horses (Eriksson, 1996).

That being said, scientists and trainers need to evaluate what is now known about conditioning the Standardbred racehorse. At the 2006 International Conference on Equine Exercise Physiology (ICEEP), a workshop was held on workload and conditioning. Although the focus was on the Thoroughbred racehorse, lessons learned at the workshop have application to the Standardbred as well. It was proposed that workload could be quantified by using several selected parameters commonly recorded in the industry, such as velocity and distance, to produce a workload index. What needs to be asked is what can readily be modified with training, and how training programs can be tailored based on scientific methods with easy application for the field (Rogers et al., 2007). The most likely reason for the reluctance of horse trainers to adopt quantitative measurement of workload is the difficulty in identifying suitable field measurements.

The goals of trainers interviewed for this chapter are very simple: to condition a horse to reach its true genetic potential; to lengthen time to fatigue; to improve the horse's skills; to minimize injury incidence; and to maintain a good attitude and willingness to work. The main goal of any conditioning program is to stimulate physiologic adaptations within the animal's body to improve performance. To reach this goal, periods of exercise and rest must be balanced.

From a basic physiology standpoint, the respiratory, cardiovascular, and musculoskeletal systems of the horse's body are responsible for performance. Research indicates that maximum oxygen uptake ($\dot{V}O_{2max}$), continues to increase with training for several weeks to months (Rogers et al., 2007) (Figure 21-1). One would think, from this observation, that long jog miles or endurance training is more important in changing the horse's aerobic performance than speed training (Tyler et al., 1998). More recently, however, it has been shown that speed training is a better choice for changing $\dot{V}O_{2max}$. Other benefits of speed work are increased volume of blood being pumped per beat (stroke volume [SV]) and a lower heart rate for a specific blood flow rate (Evans et al., 1995). The heart and lungs are not the only organs that

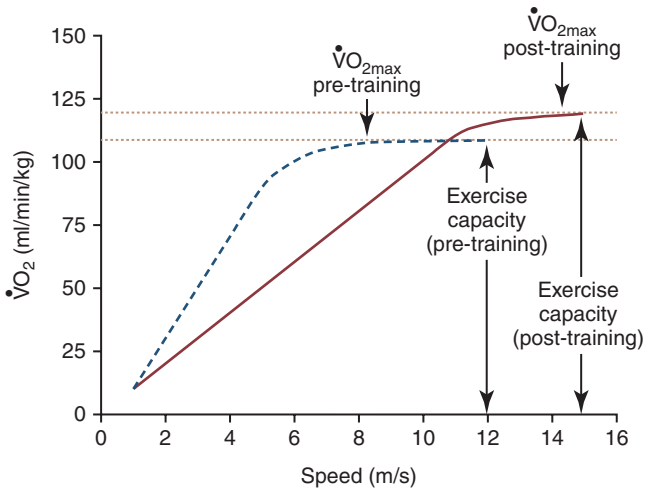

FIGURE 21-1 Maximum oxygen uptake ($\dot{V}O_{2max}$) and exercise capacity are improved with training. Note that exercise intensity can continue to increase after $\dot{V}O_{2max}$ is reached. Exercise capacity represents maximum speed reached at the point of fatigue. *(Adapted from Marlin D, Nankervis K: Training principles. In* Equine exercise physiology, *2002, Figure 15.1, p 181.)*

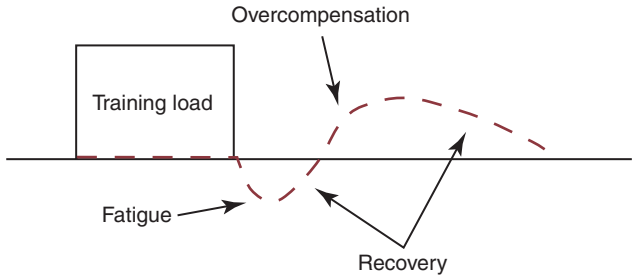

FIGURE 21-2 The adaptive response to an acute bout of exercise (*dashed line*) is shown in comparison with the basic performance level. During exercise, performance capacity is conserved until the horse reaches fatigue, at which point the level drops. After exercise, performance capacity increases past the baseline level (overcompensation). As the rest period continues, performance capacity returns to baseline. *(Adapted from Rogers CW, Rivero JLL, van Breda E, et al: Describing workload and scientific information on conditioning horses,* Equine Comparat Exerc Physiol *4(1):1–6, 2007.)*

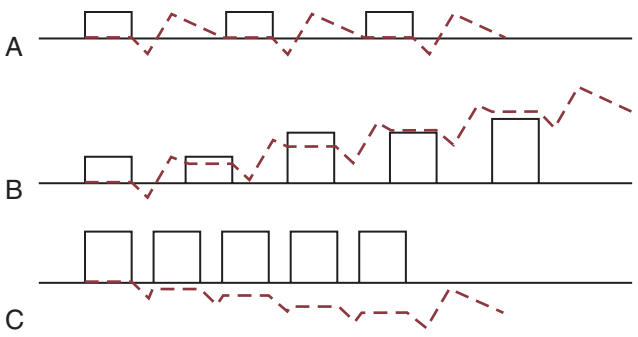

FIGURE 21-3 Adaptations in performance capacity (*dashed lines*) in response to different training strategies. **A,** Regular training sessions with the same load and relatively long rest periods do not increase performance. **B,** Regular training sessions of increasing training loads, with adequate rest periods between acute exercise bouts, increases performance. **C,** Intense training sessions with inadequate rest between acute exercise bouts will decrease performance capacity. *(Adapted from Rogers CW, Rivero JLL, van Breda E, et al: Describing workload and scientific information on conditioning horses,* Equine Comparat Exerc Physiol *4(1):1–6, 2007.)*

improve with training. Skeletal muscles and skeletal tissues such as bones, cartilage, and tendons also adapt to training. Muscles adapt to training by remodeling muscle fibers to increase aerobic capacity. Speed can also be improved with training by increasing the horse's anaerobic capacity, but exercise at an intensity of 140% to 165% $\dot{V}O_{2max}$ must be applied during the last phase of training (Rogers et al., 2007).

The metabolic demands of most equestrian disciplines are largely aerobic in nature. Conditioning programs should be designed to develop muscle properties that optimize stamina, speed, and strength. In Standardbreds, an improvement in both aerobic capacity and strength can be induced with training of low-to-moderate intensity (60%–80% $\dot{V}O_{2max}$) and relatively short sessions (6- to 12-minute duration) five times per week for 16 weeks. Prolonged training beyond this period with training at higher intensities (100%–110% $\dot{V}O_{2max}$) increases aerobic capacity but does not improve muscle strength; instead, it reduces speed and simultaneously increases the risk of overreaching and overtraining (Tyler et al., 1998).

Regardless of whether horse people are trainers or conditioners, they all need a basic understanding of exercise training and conditioning. An excellent overview of these concepts is presented in a 2007 review article titled, "Describing Workload and Scientific Information on Conditioning Horses" by Rogers et al. (2007). Their description of designing training programs based on scientific methods is discussed below.

Single exercise bouts lead to fatigue and mild cellular damage, which, in turn, result in short-term adaptive responses (Figure 21-2). Performance capacity is not increased if rest periods between acute exercise bouts are too long or if training sessions are not sufficiently challenging (Figure 21-3, *A*). With regular and gradual increases in training, the adaptation that occurs during the recovery period of a single training session leads to overall improvement in performance (see Figure 21-3, *B*). Thus, trainers of harness horses must continually provide increased levels of stress to improve performance. This "overload" principle has been used for decades. The fine point is to know what the upper limit is for these adaptations and to be aware that each horse differs in its ability to cope with this stress. It is this trait that singles out the great horse trainers and conditioners. When training is too vigorous and rest periods too short, performance is reduced because of an imbalance between training stress and recovery (see Figure 21-3, *C*). Trainers need to consider the following when designing a horse's training program: nature of the race; the current status of the horse's level of fitness and past history; the total period available for training; and the training facilities and climate. Trainers can alter levels of stress by the type of exercise; the length of training program; and the intensity, duration, frequency and timing, and length of recovery periods (Rogers et al., 2007).

Although most of the trainers interviewed utilized the swimming pool and the motorized free stall equine exerciser, they used sloped training tracks, interval training, and resistance training less. The length of the training program is critical. Despite some immediate adaptations that occur when horses are put into training, the majority of benefit can be seen within the first 10 to 15 weeks.

Intensity refers to the speed of the exercise. Fortunately, because today's Standardbred has innate speed, the amount of speed work required for race performance is less, which has decreased the incidence of injury caused by training. That being said, trainers interviewed agreed that there has been an increase in joint injuries in harness horses as race speeds continue to increase. As in the case of their Thoroughbred counterparts, with increased race speed, harness horses also can be expected to experience an increase in joint injuries, possibly catastrophic ones. When beginning a horse's training program, the minimal exercise intensity every other day should be approximately 50% to 60% $\dot{V}O_{2max}$ to improve aerobic capacity, but higher intensities are needed to improve strength (approximately 80% $\dot{V}O_{2max}$) and anaerobic capacity (up to 165% $\dot{V}O_{2max}$).

Duration refers to the total length of time of the training session. At a constant speed or intensity, duration is primarily responsible for increasing aerobic capacity or stamina. Duration of the workout should be increased gradually by approximately 10% per week over the first 10 weeks. This can be achieved by increasing the number of jog miles. Working the horse for long durations at low speeds is more effective in increasing aerobic capacity than working them for short durations at high speeds.

Finally, the number of training sessions equates to the *frequency* of the application of stress to the horse. In high speed workouts or "training miles," the horse's muscle metabolism is 100 times more than at rest, and the increased oxygen consumption results in the formation of free radicals or oxidative stress (discussed later in this chapter); this causes muscle damage that lasts for 48 to 96 hours. Subjecting the horse to further hard work or speed miles during this period is likely to induce further damage and delay recovery (Rogers et al., 2007). All trainers interviewed agreed that it takes longer for horses to recover after a race today compared with 5 to 10 years ago. This is most likely caused by the current fast race times for the Standardbred racehorse. Like their Thoroughbred counterpart, the harness horse competing at the elite level needs more than a 1 week rest period to remain competitive, and its races need to be chosen with care to ensure ample recovery time between races.

Although training methods have changed very little in the past decade, it is a fact that horses today are not trained as hard as they used to be and are trained with much less speed work. Trainers today build a base of fitness, increasing speed slowly. Horses are not scored down at fast speeds prior to a race nowadays. Generally, horses that are currently racing jog approximately four to five miles per day, train a trip 2 to 3 days out prior to the next start, race, and then have 1 day off. Horses should be jogged at a decent rate of speed (approximately 11–13 miles per hour or a 4:30–5:30 minute mile to ensure conditioning. For conversion from meters per second to miles per hour, see Table 21-1. Using the global positioning system (GPS) technology in place of a stopwatch should be considered by trainers to monitor speed and distance traveled. Training miles are performed at slower speeds than in the past, such as 26 miles per hour or a 2:20 minute mile. Hard last quarters are not performed anymore.

All of the conditioners interviewed agreed that horses are individuals, that every horse's schedule needs to be altered according to the animal's individual needs, and that once a horse gets into a routine, nothing should be changed unless a horse has a problem. The trainers interviewed emphasized careful selection of races, patience in training, and waiting for the "big" race. This is easier to achieve earlier in the season. Horses usually peak after five races, achieving their best performance in races six and seven. It is the trainer's responsibility to train horses to be competitive in the category in which they race. If a horse has a chance to be a moderately competitive 3-year-old or a raceway horse, the Meadowlands Racetrack in East Rutherford, New Jersey, for instance, is not the place to be. If you have a horse that is borderline, with the potential to be a decent horse, you do not want to "chase" the horses at big tracks, such as the Meadowlands; you will only end up breaking your horse's spirit and heart.

A decade ago, horses raced in multiple heats; with warmups and speed trips between heats. Similar to conditioning protocols, the warmup strategies of the harness horse before a race have changed as well. Today, a horse will have a low intensity warmup of approximately two miles prior to the race and then perform the actual race. Previous scientific work has shown that a warmup prior to intensive exercise such as a race accelerates O_2 kinetics, augments aerobic energy metabolism, and reduces time to fatigue, hence the preference for the warmup (McCutcheon et al., 1999). Although the Thoroughbred and Standardbred perform a similar exercise test of work at maximal heart rate (HR_{max}) and maximal speed, at distances that take 1 to 3.5 minutes to complete, the trainers of these breeds utilized different warmup strategies in the past (Jansson, 2005). Trainers of Thoroughbred racehorses favored shorter, less strenuous warmups, whereas Standardbred trainers preferred several warmup heats. Today's Standardbred trainer is warming up the horse less prior to a race and at a slower speed. A recent study in Sweden aimed to look at the advantage or disadvantage of a long warmup, especially as it related to time for recovery. Findings indicate that a short warmup, which would equate to one prerace warmup of one mile, approximately 1 hour before

TABLE 21–1

Speed Conversion Chart

Meters per Second	Miles per Hour	Approximate Mile Time (Minutes : Seconds)
1	2.237	26:49
2	4.474	13:25
3	6.711	8:57
4	8.948	6:42
5	11.185	5:22
6	13.422	4:28
7	15.659	3:50
8	17.895	3:21
9	20.132	2:59
10	22.369	2:41
11	24.606	2:26
12	26.843	2:14
13	29.080	2:04
14	31.317	1:55
15	33.554	1:47

the race, would be most beneficial. With regard to the "score" before a race, it has been scientifically documented that a warmup prior to a race, including sprints, near maximal $\dot{V}O_2$ does not improve total running time to fatigue compared with a lighter warmup (Jansson, 2005).

The principles described throughout this chapter apply to both trotters and pacers; however, trainers agreed that trotters are more of a challenge in that they need to be relaxed to be successful. Trainers want pacers to "feel good" and be overly confident and competitive on race day. Trotters get more work to be kept calm and flat. An early foundation of jog miles will help balance and even out the trotter. Although some traditionalists disapprove of trotting hobbles, most trainers find them to be an absolute advantage. Regardless, judicious use of equipment and shoeing, in addition to an appropriate exercise regimen, will improve the locomotion of the equine athlete.

BREAKING YEARLINGS

In the present day, newly purchased yearlings have experienced much human handling and contact since birth, so much so that by the time they are sold, trainers usually need to just "harness and go." Young horses currently have so much innate talent that trainers typically have to wait a long time to see which ones are special. As mentioned earlier, patience is of utmost importance because young horses (especially pacers) want to go fast right off the bat. If the trainer allows these horses to consistently go "all out," it is unlikely that these horses will last to the end of their freshman year.

The second edition of the popular *Care and Training of the Trotter and Pacer* provides insight into breaking yearlings in the chapter by Charles Sylvester, and the text concurs with the trainers interviewed. Yearlings are line driven before being hitched to the jog cart, even though the sales preparation process has already had them used to human contact. Yearlings are started in a blind bridle with a latex-covered snaffle bit and a plain overcheck, or just a chin strap with a very long overcheck. Yearlings start jogging two miles for about a week, increasing to three miles a day after that. Horses are jogged both ways of the track to acclimate them to move and visualize their surroundings in both directions. Horses are jogged at a speed of 13 to 14 miles per hour (4:00- to 4:30-minute mile) to build up their condition and to keep them alert and focused. After 4 to 6 weeks of jogging three miles per day, a fourth jog mile is added, and horses begin doing training miles 2 days per week. The first time a young horse trains, it might go a 3:00 minute mile, or approximately 20 miles per hour. In subsequent weeks the trainer will bring the horse down 5 seconds for the mile each week until reaching a 2:40 minute mile, or speed of 22 miles per hour. After this point, the total time for the mile might remain at 2:40, but a focused effort should be made to ensure that the last half and quarter will be faster. If the horse can go a mile in 2:40 minutes with a half in 1:15 minutes and quarter in 35 seconds, it is ready to proceed. When a young horse has reached a 2:40 minute mile comfortably, it is ready to begin the second phase of training with repeating workout miles (Sylvester, 1996).

It is paramount that young horses are jogged both ways of the track and in groups. Jogging in groups keeps yearlings calm, they learn to pass and switch places, making their work fun, enabling them to train down within themselves and "get to like it." Once the yearlings are broken, gaited, and have trained a mile in 2:50 to 3:00 minutes, many trainers turn the horses out for a month. Once brought back, the horses return to form very quickly. European trainers put sulkies on their horses earlier in training. The sulky takes drag off a young horse and makes its work easier. A good way to get a foundation on a horse is by going double-headers. A double-header is going two training miles without returning to the barn. The horse remains on the track between trips. It builds stamina and wind in the horse and saves time. The first mile might be in 2:50 to 3:00 minutes with a jog mile in between before turning again to go in 2:30 minutes.

Most trainers agreed that it is very tough to have a horse ready in June. It is more realistic to try for the "baby" races in July. What is paramount is that the young horse should not have a bad experience. It is too easy to break the spirit of a 2-year-old. By all accounts, *they should not be rushed*. Trainers today find it extremely difficult to have young horses in top form at both the beginning and at the end of the racing season. It is tough to get a horse ready early and still be good late in the season.

ALTERNATIVE TRAINING METHODS: USE OF RESISTANCE CART, HEART RATE MONITOR, AND LACTATE ANALYZER

Many trainers today are still not utilizing the tools available to them to help condition their horses. The primary goal of the training program does not change; it is to achieve maximum speed for the entire duration of the race. Heart rate monitoring gives the trainer the ability to stimulate the muscle fibers desired (slow-twitch, aerobic; or fast-twitch, anaerobic). Slow-twitch fibers are stimulated at a heart rate of 150 to 175 beats per minute (beats/min) or 50% to 60% HR_{max}. Fast-twitch fibers are stimulated at heart rates over 200 beats/min or at 80% to 100% HR_{max} . In other words, although the cardiovascular and energy-generating systems of the horse's body adapt quickly to exercise training, conditioners of horses must design training programs that allow sufficient time for adaptation of supporting structures, at the same time working the horse hard enough to continue to improve fitness (Geor, 2000). Heart rate monitors should be used by trainers as a means of assessing the horse's work effort and improvement in level of fitness. These monitors are simple to use and are reliable and accurate. At rest, a relaxed horse will display a heart rate in the range of 20 to 40 beats/min. A linear relationship exists between heart rate and speed—heart rate continues to increase, as does speed, until the horse's highest heart rate is reached (210–240 beats/min). At top speeds, the heart rate–speed curve flattens out as the horse approaches its HR_{max} (Figure 21-4). HR_{max} is intrinsic to the individual horse and does not change with increased fitness. However, submaximal exercise does result in a decreased heart rate, enabling the horse to perform at higher speeds before reaching its HR_{max}. An improvement in fitness is reflected in a lower heart rate at a given speed or velocity and a more rapid decline in heart rate after the exercise. When plotting heart rate versus speed, the relationship drawn moves to the right (Evans, 2000) (Figure 21-5).

Percentage maximal heart rate (%HR_{max}) also has a relationship to percentage maximal aerobic capacity (%$\dot{V}O_{2max}$), which can be utilized when assessing the horse's training program (Figure 21-6).

In the early part of training, when breaking yearlings or bringing a horse back from a lengthy lay-up, the horse should

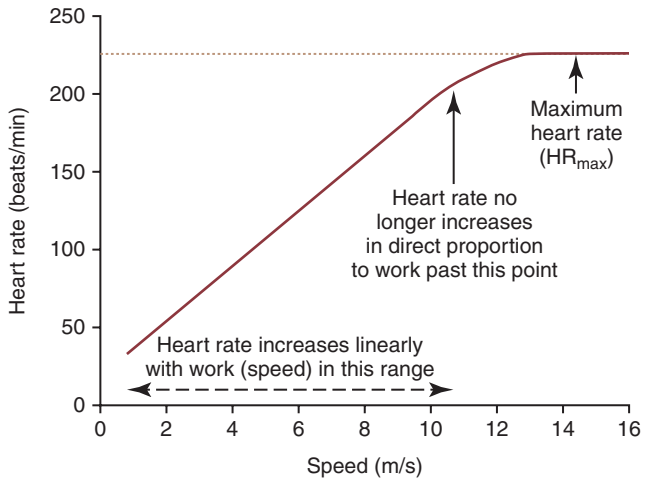

FIGURE 21-4 The relationship between heart rate and speed, with three stages of the heart rate–speed curve illustrated. *(Adapted from Marlin D, Nankervis K: Cardiovascular responses. In* Equine exercise physiology, *2002, Figure 10.6, p 119,* Blackwell Science LTD, Oxford.*)*

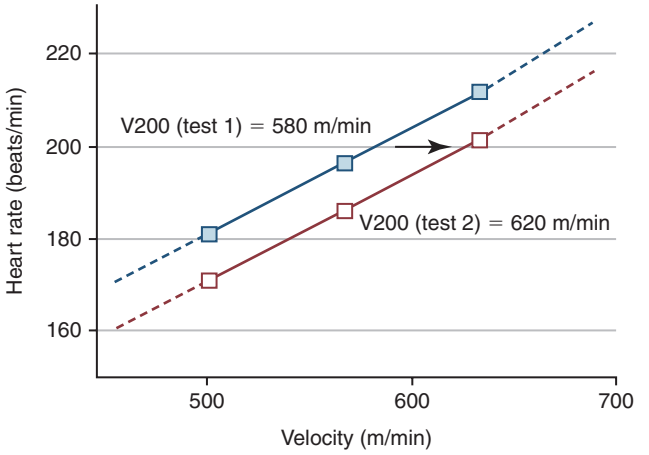

FIGURE 21-5 The relationship between heart rate (beats per minute [beats/min]) and velocity (meters per minute [m/min]) before (Test 1) and after (Test 2) 6 weeks of training. Improvement in fitness is represented by a shift to the right of the curve. *(Adapted from Evans D: Exercise testing in the field. In Hinchcliff KW, Kaneps AJ, Geor RJ, editors:* Equine sports medicine and surgery: basic and clinical sciences of the equine athlete, *2004, Figure 3.8, p 119,* WB Saunders, Philadelphia.*)*

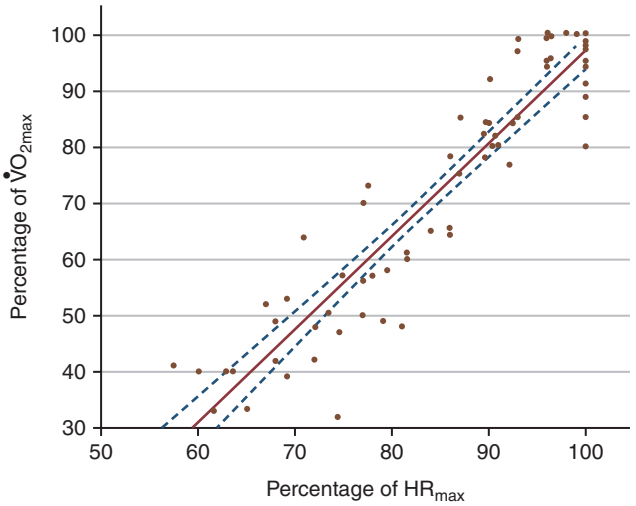

FIGURE 21-6 Data points represent horses at varying levels of fitness. There exists a positive, linear relationship between percentage maximal heart rate (%HR_{max}) and percentage maximal oxygen uptake (%$\dot{V}O_{2max}$). As heart rate increases, aerobic capacity increases. Measuring a horse's heart rate during conditioning can infer its aerobic capacity. *(Adapted from Poole DC, Erickson HH: Cardiovascular function and oxygen transport: responses to exercise and training. In Hinchcliff KW, Geor RJ, Kaneps AJ, editors:* Equine exercise physiology: the science of exercise in the athletic horse, *2008, Figure 4.1.26, p 229,* WB Saunders, Philadelphia.*)*

be exercised at workloads equivalent to 40% to 50% $\dot{V}O_{2max}$, which equates to 60% to 70% HR_{max}. If one assumes that the HR_{max} is 220 beats/min, then the target working heart rate should be 135 to 155 beats/min. After completion of this base training, speed work is introduced, and it will elicit HR_{max} and stimulate adaptations in the anaerobic energy system, as described previously. At this stage, heart rate measurement becomes less useful in indicating work effort. However, heart rate monitoring is still valuable for evaluating recovery after a training mile or race (Geor, 2000).

Although trainers in the field do not have access to a high-speed treadmill to ensure that horses are truly getting an adequate workout to continue increasing condition, by working a horse over a set distance at different speeds, it is possible to plot data on the horse's heart rate response to exercise of different intensities. To make comparisons between exercise sessions, it is important to keep the conditions as constant as possible, since environmental and track conditions do affect heart rate.

Heart rate recovery is also useful in assessing a horse's fitness level. As fitness improves, heart rate declines more rapidly following the training mile or race. Assessing the horse's heart rate at 2, 5, and 10 minutes after exercise will tell the trainer if the horse truly worked at its maximum effort and if the horse was actually capable of handling the exertion (Geor, 2000).

Field tests at multiple speeds and blood collections have suggested that the velocity to induce a blood lactate concentration of 4 millimoles per liter (mmol/L) (VLa4) is a valid measurement for the evaluation of fitness in Standardbred trotters and pacers. In determining VLa4, speed versus blood lactate is plotted. A line is drawn horizontal to the 4 mmol/L concentration. There is a shift to the right with improved level of fitness (Figure 21-7). It has also been demonstrated that superior race horses have a lower blood lactate response to an exercise test compared with poor performers. This measurement is potentially useful for race horse conditioners as they evaluate horse performance and manage the racing careers of horses in their care.

Measurements such as heart rate during exercise and blood lactate concentrations after exercise can be measured on the track. This enables trainers to calculate a horse's physiologic state by measuring variables such as the velocity at which the horse's heart rate reaches 200 beats/min, or the velocity at which blood lactate concentration reaches 4 mmol/L (Evans, 2000).

Racehorse Conditioning Systems, Inc. is trying to simplify the science for use by horse trainers in the field with the use of the resistance or power cart, the heart rate monitor, and the lactate analyzer.

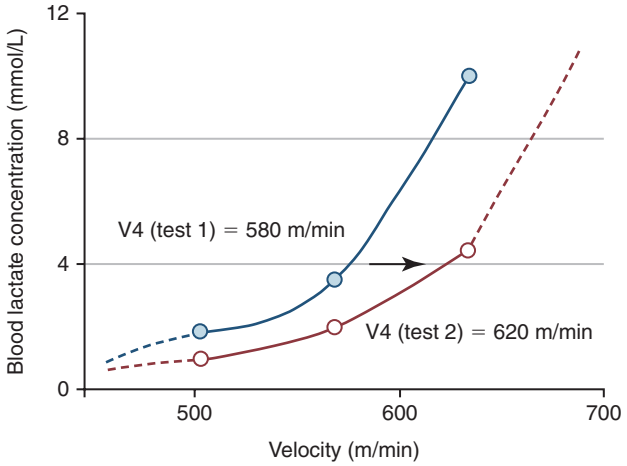

FIGURE 21-7 The relationship between blood lactate concentrations and velocity before (Test 1) and after (Test 2) 6 weeks of training. Improvement in fitness is represented by a shift to the right of the curve. *(Adapted from Evans D: Exercise testing in the field. In Hinchcliff KW, Kaneps AJ, Geor RJ, editors:* Equine sports medicine and surgery: basic and clinical sciences of the equine athlete, *2004, Figure 3.8, p 119,* WB Saunders, Philadelphia.*)*

The resistance cart is a modification of the traditional jog cart with the addition of a mechanism that provides a drag created by tension generated from gears located on the wheel hub. Different settings allow the trainer to change the drag or resistance to be used on an individual horse (Racehorse Conditioning Systems Inc., 2012).

Resistance training is wonderful for working horses at slow speeds, building strength and endurance by forcing the horse to work its back, stifles, and hocks. Resistance training can be used to supplement traditional jog miles, alone with horses that have soundness problems that make them unable to train in traditional fashion and stay sound, or in conjunction with interval or training on the "strip." When using the resistance cart, a horse should have its head free or unchecked, allowing the horse to relax and use its back and stifle muscles fully (Eriksson, 1996).

The science behind using both the heart rate monitor and resistance training is not new, but what is new is for Standardbred trainers to use both these tools in their conditioning programs. Plotting different exercises and baseline or resting levels at different work intensities provides invaluable information for trainers to track the entire course of a horse's conditioning program. With all horses, high-intensity workouts are desired but not at great speeds. This can be accomplished with the resistance cart.

There are advantages to resistance training. Horses can be worked at slower speeds while conditioning at higher heart rates or workloads. This reduces the potential for injury while ensuring that the horse's cardiovascular and musculoskeletal conditions continue to be enhanced. Valuable levels of aerobic and anaerobic conditioning can be produced by applying different degrees of pressure at slower speeds. Monitoring, recording, and analyzing heart rate data for each horse enables the trainer to assess how hard the horse is working and to objectively assess the training program for each horse. Heart rate recovery after a training bout or race can reveal the condition of the horse, how much "effort" the horse puts into the session, and, in the case of an abnormally high heart rate, if the horse is in pain or has been traumatized in some fashion. Lactate analysis can determine the level of anaerobic fitness of the horse (Racehorse Conditioning Systems Inc., 2012).

Interval training or training on the "strip" has been used successfully by Standardbred trainers in recent years. The strip is a straight, all-weather track, usually a quarter mile in length, with turnarounds at each end. Horses are put through a series of quarter-mile intervals in times ranging from 35 to 45 seconds. Horses seem to like the change in routine and appreciate the opportunity to go forward with speed without interference, as often happens in the turns. Work on the strip is to be used to complement, but not replace, traditional training on the track, where a horse needs to learn to be able to train on the turns (Eriksson, 1996).

ADVANCES IN VETERINARY CARE

Since the last edition of this text, significant advances have been made in the veterinarian's ability to treat equine joint disease and soft tissue injuries to tendons and ligaments.

Most Standardbreds can compete for many years with multiple rehabilitated injuries. A pacer may continue to race in spite of certain types of lameness more successfully than a trotter can because of the nature of their respective gaits. Horses get injured because of the mechanics of locomotion. Energy imparted to the horse's leg is from body weight and speed. As Standardbreds continue to go faster, they will get more injuries. Less catastrophic injuries are seen in Standardbreds compared with Thoroughbreds because the former race at slower speeds and at a different gait. Harness track surfaces are another reason for less catastrophic injuries seen in Standardbreds because these tracks are easier to maintain (not multilayered), less sensitive to moisture, and very compact.

Overall, veterinarians interviewed reported seeing more injuries of all kinds now than in previous years. Trainers stated that they have seen an increase in articular or joint injuries over soft tissue injuries, but they may just be managing and preventing soft tissue injuries more effectively. The introduction of diagnostic ultrasound to detect and monitor equine tendon injuries has greatly improved the veterinarian's ability to accurately diagnose the presence and severity of soft tissue injuries (Gillis, 1997). Before ultrasonography, veterinarians evaluated tendons by the presence of heat, gross swelling, and tenderness (palpation). Decisions were made on the basis of those clinical findings and anecdotal information on how long these injuries took to heal. This system did not work well because the veterinarian could not assess the healing process variability or the degree of the injury. Ultrasound enables the equine practitioner to objectively assess the severity of the injury, make the prognosis for return to work, and determine when the horse is ready to return to race training.

Current trends in human athletic rehabilitation may be appropriate for use in equine practice as well. These include rest, nonsteroidal anti-inflammatory drug (NSAID) administration, and ice and heat therapy using ultrasound, followed by early mobilization and return to a revised training schedule (Gillis, 1997). The ultimate goal is to get the horse back in training as quickly as possible with a minimal chance of recurrence of the injury.

With regard to joint disease, advances made in human medicine have been adapted successfully to horses. Veterinarians have been able to improve the treatment of articular disease because of an increased understanding of joint

pathophysiology, novel surgical equipment and techniques, and more sophisticated imaging modalities such as digital radiography. For an excellent review of the advances in the treatment of joint disease in horses, see the paper by David Frisbie (2005). Highlights are summarized below.

NSAIDs continue to be utilized by veterinarians and horse trainers for treating musculoskeletal pain. Of the popular NSAIDs, phenylbutazone (bute) remains the agent of choice because it is relatively inexpensive, is easy to administer, and has few side effects in acute therapy. Flunixin meglumine (Banamine) and ketoprofen (Orudis) are also used in the treatment of equine osteoarthritis but at a higher cost to the trainer and the owner.

The administration of intra-articular (IA) agents in the treatment of joint disease ranges from corticosteroids and hyaluronic acid (alone or in combination) to advanced therapies such as recombinant proteins that target specific cells to improve cartilage healing and treatment of osteoarthritis.

Many oral supplements that promise to maintain the integrity of the joint cartilage or synovial fluid are available to the horse trainer without a veterinary prescription. Most of these formulations contain chondroitin sulfate and glucosamine with or without hyaluronic acid. Anecdotal reports suggest that glucosamine, chondroitin, or a combination of both has a positive impact on maintaining joint health in the horse. Testing for supplements containing hyaluronic acid is currently underway. Research studying the efficacy of these supplements in horses needs to be conducted.

Extracorporeal shock wave therapy (ESWT) is a modality that shows promise in treating chronic joint injuries in harness horses. There is no therapeutic value in the case of acute injuries, besides easing pain for a short period (the analgesic effect lasts for 3–4 days). ESWT may work by increasing circulation and the subsequent release of growth factors in the injured area. It has also been suggested that ESWT causes osteoneogenesis (new bone formation) (Gillis, 1997).

ADVANCES IN NUTRITION

Scientific advances in training harness horses have been limited in the past decade; however, there has been much investigation into the impact of nutritional supplementation on equine performance. Once a horse reaches a training level close to its maximum effort, it becomes very difficult to increase performance with training alone. Also, relatively large increases in training effort to achieve small increases in performance significantly increase the possibility of physical injury to the horse. However, the use of a nutritional supplement that has ergogenic properties could mean the difference between finishing first and losing. An ergogenic supplement can be defined as any agent that has the ability to increase athletic performance, by increasing speed, stamina, or strength (Harris and Harris, 2005).

Although it is not the purpose of this chapter to discuss, in detail, the many factors (legal or otherwise) that can improve the exercise performance of the horse, it was interesting to find, from the trainers interviewed, that feeding racehorses has not changed to a great extent. Nutritional ergogenic agents range from normal food components to substances that clearly are illegal to administer to the competing racehorse. Conditioners of horses should be aware, however, of changes in equine nutrition that might give them a competitive edge because of the provision of increased energy for work, delayed onset of fatigue, or both, as well as protection from disease.

Data suggesting that nutritional management leading up to the day of competition can influence the horse's performance are insufficient. The Standardbred racehorse needs immediately available power, for a short duration, giving maximum effort for under 2 minutes. Energy requirements are huge and are restricted to blood glucose and muscle glycogen, which are rapidly converted to energy. The goal for feeding on race day should be to minimize weight, maintain optimal hydration, and promote increased availability of glucose and glycogen.

The feeding strategy for the harness race, which is primarily an anaerobic effort, should be to provide the normal ration of concentrates five or more hours before the race, with restricted forage consumption on the day of the race to reduce gut fill and body weight. Unlike humans, horses do not benefit from high carbohydrate supplements in feeds prior to a race. In other words, "glycogen loading" is not beneficial in equine athletes. Horses do not benefit from high-starch diets and, in fact, might be at risk for gastrointestinal distress because of the horse's limited ability to digest starch in the small intestine.

Water and electrolyte requirements on race day are not dramatically different from normal requirements because of the short duration of the effort and minimal sweat loss. It is important to be certain that horses treated with furosemide (Lasix) are well hydrated because increased water loss occurs through increased urination. Horses administered Lasix should be given electrolyte supplementation and plenty of water after the race to ensure adequate hydration. Because body weight makes a significant difference in speed, increased water consumption prior to a race is not desirable, hence the need to supplement electrolytes after the race (Ralston, 2004).

There are advantages to adding oil to a horse's diet. Fat is more calorie dense compared with concentrate, so the amount of concentrate necessary to maintain caloric intake needed for energy is reduced. Feeding supplemental oil is beneficial to the racehorse because of its increased caloric requirements and also because many high-performance horses will go "off feed." Edible oil such as corn oil should be introduced slowly and comprise no more than 10% of the total ration (1–2 cups per day). Research has shown that metabolic regulation in oil-adapted horses is improved. The high-power energy source needed for sprinting is, thus, "turned on" with the enhancement of the high-intensity exercise capacity arising from the increased anaerobic metabolism of glycogen to lactate (Harris and Harris, 2005).

Exercise-induced oxidative stress is another topic that has received attention in the arena of equine exercise physiology. Exercise such as racing causes an increase of reactive oxygen species (ROS), or free radicals, in the horse's body. Although ROS are necessary for proper functioning of the immune system, an excess can contribute to accelerated muscle fatigue and muscle fiber damage. Antioxidants have a role in protecting the horse from the potential damage of ROS. These include vitamins C, E, and A; selenium, copper, and zinc; super oxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GSH). In a 2004 study of Standardbred horses, which looked at the impact of training and exercise intensity on blood antioxidant markers, a single exercise bout significantly affected the levels of vitamin C, uric acid, vitamin A,

and glutathione reductase. Vitamin E, SOD, GPx, selenium, copper, zinc, and GSH remained unchanged. The antioxidant markers uric acid, SOD, GPx, selenium, GSH, and vitamin E underwent training-related modifications. There was a positive correlation between $\dot{V}O_{2max}$ and SOD activity, suggesting that the increased activity of this antioxidant enzyme has potential as an indicator of improved training level by blood analysis (de Moffart et al., 2004).

Vitamin E is the most important antioxidant. Vitamin E is a fat-soluble vitamin, which protects cell membranes from damage by free radicals. Cell membranes, which are comprised of lipid molecules, are highly reactive to ROS, making them extremely susceptible to damage. Vitamin E is essential in the horse's diet because a deficiency can cause lack of coordination and various muscle and nervous disorders. Vitamin E has been shown to protect against equine protozoal myeloencephalitis (EPM), equine degenerative myeloencephalopathy (EDM), and "tying up" in exercising horses. Vitamin E supplementation may be especially important for race horses, as intense exercise induces oxidative stress and ROS damage and decreases vitamin E levels in blood. Vitamin E is found in forage and pasture grasses. However, vitamin E content decreases with the age of the plant, as well as with processing such as sun curing, heat, baling, and grinding. Because vitamin E is fat soluble, it needs to be provided with fat in the diet so that it can be absorbed and utilized. If supplementing with extra vitamin E (2000 to 5000 international units per day [IU/day]), the horses should be fed a commercially available feed with added fat (10%) or a separate fat source such as oil or rice bran (Williams, 2007).

As a fat soluble vitamin, vitamin E can be stored in the body and is available for long periods, such as during the winter months when access to adequate forage or pasture is limited. Evidence suggests that horses undergoing continuous exercise conditioning may require levels of vitamin E as high as 300 IU/kg body weight daily (Siciliano et al., 1997).

Another important antioxidant is vitamin C. This is a water-soluble vitamin, so unlike vitamin E, it is not localized to the cell membrane. Vitamin C in its active form, ascorbic acid, is usually found inside and outside of cells where it interacts with free radicals. Ascorbic acid also aids in the regeneration of the vitamin E radical, restoring its antioxidant capacity. Vitamin C and E work together to protect the vital tissues of the horse. There is usually no reason to add vitamin C to the horse's diet because it is synthesized adequately by the liver. However, with intense exercise, the production of vitamin C may not keep up with the demand. Supplementation of ascorbic acid in the short term, close to the time of race day, may benefit the horse in the amount of 7 to 10 grams per day.

Selenium is a trace mineral found in plants. By itself, selenium does not have much antioxidant capacity. When selenium is paired with vitamin E, it becomes a strong antioxidant by stopping nerve cell damage caused by free radicals, thus preventing nerve disorders that are caused by nerve damage and degeneration and alleviating tying-up syndrome. Soil selenium levels vary in different regions of the United States. Many hays harvested in the alkaline soils of the Rocky Mountain region, for example, may be very high in selenium. Because of this variation, it is extremely important to determine the selenium status of the region from which the hay being fed is harvested so that toxicity does not occur. Most commercial feeds also have additional selenium in them, so additional selenium is not necessary.

Glutathione has antioxidant properties, which include reactivating vitamin C and vitamin E metabolites that have been oxidized by free radicals. Glutathione depletion in cells suppresses the immune response of white blood cells, prevents normal functioning of lymphocytes, and inhibits antibody activity. Glutathione is commonly included in many "immune boosting" supplements on the market. Its supplementation may enhance antibody activity in immune cells, but this has not been documented in horses specifically.

Besides vitamins and minerals, other types of antioxidants exist in the form of enzymes. SOD, GPx, and GSH all serve as quenchers of free radicals, reducing their charge through addition of electrons. These enzymes work within cells, rather than on the surface like vitamin E and selenium do. These enzymes are universal and can be found in many tissues, including the liver, muscle, and brain. SOD is found in a multitude of organs; its activity is highest in the liver, followed by the kidney, brain, heart, and muscle.

GPx is primarily found in the red and white blood cells of mammals and helps prevent oxidation of cell membranes by consuming free radicals in the cell. Selenium is important to the structural integrity of GPx, and without adequate selenium, its activity is severely handicapped. GSH is essential for GPx to effectively stabilize free radicals and protect tissues from damage. It then reduces the oxidized glutathione to complete the cycle.

The main point of concern is that oxidation increases as the need for energy increases, such as during exercise and pregnancy. As oxidation increases, so does the production of ROSs, including free radicals, which can damage vital tissues in the horse. Horses do have internal mechanisms such as vitamin C synthesis and antioxidant enzymes to keep up with the increased production of ROS, but these internal mechanisms may not be sufficient when ROS levels rise. The best way to prevent serious damage is to provide the horse with a healthy balanced diet with essential vitamins and minerals while avoiding oversupplementation (Williams, 2007).

Despite scientific advances, conditioning trotters and pacers remains rooted in tradition. New tools that quantify measurements of conditioning need to be put in the hands of professionals in the field, for the welfare of the horse and the business. Regardless, training Standardbreds requires patience and passion. The odds of getting a superstar are 1 in 5000. In 2009, there were two superior 3-year olds—Well Said and Muscle Hill—from about 15,000 of 2006 foals. The guidelines for selecting and developing a young horse come down to three things: (1) pedigree, (2) conformation, and (3) environment. The harness racing sport has definitely changed from being a sport to a true business, one that is best engaged in a professional manner. Horses are driven by excellent drivers, and there is much more early movement in present-day racing. Every horse has to perform at maximum effort for the entire mile. Standardbred horses have benefitted from years of breeding for pedigree and speed and have improved much more compared with the Thoroughbred. Standardbreds have been getting faster with each generation, whereas there has been little change with regard to speed in Thoroughbreds (Harvey, 2008).

The intent of this chapter was to bring a scientific perspective to the discussion of training the trotter and the pacer and expand the scope of discussion with regard to the need to develop practical new technologies for use in the field.

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CHAPTER

22 Training Endurance Horses

ANNE LOUISE BARNES*

Endurance is one of the fastest growing equestrian sports in the world and is a competition of distance and speed. Riders must manage the horses appropriately in variable conditions and terrain to cover the set distance in the shortest time with a healthy, fit horse on completion. Ride distances range from 40 to 160 kilometers (km) in one day, as well as multiday marathon rides. Stringent rules and regulations govern the organization of the rides and the monitoring of the horses to ensure that the welfare of the animals is protected. Riders of all ages participate in endurance races, at all levels from social and training rides through to championship rides. Participation ranges from riders with one horse taking part in local competitions as a recreation through to professional trainers, riders, and stables competing for world rankings, with breeding, sale, and training of elite horses conducted as a business. Competing in endurance is, by definition, a long-term aim because it takes several years to prepare a horse to travel at competitive speeds over long distances, particularly as the welfare of the horse is always the paramount concern (Figure 22-1).

SELECTION OF ENDURANCE HORSES

A good prospect as an Endurance horse will be structurally sound, free moving, well proportioned, well grown, and mentally mature. Many successful Endurance horses are Arabian or Arabian cross-bred horses. Such animals may be more likely to have a higher percentage of aerobic muscle fibers compared with horses bred for sprint or shorter distance racing and as such, would be generally regarded as superior for the sport of endurance (reviewed in Rivero and Piercy, 2008). The type of horse selected may depend on the terrain and climate in which it is expected to perform. Animals racing in the heat and humidity on flatter tracks generally are a leaner body type than those working in cooler climates and over more hilly courses. Some of the differences seen in body type may also be the result of training adaptations.

Most national endurance associations and the Federation Equestre Internationale (FEI) require a system of qualification for Endurance horses to be able to compete over longer distances and at faster speeds. Generally, horses may not start competing in endurance events of 80 km or more until they are at least 5 years old. This allows for optimal opportunity for growth and development. Some horses may have been paddock or pasture raised and are not subject to particular training regimes until they begin training for endurance. Other horses may have been used for showing or other disciplines before being brought into endurance work. Such alternative disciplines may have a benefit in developing the capacity of a horse to cope with novel situations such as mixing with other horses, being examined, and travelling with other horses. Additionally, this training may have helped develop the horse's muscles such that it can carry a rider in a well-coordinated manner.

Animals raised in a herd situation in paddocks or pastures may have an advantage in that they are accustomed to having other horses around them and have also had the opportunity to develop their musculoskeletal system as they learn to move with confidence over varying terrain. Within a herd, a horse that is assertive and independent, being a natural leader, may often be considered by experts to have the personality to become a strong, competitive Endurance horse.

A thorough prepurchase (veterinary) examination is recommended before purchase to ensure soundness; endurance training is a long-term undertaking and the results can be very disappointing if much time and emotion are spent in training and racing a horse that has underlying structural problems.

TRAINING FOR DISTANCE

The initial training of an Endurance horse should be based on long, slow distance work. It will take several months of foundation training in a new horse that is 4 to 5 years of age. If the horse has not undertaken any formal training before, the time frame for the foundation training will likely need to be extended. Many body systems, including musculoskeletal, cardiovascular (including fluid and electrolyte balance), thermoregulatory, and gastrointestinal systems, need to be trained to cope with the demands of endurance exercise and for the building up of mental strength. Once a horse has been "started," conditioning for rides over increasing distances will be required; this is, thus, a progressive process.

Getting to know each horse and monitoring its progress are critical factors in training. Although generalizations can be made, each horse needs to be treated as an individual and particular attention should be paid to any problems that may develop, such as back pain, girth rubs, lameness and leg injuries, or mouth rubs from the bit. It is important to ensure that the animal receives appropriate care of hooves because of the impact of hoof function on other aspects of musculoskeletal function. Routine dental care is also essential for optimal performance. Some competition organizers now allow horses to compete unshod. If this is intended, conditioning and care for barefoot riding should commence at the outset

*The authors acknowledge the work of Penny Toft on this chapter in the previous edition.

FIGURE 22-1 Australian competitor, World Endurance Championships in Malaysia, November, 2008. *(Photo by Sharon Meyers, with permission.)*

of training. If any problems do develop, especially in the early stages of training, work should be stopped while the problem is properly identified and rectified; this may mean that the horse requires further rest until the issue is resolved.

After the horse has been broken in, it may require 2 to 3 months for the animal to get accustomed to being handled and ridden and exposed to all sorts of situations. This usually involves gentle introductory exercise, followed by rest over several weeks, ideally with the horse turned out to pasture for some time. When the horse returns to training, initial retraining exercise is done at a walk and trainers may use walking machines and treadmills as well as riding to develop this fitness. Leading the new horse from another ridden horse may be a useful method to increase the distance travelled without the additional stress of carrying a rider. At this stage, it is very useful to teach the horse patience and control so that it can be ridden, turned, and stopped over all sorts of tracks and terrains. Walking through all types of countryside helps accustom the horse to the different environments it may encounter during endurance events. The horse may have to traverse over and around rocks and fallen timber, trees with overhanging branches and other sorts of vegetation that it has to work through, as well as different surfaces such as sandy, slippery, firm, and rocky areas. A horse that is used to working carefully through all sorts of tracks will develop a good sense of self-preservation and when distance and speed are increased, it is better able to take care of itself and its rider.

Initial walking sessions may be over 5 to 10 km (3–6 miles), three to four times a week, with a few short periods of trotting for several minutes during the session. After 3 to 4 weeks, once the horse has recovered well following an hour or two of continuous exercise, longer rides can be introduced, with 2 to 3 hours of mostly walking and some trotting, perhaps at a pace averaging around 10 kilometers per hour (kph; 6 miles per hour [mph]), for up to 20 km (12 miles) total distance, followed by a rest day. The horse can be walked then trotted in 5-minute blocks and as fitness increases, the blocks of time can be slowly increased. For instance, the time spent trotting should be doubled, and a similar amount of time should be spent walking or until the horse has recovered from the faster pace, which would be evidenced by the return of heart rate (HR) and respiratory rate (RR) to normal. Such workouts can be performed three to four times per week in the initial stages. Most of this work is best done on flat ground, progressing to some hill work, again depending how the horse is responding to the training. Some horses may prefer cantering to trotting, in which case cantering can be substituted for trotting. At this stage of endurance training, the aim should not be competitiveness, but rather, ensuring that the horse is not overworked and has training sessions that are interesting and are not likely to induce lameness. Trotting the horse "in hand" after each training session is good practice for veterinary inspections and very useful for detecting any problems early.

As the horse's fitness increases and it is able to go further and faster, additional longer sessions can be added per week. The longer sessions can be gradually increased by 5 km (3 miles) each week, progressing up to a total distance of 40 km (25 miles), using walking, trotting and some cantering. As always, the response of the horse to this increase should be monitored closely, to ensure adequate recovery before each session.

According to most endurance race rules, training rides at affiliated events are restricted with regard to the speed the horses can travel, based on the realization that going too fast too early in a horse's career can cause damage. Training rides are generally 40 km (25 miles) and the horse should be able to cover that distance in 3 to 4 hours. Having a measured training track and progressively increasing the distance travelled in training allow the rider to monitor the progress of the horse, by checking the distance, time travelled, and most importantly, the horse's response to the exercise. The most useful measure of fitness is HR— at rest, the maximum during exercise, and the recovery after exercise.

Once the horse is able to successfully complete a training ride of 40 km (25 miles) in reasonable time and with good recovery, with some further distance training, it may be ready to attempt an 80 km (50 mile) endurance ride. The horse should be inspected by a veterinarian and be allowed to rest at least every 40 km (25 miles); this gives an opportunity to stop at a shorter distance if the horse is found to be not fit, or sound, or well enough to complete the full ride. To be deemed an Endurance horse, usually the horse must successfully complete a series of longer rides 80 km (50 miles) or more, and these rides may be restricted in pace. Once qualified, the horse will be allowed to attempt longer distances and travel at an unrestricted pace.

Championship rides are 160 km (100 miles), split into sections of no more than 40 km (25 miles), with a veterinary inspection and rest between each section and are run over 24 hours or less. Preparation for a 160 km (100 mile) ride requires much longer training sessions, progressively built over slow long-distance rides. Horses that take 2 to 3 years to be prepared for 160-km rides are much more likely to have a longer endurance career compared with horses that are rushed through their preparation.

TRAINING THE MUSCULOSKELETAL SYSTEM

Endurance exercise is primarily aerobic and appropriate training will enhance the natural aerobic capacity of muscles (D'Angelis et al., 2005; Essen-Gustavsson et al., 1989; Serrano et al., 2000). Thus, the initial training for the distance mentioned above is also well suited to improve the overall aerobic capacity of muscles (see Chapter 12), as well as associated cardiovascular and respiratory system adaptations.

Such exercise will strengthen ligaments, tendons, and bones, as this base of strength is very important before fast, more intense work is attempted. There will be gradual remodeling of bones and strengthening of tendons and ligaments in response to this conditioning and it is important not to hasten this process. Rest time is critical while the remodeling occurs (see also Chapter 13). If the horse appears listless and tired or demonstrates any signs such as swelling or filling in the legs the day after any training session, it is advisable to reduce the training session or give the horse a rest day, to give the musculoskeletal system time to strengthen and adapt to the stresses being progressively applied.

The growth plates of the vertebrae may not close until the horse is 5 or 6 years old, that is, the back of a younger horse is highly susceptible to injury and remodeling if subjected to intense pressures too early. Working the horse to strengthen the back and abdominal muscles, for instance, using dressage, long reining training, and hill work at different angles can help develop the epaxial musculature or the "top line" of the horse. Good saddle fit is vital and it is important to realize that as the horse grows and develops, the back may change shape so the saddle fit should be checked at least twice a year.

High-intensity training is required for improvements in anaerobic capacity and occasionally within an endurance ride, there may be some anaerobic exercise, for instance, a gallop finish, or hard, fast work up a hill and horses that undergo some short sprint training may be better able to perform under such situations. Short, intense sprints—for instance, cantering fast for 200 to 400 m on the flat ground or for more intensity up a hill—can be undertaken during other training sessions, after a suitable warmup with walking and trotting. The horse can then be walked until it has recovered, with lowering of HR and respiratory rate. Three to four repeats can be conducted within the session.

TRAINING ENERGY SYSTEMS IN THE MUSCLE

The aerobic training helps induce the enzyme systems to provide greater muscle aerobic capacity (D'Angelis et al., 2005; Rivero and Piercy, 2008; Serrano et al., 2000), delaying fatigue. Most of these adaptations occur after a couple of months of training and ideally coincide with training to increase the distance the horse travels.

An improved capacity of the muscles to oxidize fat is included in the changes mentioned above. Fat use for energy production has a glucose-sparing effect to delay glycogen depletion and thus increases time to fatigue. Also, increased amounts of fat in the feed increases the amount of fat oxidized by muscles (Harris, 2009). Fat is considered a "cooler" feed because it does not generate the same heat during digestion as does roughage, which is also ideal for Endurance horses. Endurance horses can have fat introduced slowly into their diets, either in various complete feeds or as added vegetable fat. It may take 1 to 3 months for muscles and the digestive system to fully adapt to the supplementation of dietary fat. As the training intensity increases, added fat can be gradually introduced in the diet, which would enhance endurance capacity as well as provide a "cool," high-density energy source to the horses at a time when their requirements are increasing.

Postexercise replenishment of muscle energy, in the form of glycogen, takes 2 to 3 days (Hodgson et al., 1983; Snow et al., 1982) and therefore, rest and recovery for several days after a hard exercise session or ride are important. This is not possible when the horse is competing in multiday rides and it can be expected that the horse's performance may be undermined after several successive days of exercise. Fatigue can, in turn, result in poor coordination and stumbling, so added care is required to prevent injury.

TRAINING THE CARDIOVASCULAR SYSTEM

The recovery of HR in an endurance horse is considered the most critical measured variable in competitions. Absolute cutoff values of HR are used to indicate whether the horse has recovered sufficiently after each phase or section of the event to be allowed to continue (Figure 22-2). Depending on the place and type of competition, the maximal heart rate (HR_{max}) may be from 55 to 64 beats per minute (beats/min) after each section of the course; the value is set before the competition. In most types of competition, the horse has 20 or 30 minutes to recover to the set maximum. These times and values have been set on the basis of initial observations of competing horses, backed up by research, which has indicated that a workable maximum ensures that few horses will be compromised if they recover to such values within the set time. Thus, development and training of the cardiovascular system is integral to success in endurance competition.

The usual resting HR of a horse should be between 30 and 40 beats/min, measured when the horse is standing quietly in the morning before any exercise or stimulation, and the HR_{max} of a horse working hard is up to 240 beats/min (McKeever and Hinchcliff, 1995). Fitness is measured not by the maximal rate but by how quickly that rate returns to the prescribed "normal" after exercise ceases.

HR will be elevated by the sympathetic nervous system and by the muscular demands for delivery of substrates and removal of wastes such as carbon dioxide, lactate, and heat. Additionally, dehydration-induced deficits in cardiac output, detected as lowered blood pressure and less blood flow to muscles, will reduce substrate delivery and removal of wastes, further elevating HR. Training to "increase the fitness" of the horse will improve cardiovascular function, including the

FIGURE 22-2 Heart rate (HR) measurement at a veterinary checkpoint during an Australian endurance ride competition facilitated by a polar HR monitor. *(Photo by Sue Crockett with permission.)*

fluid and electrolyte balance of the horse, and improve recovery rates (see Chapter 6).

HR is a reflection of cardiac output, where cardiac output is a function of HR multiplied by stroke volume (SV). The SV of the horse is influenced by the contractility and filling volume of the heart. With athletic training, both contractility and filling volume are increased, resulting in increased SV and cardiac output (Geor and McCutcheon, 1998). Consequently, improved blood delivery throughout the body will improve the athletic ability and fitness of the horse. As the body becomes better at managing the rigors of exercise, blood flow to muscles increases and the capillary network becomes developed, thereby increasing the aerobic potential of muscles. This increased circulation through muscles will also improve removal of wastes from the body. Heat is an important waste product of muscular work and it is brought via the bloodstream from muscles to the surface of the animal for dissipation.

To induce any of these changes, the workload must progressively stress the system. Thus, after the initial conditioning for distance, increasing workload can be imposed to increase the distance that the horse can work without fatigue and with adequate recovery. HR monitors may be used to assist with assessing the HR response to exercise (Figure 22-3). It is expected that a normal fit, healthy horse should be able to recover quickly from a 2-hour training session so that its HR has returned close to normal resting values within 10 minutes. Intensity of exercise, weather, and excitement can all influence HR recovery. The effects of prolonged exercise are considered to be cumulative, such that it may take longer for HR to recover to resting values after an 80-km ride as opposed to a 25-km training ride. Keeping records of completed training and HR response will allow evaluation of the fitness of the horse. The HR rules for competition provide a useful guide in that horses that have done extended distance are expected to recover to a HR around 60 beats/min within 30 minutes of completing the ride.

HR recovery can be enhanced by cooling the horse, to improve heat loss. Washing the horse with cool water helps remove heat from the body surface and aggressive cooling is used within competitions to speed HR recovery (Figure 22-4). A technique that comprises washing, walking, and washing again is commonly used so that heat from deep within the tissues is transferred to the body surface for loss; if a horse is not adequately cooled, the HR may remain high or may increase again as soon as the horse is walked, presumably because walking, rather than standing, increases blood flow to muscles thereby flushing heat and other wastes from the muscles; these, in turn, affect HR.

FIGURE 22-4 Cooling during a veterinary checkpoint World Endurance Championships in Malaysia, November, 2008. *(Photo by Sharon Meyers with permission.)*

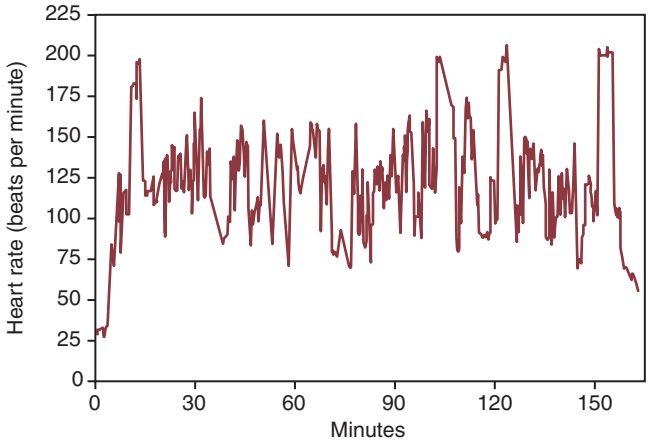

FIGURE 22-3 Heart rate (beats per minute) recorded every 5 seconds after saddling up and during the 30 km first phase of an endurance ride.

Resting HR can also give a useful indication as to how the horse is responding to the exercise sessions. If resting HR is elevated, it may indicate that the horse is unwell or dehydrated or may mean that it has not fully recovered from the last exercise bout and requires longer rest. Continuing to train with a horse that is insufficiently recovered can overstress and weaken the very systems that should be becoming stronger. Rest is a crucial part of training the Endurance horse.

TRAINING FLUID AND ELECTROLYTE SYSTEMS

Thermal load has a huge influence on the fluid balance of the exercising Endurance horse, especially in hot weather. This occurs because the removal of heat from the body results in loss of fluid through evaporation from skin and the respiratory tract (McKeever, 2008, Chapter 6). Training increases plasma volume and the percentage of total blood volume at rest (Geor and McCutcheon, 1998). Indeed, increases in

plasma volume occur after as little as 1 week of training, perhaps due to activation of hormonal mechanisms that cause sodium and, therefore, water to be retained (McKeever and Hinchcliff, 1995). This adaptation will aid in maintaining fluid volume, even with moderate fluid losses during exercise. Thus, this training-induced increase in plasma volume will likely help maintain cardiovascular function and thermoregulatory capacity during the rigors of endurance competition.

The loss of substantial volumes of sweat during exercise occurs with concurrent losses of electrolytes, particularly sodium, chloride, and potassium, as well as smaller amounts of calcium and magnesium. Replacement of these electrolytes via feed, water, and supplementation is required (McCutcheon and Geor, 1998). Training results in a decrease in total sweat losses (fluid and electrolytes) per unit time of exercise. Loss of sodium is especially decreased when horses are acclimated to heat (Geor and McCutcheon, 1998). Doubtless these adaptive responses are designed to help maintain the fluid balance of the horse.

For adequate acclimatization to working in hot, humid weather, the horse should undertake some training in such conditions so that its body temperature does increase and the sweating response is stimulated. Generally, it is considered that at least 3 weeks of exposure are required to stimulate significant acclimatization to the heat. Measurements that can help determine whether the horse is acclimatizing to the heat and, in particular, assess the state of hydration include body weight (a decrease may indicate loss of total body water), resting HR, response to exercise (speed and HR over a standard distance track), HR recovery after exercise, and general demeanor. Perhaps the most important aspect of training on fluid balance is teaching the horse to drink at every available opportunity.

TRAINING GASTROINTESTINAL SYSTEMS

Physiologic responses to exercise are stimulated, to a large degree, by the sympathetic nervous system and one of the usual results of that is decreased blood flow to the gastrointestinal system and decreased gut motility (Lorenzo-Figueras and Merritt, 2008). However, it is essential for an Endurance horse competing over long distances to eat and especially drink to replace losses during the event; a horse may lose 10 to 15 liters per hour (L/hr) of fluid during competition (Carlson, 1983), and therefore must be trained to drink frequently and to consume appropriate amounts.

Water on course and at base may be supplied in buckets or drums or may be available in dams or natural water sources, so the horse should be exposed to a wide variety of sources. It should preferably be trained to drink from almost any source of fresh water. Initially, many horses may not drink early in the ride, but in later sections, when they are thirsty, they may start to drink. Because of the extensive fluid losses that may accrue during the ride, it can be impossible for a horse to make up for not drinking at the start. McKeever (2008) discusses reasons why horses might not drink during exercise. Various tricks can be used to persuade the horse to drink, for example, taking water from home to the ride or out on the training track, mixing molasses or other substances in the water so as to hide the different taste, or carrying a collapsible bucket that the horse is used to drinking from.

Teaching the horse to drink water with electrolytes may be of benefit in that the presence of electrolytes helps disguise the taste of unfamiliar water and helps with replacement of fluids and electrolytes; it has been shown that horses given an electrolyte mixture to drink immediately after exercise, followed by free access to plain water, were ultimately better hydrated than those that drank only plain water.

It is important that the horse drinks and eats well on the day before the ride. This will ensure that it commences the ride in an optimal state of hydration. A good source of roughage such as high-quality hay will provide a reservoir of water and electrolytes in the large intestine. At many events, the horses are stabled overnight before the ride and they can be given various feeds during that time. Provision of high-energy concentrates in the last couple of hours before the event may cause an increase in circulating insulin concentrations, which, in turn, decreases blood glucose and nonesterified fatty acid concentrations, thus having deleterious effects on endurance performance (Harris, 2009). Therefore, most experienced competitors only provide roughage and water in those last few hours prior to competition.

The type of food to offer during training and at rides depends what the horse will eat. Generally, the best food is that which the horse will consume, especially in novel environments as at competitions. However, it is important not to overfeed the horses with concentrates or grain, especially in situations where the horse might have reduced gut motility. Retention and fermentation of high-energy feeds may result in adverse consequences such as diarrhea and possibly laminitis. Most horses seem to prefer to eat grass, hay, and moist foods such as carrots at rides, but a variety of foods can inspire them to eat. Usually, horses like to eat, so if the horse has stopped wanting to eat during or after a ride, this can indicate slowed gut motility, which can lead to colic and this should be a warning sign that the horse is not recovering normally. The food offered at hold or rest periods during the ride can be moistened to prevent a hungry, dehydrated horse choking on a mouthful of dry feed. Wet mashes can also contain electrolytes and sweeteners, which the horse may more readily accept than dry food. Provision of electrolytes in the form of concentrated pastes after the horse has eaten and drunk well should be done judiciously because the pastes can draw moisture into the gut and also cause some gastric and oral irritation, which may discourage the horse from eating or drinking any more.

The fluid, cardiovascular, and gastrointestinal systems are interlinked such that reduced fluid volume and poor circulation to the gastrointestinal tract can result in reduced gut motility. Thus, training for increased fitness, better heat removal, better circulation to the gastrointestinal system, and enhanced drinking when on track in competition all contribute to the health of the well-trained endurance horse.

MENTAL TRAINING AND CROSS-TRAINING

Keeping the horse interested in going long distances can be a challenge. Different training tracks, training with other horses, altering speed when the horse is ready for some fast work, and keeping it "fun" will help keep horse and rider interested. Within the training program, it is important for the rider to have set goals and records so that the progress can be planned and monitored, but it is doubtful that the horse appreciates the achievement and will need other incentives to perform, as

required. Horses are herd animals, so working along with other horses can be important and useful, as well as perhaps comforting when novel or strange stimuli are encountered.

Doing different exercises can also keep the horse mentally stimulated and also result in very useful development of different muscles and locomotion patterns. Arena work such as dressage training, long rein work, jumping or cross-country courses can all be extremely beneficial for the endurance horse and rider, helping to develop different muscle groups, in particular the back, and improving coordination and responsiveness to the rider.

It is also important to train the endurance horse for repeated phases of the competition such that they are willing to accept being saddled and returning to the track after the hold or rest period. Riding the horse twice in one day in training can be a useful first step, even if the second ride is short. In 80-km (50-mile) events, there will be at least two loops; in some championship events, there may be up to seven loops and it can become challenging to have the horse continue returning to the track.

TRAINING FOR VETERINARY INSPECTIONS

The endurance horse is subjected to a number of veterinary inspections during the course of an event: before, at points during, and after the horse has covered the distance (see Figure 22-2). At each inspection, the horse must be judged fit to continue and this is based on HR recovery and evaluation of metabolic variables, with particular emphasis on hydration status and gait. Thus, it is a requirement that the horse submit to these inspections by standing calmly while the veterinarian listens, looks, and counts. The Endurance horse should be trained to stand quietly while other horses may be coming and going and while someone handles them all over. Because the HR during the ride is such a critical measurement, a horse with HR elevated due to excitement, not due to poor recovery, may still be eliminated from the event. Taking a novice horse to rides or shows even if the horse is not competing and allowing it to acclimatize to the conditions and the excitement around other horses and people can be a useful training method. Having other individuals handle the horse at home under various conditions may also accustom the horse to these interventions.

Training the horse to "trot out" appropriately for examination of its gait is essential. The general scheme for the gait examination is to have the horse trotted out under loose lead: either straight out and back or in a pattern such as a triangle. Therefore, practicing is useful and likely essential. The horse should trot in a relaxed way while being led from its left shoulder, turn under control, and stop without undue slowing. Each horse may have a speed at which it looks best and practice can determine that speed. Such training does not seek to hide lameness but allows each horse to be displayed at its best.

TRAINING FOR SPEED

Some elite competition rides are being won at speeds of over 20 kph (12 mph) for the 160-km (100-mile) race. Performance at this level requires another plane of fitness and athletic ability. It is very important not to attempt to progress to this level without a sound base of distance conditioning, which may have taken 2 or more years to build. If the terrain allows it, the horse can be allowed to naturally increase its efforts during training, by trotting and cantering up hills. On flat tracks, it is easier to have a more structured training program that may require set amounts of time or distance at a particular speed and HR. Newer technology such as global positioning system (GPS) devices can be used to help train horses at particular speeds over set courses.

Initially, speed work should occur for only short bursts over a minute or two. "Longer sessions of fast work can be conducted with the older, conditioned Endurance horse, taking care to monitor the HR to ensure rapid recovery after cessation of work. The speed of HR recovery after work will give the best indication of the fitness of the horse, which is why measurement of this variable is used in the veterinary inspection. Indeed, in many forms of competition, fast HR recoveries confer a competitive advantage to the horse, as the horse can be returned to the competition more rapidly compared with those horses with slower HR recovery.

Once a horse is comfortably able to complete an 80-km (50-mile) event in 8 hours riding time, with good HR recoveries and no lameness, then it should be ready to progress to extra speed training. This may involve at first one session per week of around 10 km (6 miles) fast trotting or cantering, at 16 kph (10 mph). Often, a loping canter, as opposed to a fast trot, can be a more efficient and practical gait when working toward these increased speeds. There will usually be a transition from trot to canter such that energy use in a medium canter will be less than that in a fast trot and HR will be correspondingly lower. Note, however, that different horses will have different preferred gaits for a particular speed. Once the horse is able to recover quickly and easily from the more intense session, speed or distance can be increased (but not both at the same time). For example, one longer or faster session (e.g., 20 km [12 miles] at 15 kph [9 mph], or 10 km [6 miles] at 20 kph [12 mph]), or two shorter sessions can be conducted per week, with rest days, walking, and arena and dressage work as possible intervening sessions.

Interval training can be of benefit in increasing the workload to induce a response without causing training overload. This can be accomplished by repeated intervals of fast trotting, progressing, as fitness increases, to cantering and galloping up a hill or over a set distance or for a set time with cool-down periods after each interval. The horse should be adequately warmed up before commencing the intervals and monitored carefully for recovery afterward. The number of intervals done in any session will vary, depending on the horse's fitness and stage of training, but generally, three to six fast bursts per session are completed and such intense sessions are only done once per week. The distance and time for each interval can also vary. Generally, 1 to 2 km (0.5–1.5 miles) at a fast pace, interspersed with time for recovery equal to that taken to do the fast work, will provide a hard workout. Monitoring HR during fast work and then during recovery will allow closer scrutiny of the intensity at which the horse is working and whether it is recovering sufficiently and appropriately. During recovery, the horse can be walked, for instance, back to the start of the hill or track.

For horses to complete championship length (160-km [100-mile]) rides in less than 8 hours, they may be required to complete some sections of the ride at 25 kph (15 mph) or more. Not every horse is athletically capable of doing this and not every trainer has the resources to keep a horse healthy

while training the horse to this intensity of racing. Flat, measured training tracks of 20 to 30 km (12–18 miles) can be used for fast canter work such that the horses at this level may do 20 to 30 km (12–18 miles) at 30 kph (18 mph) once or twice a week, again interspersed with lower-intensity sessions once the horse has recovered from the fast session. Continual monitoring of the horse during the training and later, to ensure recuperation and no lameness, is critical. Once a horse is able to perform at this level, increasing speed even further may be done in actual races, using the competitions to get the horse to do the fastest work. In such cases, it is crucial that the rider keep a balanced, achievable approach (i.e., a cool head) rather than push the horse past its capacity. Competitions that are split into multiple shorter sections do allow for repeated fast efforts, with recovery in-between, and can result in faster times but also may result in greater fatigue.

RETURN TO WORK AFTER TIME OFF

Rest is an important component of the training, with rest periods of several days after a hard or long event. As mentioned above, resting HR can be a useful indicator of recovery and a return to normal resting HR may coincide with the observation that the horse is again eager to move around energetically. Having one to several months off work at various times during the training program will further allow recuperation and development, especially in young horses. Such points occur typically after the first successful 40-km (25-mile) ride, after three 80-km (50 mile) rides, and in between event seasons.

The horse does retain some fitness for several weeks, the level retained depending on what had been achieved before the rest period. The trained muscles maintain the changes for 5 to 6 weeks of inactivity (reviewed in Rivero and Piercy, 2008). However, return to work after a month or more of rest needs to be followed by a gradual reintroduction of distance and then speed over several weeks. The exact timing and nature of the exercise program after these longer rest periods will depend on how the horse responds again to the work, but a truncated version of the initial training process should be appropriate, starting with walking and trotting over 5 to 10 km (3–6 miles), three to four sessions per week for 2 to 3 weeks building up again to the longer and faster sessions. Generally, experienced competition horses will have returned to doing 80-km (50-mile) rides 2 months after returning to work from a layoff.

OVERTRAINING

Overtraining is a notorious problem in Standardbred and Thoroughbred racehorses. This syndrome may also occur in Endurance horses, particularly if the horses do not receive sufficient rest after longer or harder rides. HR recovery and resting HR are useful measures to determine whether the horse is sufficiently recovered or, indeed, may be overtrained. For example, resting HR may remain elevated for several days, indicating that the intensity of training should be reduced.

Overtraining can also cause damage to musculoskeletal tissues, resulting in lameness. Repetitive concussive injury to legs can cause hoof or joint problems that can be difficult to diagnose, as the lameness may only manifest after 40 km (25 miles) or more of work. Training on different surfaces, use of hoof pads, and nonconcussive exercise such as swimming may be useful for horses with such problems; sometimes, prolonged rest is required for remodeling and repair. Heavily trained horses can also develop back problems and it is important to ensure good saddle fit, change the lead leg when riding, and work on strengthening of the epaxial muscles of the back, particularly by performing dressage or arena exercises.

Horses on heavy training schedules will require more careful nutritional management; overtrained horses may suffer from decreased appetite, perhaps associated with physiologic or pathologic changes to the gastrointestinal tract. Weighing or body condition scoring the horse each week can be useful in monitoring nutritional requirements.

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CHAPTER

Training the Event Horse

23

WENDY SCHAEFFER

THE SPORT OF MODERN EVENTING

THE MODERN EVENT HORSE

The modern event horse is a unique athlete, in fact, a triathlete. Event horses must perform in the three different disciplines: (1) dressage, (2) cross-country, and (3) show jumping, each to a high standard to be competitive on the world stage. They must be absolutely sound of body and mind and possess a natural athleticism on flat ground and over fences to excel in the demanding sport of eventing. Although they may not possess the same scope of movement on the flat surface compared with their pure dressage counterparts or have quite the same level of excellence in their jump as the pure show jumper, event horses must be good to excellent gallopers and brave, careful jumpers to succeed in the cross-country phase, which is the core discipline of the sport.

Official eventing competitions begin at the pre-novice level and increase in level through *novice* (called *preliminary* in the United States, *one star* in Australia), *intermediate* (two-star 3-day event), to *advanced* (three- and four-star 3-day event). "Star" levels are typically denoted as CCI/CIC* and CCI/CIC** with asterisks representing the star level. Moves are planned at the Federation Equestre Internationale (FEI) level for the pre-novice level to become 1*, with current star levels moving up to reach 5*. CCI is the abbreviation for the French "Concours Complet Internationale" and refers to a "long" format event. CIC (Concours International Combiné) is a "short" format event, which is more like a regular one-day event or horse trials (also termed CNC, with the N standing for National).

DRESSAGE, CROSS-COUNTRY, AND SHOW JUMPING PHASES

The dressage phase in modern eventing has reached a very high standard; the world record score in international championship competition of 20.8 (approximately 86%) set by Bettina Hoy at the 2002 Jerez World Equestrian Games reflects the excellence that can be attained by an event horse and the competitor's degree of focus in this first phase. The difficulty of an eventing dressage test increases with each consecutive level of eventing, the highest level using a test that equates to a medium level in pure dressage. Although the standard of dressage performance and the difficulty of the tests have certainly risen in recent years, the movements required during this phase must still reflect the training to make a horse an event horse and not a pure dressage horse.

The cross-country phase has increased in technical difficulty in recent times (Figure 23-1), as fixed fences are designed to be more natural than the pure jumping fences and are built over variable, mostly undulating terrain. Although the actual sizes of the fences have not increased, the variations of terrains where the fences are placed have been cleverly utilized by course designers to create challenges for the horse-and-rider team. Essentially, course designers are aiming to test a horse's training and rideability as much as its courage since the jumping effort asked of the horse may be potentially dangerous. The current courses demand absolute accuracy of stride rhythm and line, which would enable the horse to reach the correct take-off point and successfully negotiate the obstacle. Major championships, for example, the 2006 Aachen World Equestrian Games and the 2008 Olympic Games (where the equestrian events were held in Hong Kong), have developed cross-country courses of high technical difficulty combined with a tight course time to provide a discriminatory phase of the competition.

Again, the standard of the show jumping has improved markedly in recent years. Gone are the days when an average jumper could get lucky to have a pole or two down in the final phase. The occurrence of eight or nine fences down is not unheard of at an international-level competition. At the 1992 Barcelona Olympics, New Zealander Andrew Nicholson's "Spinning Rhombus" had nine fences down in the show jumping phase, which led to the Australians winning the gold. At the 2004 Athens Olympics, Australian rider Stuart Tinney lowered nine rails on his horse "Jeepster," which had won gold at the 2000 Sydney Olympics. On many occasions, after the round begins, the horse may panic and lose its jumping technique; penalties can mount quickly! Being the final phase, the pressure on competitors can be intense, as it was at the 2008 Olympics, where just 10 penalties separated the top 15 competitors, with 4 penalties separating the top 4 in the show jumping phase.

WEIGHTING OF PHASES AND SCORING SYSTEM

The traditional weighting of phases of 3:12:1 among the dressage, cross-country, and show jumping phases of any event has, for the most part, changed significantly as the sport has become more professional and the standards of riding and training in the dressage and show jumping phases have improved. Although it is difficult to reassign the weighting accurately, the author of this chapter recommends that the dressage and show jumping phases be at least as influential as the cross-country phase and sometimes more so, especially at the lower levels of the sport. Each individual competition has a slightly variable weighting of phases, which may be influenced by weather and the resultant footing on the cross-country course, the terrains of the venues, and the variable difficulty of the cross-country course itself from

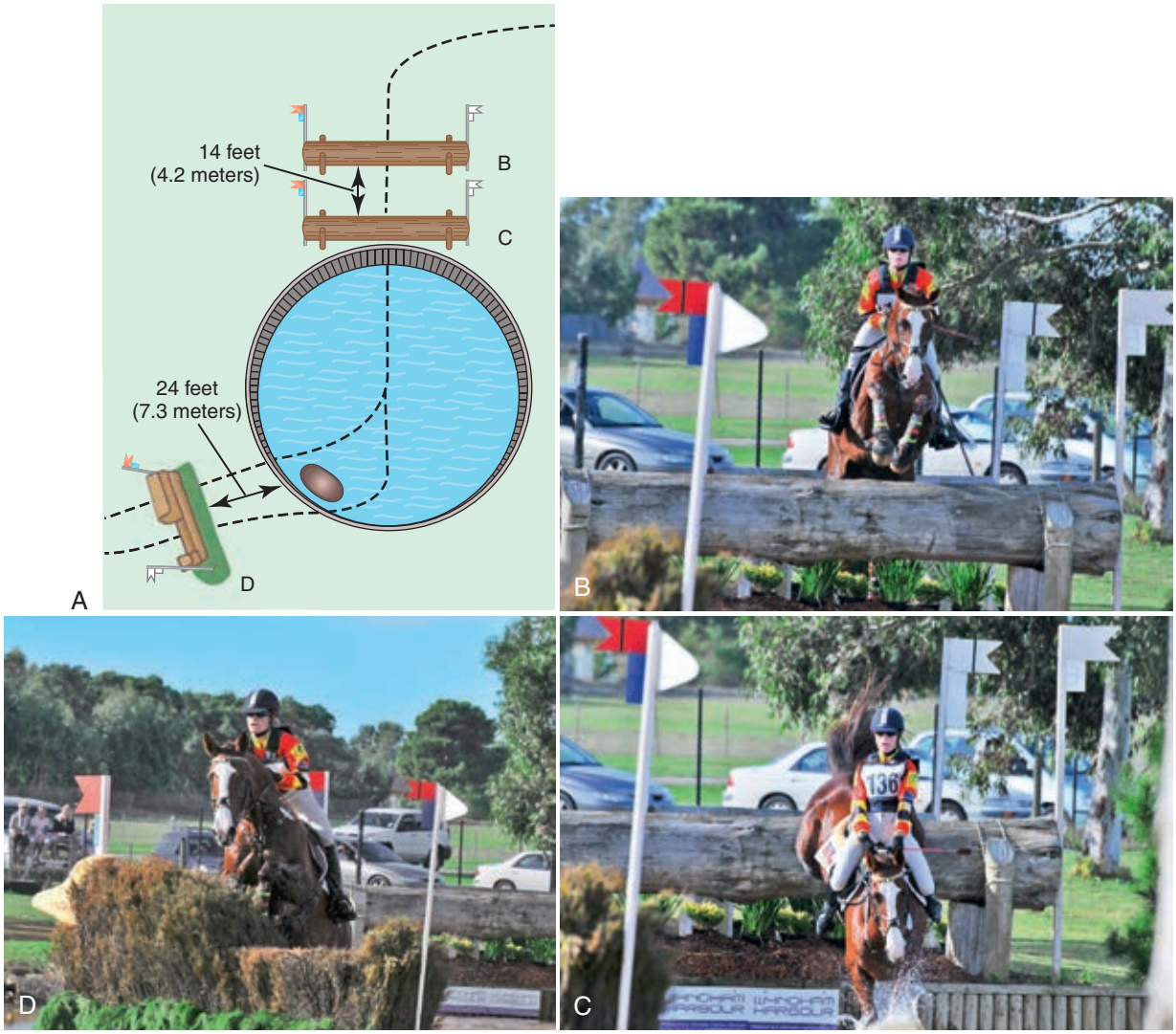

FIGURE 23-1 Sequence of images taken from a combination water jump at a CCI*** event in Melbourne, Australia, 2008. **A,** Aerial overview of three jumps; **B** and **C,** prior to entering the water, and **D,** taken on leaving the water.

year to year at the same venue. In general terms, the higher the level of the competition, the more influential the cross-country phase becomes; a higher proportion of jumping and time penalties and eliminations or withdrawals are expected at the CCI/CIC**** competitions compared with those at CCI/CIC***, CCI/CIC**, and CCI/CIC*.

The scoring system of the event itself rewards dressage excellence more than it does jumping excellence. In dressage, a mark out of 10 is awarded for each movement in the dressage test (up to approximately 25 movements) as well as 4 collective marks at the end of the test for paces, impulsion, submission, and rider position or effectiveness. These are added up and converted to a "good marks" percentage, which then has a coefficient of 0.6 applied to give a penalty score.

Jumping excellence is rewarded to a greater extent over the tough cross-country and show jumping tracks in that the better jumping horses are less likely to accrue jumping penalties; however, no penalty difference exists for the round where a horse just scrapes through compared with another horse that performs a foot-perfect round. Cross-country jumping penalties are, however, significant—20 penalties for the first refusal or runout at a fence, 40 penalties for a second refusal or runout at the same fence, and elimination for a third refusal, either at the same fence or a total of 3 refusals or runouts on course. A fall of horse or rider also now constitutes elimination, as does a course error (jumping a fence or parts of a combination out of order). Time penalties can also accrue quickly with 0.4 of a penalty per commenced second over the optimal time.

The show jumping phase has also become more demanding and influential as the heights and technical difficulty of this phase have increased. Lowering of the height or width of the fence—a "rail down"—results in 4 penalties, as does the first refusal. The second refusal, fall of horse or rider, or a course error constitutes elimination. Time penalties can also become very expensive with each second or part thereof over the optimal time resulting in 1 time penalty. The intense pressure of the show jumping phase, where riders enter the competition arena in reverse order of merit, has caused many riders to plummet down in the final standings or even become

distracted and lose their way on course, resulting in elimination. The show jumping is especially important at the Olympic Games, where, since 2004, a second show jumping round has been used to decide the individual medals.

FENCE CHARACTERISTICS AND COURSE DISTANCES

At the pre-novice level, the maximum height of fences is 1.05 m, with a moderate level of difficulty. Combinations such as water, banks, and sunken roads are included in a relatively encouraging manner, although the difficulty of these fences does vary from event to event. Straightforward narrow fences and apexes or corners are also used at this level. The cross-country speed is set at 500 m/min for a distance of about 2500 m, which requires a good gallop, efficient lines, and confident jumping.

CCI* and CIC* cross-country fence dimensions increase in height to 1.1 m and width to 1.4 m, with a corresponding increase in the difficulty of the fences. Water and bank complexes may include surrounding fences that are closely related to test a horse's balance and its ability to hold a line. The cross-country speed increases to 520 meters per minute (m/min) with course lengths from 3640 to 4160 m in CCI* classes, with a maximum of 30 jumping efforts. CIC* classes run over approximately 2800 to 3200 m. The show jumping phase has the maximum height of fences at 1.15 m and width 1.4 m or 1.9 m for triple bar fences, with the speed being 350 m/min. The course must have 10 to 11 fences, with a maximum of 13 jumping efforts.

CCI** cross-country courses have maximum fence dimensions set at 1.15 m high and 1.6 m wide, with increased technical difficulty of both the fence types and placement of the fences in relation to the natural or man-made terrain. The speed on course increases to 550 m/min for 4400 to 4950 m, with a maximum of 34 jumping efforts. The show jumping phase has the maximum height of fences at 1.20 m and width 1.5 m or 2.1 m for triple bar fences with the speed being 350 m/min. The course must have 10 to 11 fences, with a maximum of 14 jumping efforts.

CCI*** cross-country courses have maximum fence dimensions set at 1.20 m high and 1.8 m wide, with a further increase in the size and technical difficulty of the fences. The speed on course increases to 570 m/min for 5700 to 6840 m, with a maximum of 40 jumping efforts. The show jumping phase has the maximum height of fences at 1.25 m and width 1.6 m or 2.3 m for triple bar fences, with the speed being 375 m/min. The course must have 11 to 12 fences, with a maximum of 15 jumping efforts.

CCI**** cross-country fences remain with a maximum height 1.20 m but the width increases to 2 m. The fences, in particular combinations such as the water jumps, become more demanding of a horse's bravery and trust in its rider. The speed on course increases to 570 m/min for 6270 to 7410 m, with a maximum of 45 jumping efforts. The show jumping phase again maintains the same maximum height of fences at 1.25 m and width 1.6 m or 2.3 m for triple bar fences, with the speed being 375 m/min. The course must now have 11 to 13 fences, with a maximum of 16 jumping efforts.

SHORT VERSUS LONG FORMAT

Short-format events are one-day events and CIC classes that are generally considered preparatory for the long-format or CCI classes. Long-format (CCI) classes, which maintain a longer cross-country course, are held over 3 to 4 days and can be run with or without Steeplechase. Very few CCI classes have been run with Steeplechase to this author's knowledge, since the tragedy of a rider fatality at Burghley CCI**** in 2004, where the course was run over maximum length in sticky going that proved very tiring for the horses. This was in direct contrast to the 2004 Athens Olympic Games, where the cross-country was under 10 minutes for the first time.

Although the new long format of the 3-day event, without the Roads and Tracks and Steeplechase phases, demands less endurance from the horses, the longer cross-country courses, especially at the championship level, remain taxing, requiring significant speed and stamina from the horses.

FUTURE DIRECTION AND POTENTIAL IMPLICATIONS

The fact that the sport has changed much in recent years is undeniable; the level of professionalism shown by both individuals and their country's teams at international competitions has reached new heights. The dressage and show jumping phases are far more influential than they have been in the past and are likely to continue to become even more demanding, as these phases are least likely to impact negatively on the welfare of the horse. Although it is hoped that the sport will retain the integrity of its core phase, the cross-country, with reasonable length courses that require true horsemanship to ride and to train horses to compete successfully, the new reality of the sport is that the standard of riding in all phases of competition is so high that a competitor needs to be in the top few after dressage to have a realistic chance of winning.

SELECTING THE IDEAL HORSE

CONFORMATION

Strong conformation of the event horse is paramount given both the amount of preparatory fitness training necessary to perform successfully in the cross-country phase and the varying ground conditions that they will compete over during their careers (see also Chapter 15).

On physical assessment, a potential event horse should look naturally balanced—its front end (neck, shoulder, and forelimbs) should mold with its back end (hindquarters and croup), both ends joined by a strong moderate length back. All limbs should be straight, with large, clean joints that possess a full range of free movement. To assess limb straightness, one should imagine a plumbline dropped down the limb; assess the balance of the limb on either side of midline and look for any rotation or torsions or medial or lateral deviations. Although very few horses have a perfect conformation, some traits are less desirable than others, and the significance of such conformation faults is often a matter of degree. This author would prefer toe-out than toe-in forelimb conformation from the view of long-term soundness and would avoid overt sickle or cow hocks, as these are likely to represent a biomechanical disadvantage to hindlimb strength and impulsion and may predispose to injury in the long term. The slope of the shoulder and the corresponding slope of the pastern should not be too upright, as these may predispose to greater impact forces, leading to degenerative joint disease, whereas too great a slope of the pastern places increased strain on the suspensory apparatus, predisposing a horse to tendon strain, suspensory ligament strain, or both. The old adage "No foot, no horse" is also well worth remembering; feet should possess adequate width and depth of heel with a well-developed frog and a reasonably thick, concave sole.

The tubera sacrale (the bony prominences that make the "jumpers bump") should be level; a steep angle between the tubera sacrale and tubera ischii may reflect potential good jumping ability. An excessively long back should be avoided because this may predispose to back muscle problems. Although this is not related specifically to soundness, many good event horses have had a very prominent wither. Ideally, an event horse should also have a well-conformed neck of reasonable length. This will help the horse move with the correct outline for dressage and avoid the criticism of "neck too short."

TEMPERAMENT

Trainability of the event horse is paramount as it must perform to a high standard in all three disciplines. A calm, relaxed attitude for the dressage phase is ideal so that the atmosphere of the competition raises a horse to produce its best work, without causing overexcitement and the resultant tension to spoil the quality of the paces or lead to disobedience. Harmony between horse and rider leading to a soft, pleasing picture is generally rewarded in contrast to tension with outstanding paces in eventing dressage. Enthusiasm and a genuine desire to gallop and jump are important traits for the cross-country phase as the horse must "dig deep" to finish the course at full pace, whereas the jumping phase primarily requires the horse to be careful. A horse with an exceptional attitude and rideability will often outperform the talented but difficult horse which leaves its best work in the warmup arena or the home environment.

BREEDING

As the sport has become more professional, we have seen a significant increase in the number of purpose-bred event horses. With speed and stamina remaining essential traits for the modern event horse, Thoroughbreds (TBs) tend to dominate. Breeders have recognized this and have utilized many "jumping line" TBs (i.e., sires that have produced a number of progeny with success at jumping, jumping being a consistently inherited trait) in conjunction with both the Warmblood and Irish Draught to produce the modern sport horse. German Olympic gold medalists from Hong Kong (2008 Beijing Olympics) were mounted on horses that were up to 63/64 TB; Gina Miles (USA) on her Individual Silver Medal horse "McKinleigh" and Megan Jones on her "Kirby Park Irish Jester" (fourth individually in Hong Kong) represent the best of the Irish Sport horses with approximately 75% TB blood. Pure TBs have still been successful at the highest level, Kristina Cook's "Miner's Frolic" (individual bronze medalist in Hong Kong) being an exquisite example of the modern event horse. The Selle Francais have also produced some impressive event horses; Jean Teulere's 2002 World Champion Espoir de la Mare and Nicolas Touzaint's European and World Cup Champion Galan de Sauvagere are prominent examples.

A great example of a jumping line TB is Brilliant Invader which sired Blyth Tait's "Ready Teddy" (individual Olympic gold medalist, Atlanta, 1996; and World Champion, Pratoni Del Vivaro, 1998). Many Brilliant Invader horses have been successful eventers in Australia up to the 4* level.

TRAINING THE YOUNG HORSE

STARTING THE YOUNG EVENT HORSE

A varied training education is important for all young horses, especially the event horse that needs to be multiskilled. Ideally, the horse will begin regular, structured training as a 4-year-old, progressing according to its physical strength and balance and how well it accepts the training mentally. Young horses will progress at different rates; the experienced trainer appreciates that in the long term, some horses will benefit from more time, but some others may need to be constantly challenged to avoid their becoming disinterested or ceasing to improve in performance. A variety of training sessions should be included in the young horse's program to prevent its becoming bored or overtrained; riding out on roads or in fields should be interspersed with arena work. Informal cross-country schooling can also begin as part of these riding out sessions; an older horse providing a lead over natural ditches and through waterways may help the young horse have positive training experiences. Working on slopes while riding out may be as beneficial for the development of a young horse's balance as more formal schooling sessions on a flat arena.

FLAT AND JUMP TRAINING

As the horse's ability to successfully carry itself and rider in a balanced way is central to its performance in all three phases, flat work forms the basis of the young horse's training. The easier the horse is to ride on the flat and the greater its suppleness, maneuverability, and general obedience, the greater the likelihood is that its jumping performance will be rewarding. Primarily, the young horse must be trained to travel freely forward from the leg into the hand in a straight, rhythmic manner in all three gaits. Preliminary lateral work such as leg-yields and shoulder-fore exercises are often best begun at an early stage. These establish movement away from the rider's inside leg and to help engage the horse's hindquarters by requiring its inside hind leg to step under its body. With consistent flat training the young horse will gradually develop improved postural strength and coordination of its body, which lead to better balance and movement in its paces.

Pole work is an excellent training tool, as it can provide both an interesting variation to flat work and a degree of gymnastic work; sequences of trot poles, especially when raised, require a horse to move with greater suspension in its gait. The introduction of canter poles is a precursor to jumping with the pole, in effect, being a very small jump. Repeated cantering over a single pole helps the horse and the rider develop judgment with regard to the distance to the fence and to be able to adjust the canter stride, making small corrections on the way to the pole. More advanced canter pole exercises, where a sequence of poles are set a certain distance apart, provide an objective measure of how successful the lengthening and shortening of the horse's stride is. With careful progression in the degree of technical difficulty, these exercises teach the horse to remain relaxed and travel in a constant rhythm to the fences. The added advantage of pole work is that even with mistakes, injuries are rare.

Jump training is then a natural progression from pole work, with the jump being just an elevated canter stride. However, it is best that the young horse begin jumping out of trot so that it can remain balanced and straight on its approach to the fence. Once the horse has been convinced that it is best to go over, not around, these new obstacles in its path, then cantering over small single fences and straightforward related lines can begin. When these exercises are performed consistently with good rhythm, then combinations

and more complex lines can be included in the training in preparation for show jumping and preliminary competition days. In conjunction with jumping single fences and lines to simulate those encountered in competition, gymnastic or suppling jump exercises should be introduced to encourage the young horse to fully use its body to jump the fence. These exercises generally involve jumps that are much smaller in size; in effect they are low-impact exercises and so can be performed with higher repetition. Small, narrow corners and relatively wide-faced arrowheads (narrow-faced fences with wings) can also be introduced as part of the young horse's jump training. These fences are best set up in an arena starting with the use of plastic drums or blocks. It is a sound training principle to train the young horse at a higher level than that which is expected in the competition arena; extra time spent in training should provide the young horse with a solid foundation prior to beginning its competition career.

PREPARATORY TRAINING DAYS AND COMPETITIONS

Prior to competing at its first event, it is ideal to gradually expose the young horse to the new world of competition—the transport, the atmosphere of the venue, and the number of horses out. Group training days or clinics with a small number of horses in a session are preferable for a first "outing" off the home property. Alternatively, taking a young horse to a low-key competition for an outing with an older horse as a role model is beneficial. The young horse learns to remain calm while stationed at the vehicle and to experience the different sights and sounds of the competition atmosphere without too much pressure to train at its best or compete in the new surrounds.

Following such outings, the number of which is determined by how relaxed or how quickly the young horse settles on the competition or training day, a club dressage day or a low-key official dressage day should be undertaken. It is ideal to get the young horse in the competition arena on more than one occasion to reinforce that the competition arena is just a different training venue. In these initial forays into the dressage arena, it is important not to place the young horse under too much pressure to perform; a consistent relaxed test, where the young horse stays with the rider mentally, is preferable to a test that displays significant resistances even while performing excellent work. A show jumping training or competition day at a jump club, where the young horse can jump several rounds of progressive heights, should also be used prior to the first event where all three phases are put together for the first time. Again, the aim of this preparatory competition day is to keep the young horse happy in its work while it is being exposed to progressively greater challenges that are more of an environmental nature rather than of higher technical difficulty.

THE FIRST EVENT

Usually, young horses begin their eventing careers at the unofficial introductory (height of fences approximately 80 cm) or preliminary (approximately 95 cm) level for a straightforward introduction to the three-phase event. They may well have trained and competed at higher levels in the pure dressage and show jumping competitions previously attended; stepping them down a level when they combine all three phases for the first time may be a safeguard to overfacing the young horse early in its competition career.

PROGRESSION OF THE YOUNG EVENT HORSE AND TRAINING AT THE PRE-NOVICE AND 1* LEVEL

THE FIRST SEASON AND PROGRESSION THROUGH THE GRADES

Depending on performance at the first event, age and maturity, and rider experience, young horses can either be advanced straight into official competition or made to do some miles in the unofficial ranks to bolster confidence and gain necessary experience.

A professional rider on a purpose-bred youngster that has had the required preparatory training and competition background, as detailed above, can comfortably progress to the pre-novice level after initial exposure to eventing at the unofficial level. Although the young horse may be given a couple of runs to gain experience at this first official level, it will soon be expected to be competitive if it is to be a serious event prospect. If the young horse remains relatively unsuccessful in a set period, it may be determined that the horse should either focus on other disciplines such as dressage or show jumping or participate only in amateur competitions or pleasure activities.

The number of starts at each level before upgrading may well be dictated by circumstances other than a young horse's actual performances, such as the timing of appropriate events, the number of horses its rider is also campaigning at other events, or disruptions caused by injuries. Ideally, three to four starts at short-format pre-novice events will expose the young horse to a variety of different fences and ground conditions and equip it with the necessary skills to upgrade successfully to the 1* competition. Whether all of this happens in the horse's first season is again dependent on many factors. It is often beneficial to start in two to three pre-novice events in either the autumn or spring season before further progressing the horse's training and dressage and show jumping competition in the next preseason period. During the following eventing season, a final start at pre-novice level before upgrading can be a great confidence-boosting way to start the new season.

The young horse that has successfully completed three to five short-format 1* competitions will usually compete in a long-format competition (a CCI*) at this level before upgrading to 2*, where a similar number of starts at both short-format and long-format competitions will need to be successfully completed before upgrading to 3*. This process could be expected to take 2 to 4 years, depending on both the horse's competition record and non–performance-related factors, as detailed earlier in this chapter. An event horse cannot reasonably be expected to compete successfully at the 4* level until it is at least 8 years old to enable a solid grounding at the 3* level.

FLAT AND JUMP TRAINING

Although the progression of the young event horse successfully through the grades is a vital management skill, the management and progression of their day-to-day training depends on the essential skill of the rider and the trainer, as this develops the horse physically and mentally for higher level competition. Again, flat training forms the basis for the work program of the young event horse, although "flat" training on undulating ground to further challenge their balance can also be useful. Greater degree of engagement and improved quality of paces are required of the young horse as it progresses through the grades to remain competitive after the dressage phase. Regular training must be continued to instill further obedience and to

develop further muscular strength and coordination and self-carriage. Some horses are rather limited in their natural paces; therefore, special work must be done to develop the horse's stride so that a clear difference can be shown between working and medium paces. Working over trot poles, flat on the ground or slightly raised, set either in straight lines or around the perimeter of a circle can be very helpful to increase lift and length of the stride in trot. Although lateral work is not required in the dressage at the pre-novice or 1* level, it is useful to begin training these movements to improve a horse's suppleness and engagement of the hindquarters. Many horses competing at 1* events will be competing at the elementary level in pure dressage competition, which does require performance of the lateral movements, shoulder-in, and travers. The rein-back movement is also introduced at the 1* level, so the young horse must be carefully trained well before it is required to be performed in a test situation.

The young horse must also develop the ability to jump bigger fences, both across country and in the show jumping arena. A premium should be placed on straight, accurate jumping to maintain the quality of the horse's jump, which will decline if it is allowed to drift on take-off or in the air. The horse must be trained to "hold a line" to cope successfully with both combination fences as well as corner and arrowhead fences, where there is a narrower face available for the horse to efficiently jump the fence. This discipline can be taught over smaller and narrower fences, wider-faced fences, or both to begin with, but the horse must progress to jump these fences as wide corners and narrow arrowheads prior to being exposed to them in competition. Likewise, it also needs to jump larger fences to develop further the required muscular strength and coordination. Continued and progressively technical pole work and gymnastic, suppling exercises are invaluable.

CONDITIONING AND PRELIMINARY FITNESS TRAINING

There are three principal aspects of fitness and conditioning: (1) fitness of the cardiovascular system, (2) fitness of the respiratory system, and (3) fitness of the musculoskeletal system (see Chapters 9, 11, 12). The horse must be sufficiently fit so that the work of warming up for and performing the dressage and show-jumping phases and completing the cross-country do not unduly tire it. A tired horse is much more likely to make unforced errors or to injure itself. Tendon and ligament injuries are principally fatigue-related injuries.

For short-format competition at the pre-novice and 1* levels, flat and jump training undertaken to prepare the horse appropriately for these events, combined with some hill work, is often sufficient for fitness. Some horses, especially non-TBs need to learn how to gallop and will require additional training to do so. A more experienced horse provides a role model and may create a spirit of competition to motivate the young horse to try harder. Daily turnout is also important both for the horse's mental attitude and its general ambulatory fitness. If turnout facilities are limited, daily walking on a horse walker is recommended.

It is important also to ensure that the young horse learns to balance itself cantering uphill and downhill, and if the local terrain is flat, some effort must be made to find other suitable ground. This work also will benefit the horse from an overall fitness point of view. Specific fitness programs for long-format competition will be detailed in the following sections.

GPS DATA IN TRAINING VERSUS COMPETITION IN 1*- AND PRE-NOVICE–LEVEL HORSES

A study performed by the author collected data on the physiologic responses of event horses to training and competition. A hill work fitness session and a competition run for young event horses were recorded using a global positioning system (GPS) unit and a heart rate monitor (Table 23-1, Figure 23-2). It was apparent that the training heart rates of the two 5-year-old horses in the study were higher than those achieved in competition, whereas maximum speeds were higher in competition than those achieved in training. Essentially, the hill used for their preliminary fitness work was steeper than anything they had encountered in their early competition runs.

STRETCHING, CORE STRENGTHENING, AND DYNAMIC STABILITY EXERCISES

Stretching exercises include taking all four limbs through to the end of their range of comfortably available movement and holding for 15 to 20 seconds. The stretches are then best repeated to gain a greater stretch as the horse learns to relax into the stretch. It is imperative not to force the horse too far into a stretch to the point where it loses confidence and resists the movement. These are covered well in a recent text on the subject (Stubbs and Clayton, 2008). Used in conjunction with such flat and jump training as previously described, these exercises are an important means to develop a young horse's athletic potential.

MANAGEMENT OF THE YOUNG EVENT HORSE

Much time, effort, and money are put into training the young event horse to perform to its potential; it then follows that similar effort is made to ensure that the young horse has the best chance to stay sound in the long term. Quality shoeing cannot be overrated, as this has great effect on the forces that are dispersed through the horse's limbs. Efficient, thorough management of any lameness issues is vitally important to enable effective preventive management strategies to be put in place as soon as practically possible.

Icing and clay poulticing the horse's front legs after work on harder ground and following cross-country competition runs is a useful management strategy. Being selective regarding the ground at both training and competition venues is a sound long-term management strategy but one which must be balanced with the need to gain competition experience. Perhaps the most important management strategy for all horses is ensuring that the arena surface is both consistent and of optimal cushioning; too hard a surface will predispose horses to jarring injury and too deep a surface to ligament and tendon strain. Inconsistent surfaces, however, are the worst of all.

TABLE 23–1

Heart Rates (bpm) and Speeds (km/h) Attained during Training and Competition in 1* and Pre-novice Level Event Horses

	Maximal Heart Rate in Training	Maximal Speed in Training	Maximal Heart Rate in Competition	Maximal Speed in Competition
1*	223	32.8	203	41.1
PN	211	29.8	203	37.8

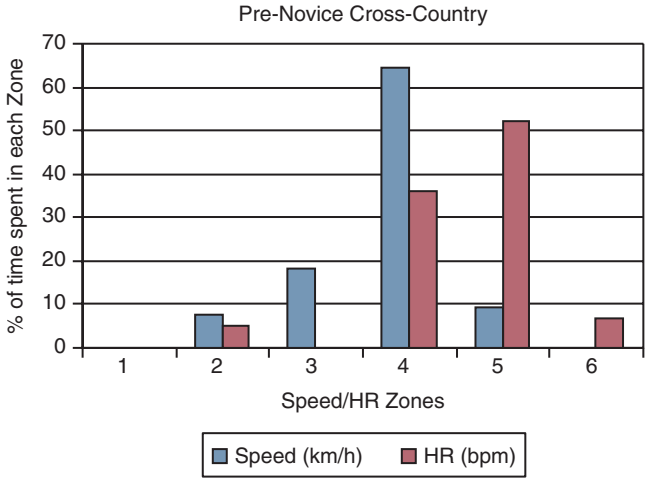

FIGURE 23-2 The percentage of time an eventing horse spent in each speed and heart rate (HR) zone during a pre-novice cross-country competition run. *Zone 1:* Speeds upto 9.9 kilometers per hour (km/hr) and HR up to 89 beats per minute (beats/min). *Zone 2:* Speeds up to 10–19.9 km/hr and HR 90–119 beats/min. *Zone 3:* Speeds 20–24.9 km/hr and HR 120–149 beats/min. *Zone 4:* 25–34.9 km/hr and HR 150–179 beats/min. *Zone 5:* Speeds 35–44.9 km/hr and HR180–199 beats/min. *Zone 6:* Speeds 45 km/hr and HR 200^{+} beats/min.

TRAINING OF THE NATIONAL LEVEL EVENT HORSE AT 2* AND 3* LEVEL

FLAT AND JUMP TRAINING

At the 2* level, the event horse performs a dressage test equivalent to elementary standard in pure dressage; medium paces in trot and canter, shoulder-ins and travers, counter-canter, rein back, canter-walk, and canter-halt transitions are performed, so the horse needs to be systematically trained well in advance of its necessary performance in competition. Ideally, the event horse has been competing for some time at the elementary level in pure dressage competitions and so is well prepared to be competitive in the dressage phase when it reaches the 2* level.

Eventing dressage at the 3* level now requires the horse to perform a single flying change in each direction as well as counter-canter, which can become quite a challenge as the horse often becomes "change happy" with the added tension produced when performing in competition. Depending on the nature of the individual horse and its natural tendency to perform flying changes, changes may be best introduced earlier in the horse's training, that is, when it is at the 1* or 2* level. The training of flying changes may well need to be started, backed off, and then revisited at intervals if the horse finds them so stressful that the overall quality of its dressage performance is adversely affected. The lateral work is further progressed with the half-pass movement included either continuously in one direction on both reins or in a counter-change of hand movement, where the horse is required to perform the half-pass in one direction for half the arena, straighten, then perform the half-pass in the other direction to the opposite side of the arena. These movements do require consistent training to be performed well in a test situation; however, it is important for the rider to remain primarily focused on the quality of the horse's movement as opposed to riding test movements. To correctly perform the lateral work in the trot and the flying changes in the canter, a reasonable degree of collection in the paces is required, and the horse must be trained both consistently and persistently. Essentially, the quality of the leg-to-hand connection is fundamental to producing quality paces that are consistent in rhythm and expression.

The event horse's jump training is also a continual process; improving the quality and consistency of the horse's jump and the rideability around courses is paramount. Continued pole work, gymnastic, and suppling exercises remain invaluable to this end. Training over corners or apexes, arrowheads or narrows, and angled lines is further progressed with the aim of training the horse to focus on jumping anything in its path as opposed to seeking to run around it. Cross-country schooling over ditches, banks, and water is ideal as a pre-season routine or, if necessary, to help a horse recover from a scare encountered in competition.

DRESSAGE AND SHOW JUMPING COMPETITIONS

Pure dressage competitions provide many benefits for the event horse. The atmosphere is generally more relaxed at a dressage competition, as it is not followed by cross-country events; the combination of these factors can give the tense horse a better chance of performing well in a dressage arena. Different tests at the same level can be ridden as opposed to a more limited choice of event tests for each level; this may help prevent the horse from anticipating movements. The test can also be ridden more in a training mode, wherein the rider may choose to sacrifice good marks for a particular movement for discipline or to better train the horse. For the horse that tends to get lazy in the eventing tests, where a whip is not allowed, the use of a whip in a test situation can be a valuable training tool. Elite event horses have certainly competed up to the intermediate 1 level in pure dressage, which provides a significant challenge to the horse in the competition arena and significantly steps up the intensity and technical demand of its training. However, the risk is that competing at such a high level of pure dressage may mentally stress the event horse or place its musculoskeletal system under unreasonable demand given that the prime objective is to best prepare the horse for continued success in the eventing discipline. The experience and judgment of the rider and the trainer is called upon when making such plans for the horse's competition career, as is their sensitivity to the horse's ability to cope, both mentally and physically, with such training and competition demands. Flexibility and preparedness to change plans in the horse's best interests is important.

Likewise, competing the event horse in pure show jumping classes can be of great training value as more jumping experience can be gained with less demand on the horse's musculoskeletal system compared with cross-country competitions. Although it may not always be desirable to press an event horse "against the clock" in a jump-off situation because of the risk of joint sprains from tight turns on landing, riding short approaches or angled lines to fences can be useful training as they place the horse under a degree of pressure to simulate that which can be expected in the show jumping phase at the bigger events. The experience and judgment of the rider are important with regard to what height-jumping classes the event horse is started in and how it is ridden in those classes to ultimately best prepare it for success in the eventing field. Some 3* and 4* event horses have competed

successfully up to the grand prix level show jumping, although not necessarily during their event season. It is perhaps more reasonable to compete event horses in classes up to 1.40 m, especially during their eventing season or in preseason competitions. Competing at such a high show jumping level does pose the risk of the horse being overfaced by the size of the fences; but when the horse is ridden well, such competition mileage certainly challenges the event horse and, in doing so, can help keep it a careful jumper, which some trainers feel is difficult to do if the horse only ever jumps up to 1.30 m as in eventing show jumping.

As the event horse progresses up through the eventing grades, the usual practice is to place it in fewer events to minimize the risk of injury and to reduce general wear and tear. Dressage and show jumping competitions then become more important to both re-create the competition environment for the more experienced horses and to further progress their training.

FITNESS TRAINING

A horse's fitness for eventing competition becomes of greater importance as it moves up to levels where the demands for speed and distance are more significant as are the size and difficulty of the fences, surrounding terrains, or both. Preseason fitness training prior to short-format competitions consists of long trot sessions interspersed with canter work, ideally on a gentle-to-moderate slope. A specific gallop program is recommended to begin some 6 weeks prior to a long-format competition, with progressively more intense gallops (except perhaps the last one or two gallops which may be tapered back a level) being performed every 3 to 4 days, adding up to 12 gallops in all. Running across country at short-format competitions also counts as a gallop workout.

Gallops can be performed in a variety of ways. Uphill gallops that require the horse to be pushing all the time are the shortest, most intense training gallops, with the incline of the slope and evenness of the camber of the ground determining the intensity. Although less mileage is required to achieve peak fitness, such intensity of work may overburden the horse's musculoskeletal system, especially in a horse with prior injuries or management issues. Gallops on the flat ground—at track or beach gallops—are less demanding on the horse's body but require the horse to spend more time galloping to achieve anything approaching a similar level of fitness. Serrano et al. (2002) compared heart rate and blood lactate responses achieved by Advanced event horses during exercise with those during CCI*** competitions, and found that only intense hill gallops provide a similar intensity of workload. For any Advanced event horse, the rider has to finely balance the need for the horse to be as fit as possible and its need to remain sound enough to compete at all. Having a clear idea of the terrain likely to be encountered at the long-format competition will help the rider tailor a fitness regime for each horse and its current program. Seasoned event horses do retain a degree of residual fitness and are much easier to return to peak fitness compared with horses reaching that level of fitness for the first time; the experienced horses may successfully manage a long-format competition with less fitness work than would be expected or required of the first-timer at that level.

GPS DATA IN TRAINING VERSUS COMPETITION IN 2*- AND 3*-LEVEL HORSES

As with the horse's dressage and jumping training, it is a sound principle to "fitness" train a horse at a higher level than is required in competition. In the author's study on the physiologic responses of event horses to training and competition, heart rates were recorded, using a GPS unit and a heart rate monitor, during a training run performed toward the end of each horse's eventing season when it had achieved full fitness and during a competition run. As Table 23-2 indicates, training heart rates were higher than those achieved in competition by the horses, whereas maximum speeds were higher in competition than those achieved in training. These horses were trained on a substantially sloped hill, which created a high intensity of training for relatively less mileage than that which would be required to produce an equivalent fitness on flat terrain (see Table 23-2; Figure 23-3).

PLANNING THE COMPETITION CAREER

Of similar importance to the planning required to successfully progress the young event horse up through the grades is the planning of the competition career of the more experienced horse through 2*, 3*, and 4* competitions. Although many factors influence the ultimate success of an event horse's competition career, careful planning of the number and type of competitions (pure dressage and show jumping competitions as well as short-format and long-format events) in which the horse is entered can be an important part of preparing a horse to be at its peak for major competitions.

MAINTAINING SOUNDNESS IN THE EVENT HORSE

In addition to the management strategies detailed in the earlier section, daily management of the event horse may include some form of physiotherapy—general massage as well as specific stretching and strengthening exercises. Such therapies as acupuncture, acupressure, and muscle tension release techniques have also been reported as being beneficial to the event horse by increasing blood flow to tissues to promote more rapid healing of any injuries sustained.

Therapy systems are also popular choices for use on the athletic horse; they can be more easily fitted in to the hectic schedule of a busy competition yard, as they can be left on a horse before or after exercise without constant supervision. The Activo-Med system utilizes a pulsed electromagnetic field and massage therapy in one rug, with or without boots, and the Equissage therapy unit provides cycloidal vibration to promote improved blood circulation. A massage device, known as the Thumper, which, in effect, helps reduce the work of the therapist's hands can provide massaging action more deeply throughout the horse's muscular system.

Another important management strategy for the older event horse is to avoid long breaks (more than 2 months) out of training, unless forced through injury. Although horses maintain

TABLE 23–2

Heart Rates (bpm) and Speeds (km/h) Attained during Training and Competition in National 2* and 3* Level Event Horses

	Maximal Heart Rate in Training	Maximal Speed in Training	Maximal Heart Rate in Competition	Maximal Speed in Competition
3*	215	39.4	207	41.7
2*	203	33.5	193	47.2

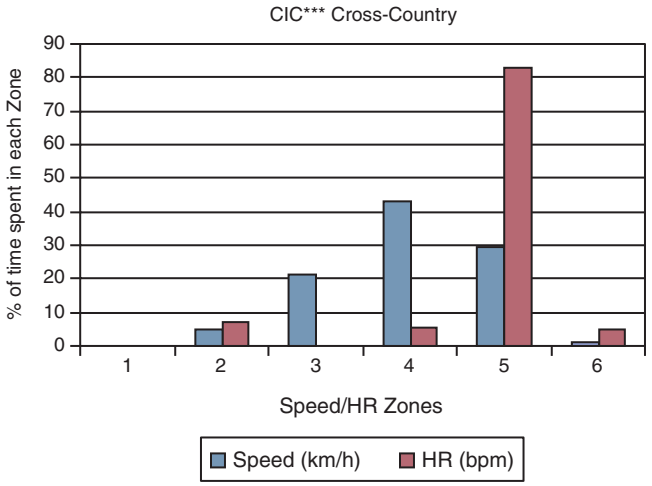

FIGURE 23-3 The percentage of time a horse spent in each speed and heart rate (HR) zone during a CIC*** cross-country competition run. *Zone 1:* Speeds up to 9.9 kilometers per hour (km/hr) and HR up to 89 beats per minute (beats/min). *Zone 2:* Speeds 10–19.9 km/hr and HR 90–119 beats/min. *Zone 3:* Speeds 20–24.9 km/hr and HR 120–149 beats/min. *Zone 4:* Speeds 25–34.9 km/hr and HR 150–179 beats/min. *Zone 5:* Speeds 35–44.9 km/hr and HR 180–199 beats/min. *Zone 6:* Speeds 45km/hr and HR 200^{+} beats/min.

some fitness when turned out to field for a prolonged period, additional exercise is beneficial, especially for the musculoskeletal system. Human athletes do not take long breaks from training, although they may modify their training programs. This is of particular importance for older horses that may have had some problems associated with the musculoskeletal system.

TRAINING OF THE INTERNATIONAL EVENT HORSE

FLAT AND JUMP TRAINING

The International event horse should be well established on the flat arena and over fences, having already spent many years in training and competition. Each horse will, however, have its strengths and weaknesses; generally, those areas which are weakest are also those that have greatest potential for improvement. Training on flat ground is a continual process; for the weaker dressage horses, the challenge is to improve the quality of their paces, their general obedience, and submission, whereas horses stronger in the dressage phase will continue to improve with further strengthening and suppling work. Many event horses will train at a much higher level than they are required to compete at to produce higher-quality work in competition. The standard of dressage has evolved to such a level that progressive training never stops. Lucinda Frederick's "Headley Brittania" produced its best test to lead after dressage at the 2008 Olympic Games at the age of 18 years.

The current 4* dressage tests are equivalent to a test of medium/advanced difficulty in pure dressage terms. It could be argued that certain movements are more difficult in an event test such as flying changes out of counter-canter at the completion of 15 m serpentine loops, shoulder-in down the center line, or an S-bend medium trot, which begins down the long side of the arena before moving on to the short diagonal line and then back down the opposite long side to finish.

Jump training is again progressive with regard to the technical difficulty of the lines and exercises utilized necessarily more so than the size of the jumps. Modern cross-country courses have a strong focus on the horse's ability to hold its straight and curving lines between fences as well as its ability to turn and refocus quickly on the next fence. Such fences are often very narrow and can be optically challenging for the horses; awkwardly shaped oxers brightly painted as houses or tall, narrow café tables are examples of non-natural fences that can be expected at the high-profile events. Angled lines, which can be as severe as 45 degrees to the face of the fence, between closely related offset fences are also popular and require extreme riding accuracy in both the line to and between fences and the distance to the first fence itself. The angled houses into the last water at the 2006 Aachen World Equestrian Games proved to be a significant test and highly influential in the final results. Hence the high-level event horse requires a significant degree of training over narrow fences and angled lines; this is most easily performed using portable fences such as those created with 44-gallon plastic drums either in an arena or as part of a training cross-country course.

FITNESS TRAINING

Fitness training comprises a major part of the elite event horse's program in preparation for a 4* competition. The intensity of the gallop program is stepped up a level; a horse competing at a CCI*** may perform three uphill gallops per training session, whereas the 4* horse will build up to four uphill gallops or an extra repetition of flat gallop, that is, 4 × 6 minutes at faster speeds. Competing at the highest international level may well involve significant travel, which can interrupt the preparation, especially if the horse suffers from any degree of travel sickness. It is best to plan for a week or so of leeway to allow the horse some rest and recovery time when it first lands in a new country or hemisphere. Horses competing at the Olympics may well have to perform under environmental conditions very different from those to which they are accustomed. Conditions of high temperature combined with high humidity have the potential to compromise the horse's normal thermoregulatory system and cause heat stress. Veterinary research prior to both the 1996 Atlanta Olympics and the 2008 Beijing Olympics, where the equestrian events were held in Hong Kong, has helped form excellent guidelines for rapid cooling and preparatory warmup techniques to ensure that the horse starts the course in optimal condition (see also Chapter 8).

WARMUP FOR CCI**** CROSS-COUNTRY EVENT

Warming up for such a demanding test as a CCI**** cross-country is of great importance but, until recently, has had limited research devoted to it and hardly any discussion between riders and coaches on the subject. Prior to 2004, CCI**** competitions were run with a full speed and endurance phase (roads and tracks; steeplechase; roads and tracks) undertaken prior to starting the cross-country course, with a compulsory 10-minute recovery break. With such a compulsory "warmup," little time or opportunity was available to consider performing a warmup that was appropriate for the jumping phases, and the steeplechase effectively became the jumping warmup for the cross-country event. With the advent of the first CCI**** without steeplechase at Kentucky in 2004, suddenly, much more interest was generated in how best to warmup for the cross-country

event. Collaborative discussions among riders, coaches, and veterinarians ensued, and it was decided that competitors should have access to a steeplechase fence and a reasonable area to gallop in the warmup period. The author of this chapter believes that a "free" warmup, where a horse can be schooled with general submission and suppling work in moving up and back through its paces and progressively jumping bigger fences at higher speeds, is far more preferable to the "old format" of the roads and tracks and steeplechase phases, where often the first fence the horse jumped for the day was the first steeplechase fence at flat gallop.

Research by Tyler et al. (1996) demonstrated that exercise on a treadmill at 70% of maximal heart rate (equivalent to 50% maximal oxygen uptake) for 5 minutes prior to completing a maximal exercise test, resulted in greater aerobic contribution to the energy demands of the horse during intense exercise. Although such intensity is appropriate physiologically, it has not typically been used in eventing horses. It would be of interest to ascertain if a higher-intensity warmup in the field does consistently increase the aerobic contribution to the energy demands of intense exercise thereby delaying the onset of fatigue. No study comparing different types of warmup has ever been conducted so far, partly because it would be difficult to demand that riders warm up according to strict research protocol. However, the author of this chapter in performing a descriptive pilot study in the field of equine exercise physiology concluded that a period of 5 minutes of a hand-gallop of approximately 450 m/min (specifically a speed that caused the horse's heart rate to reach 70% of maximal heart rate) was beneficial to the horse. As a professional rider as well, the author felt that her horses were better prepared after completing such a physiologic warmup exercise before jumping during their warmup to cross-country events. The excitable horses became more settled, and the lazy ones became more motivated.

GPS DATA IN TRAINING VERSUS CCI**** COMPETITION

Using data obtained by the author, gallops were recorded during successive weeks of a horse's preparation for an international 4* competition. The horse in the study had not competed at a long-format competition for some 8 months prior to commencing this gallop program but had undergone 8 weeks of conditioning work. Table 23-3 lists the heart rates and speeds attained during the 6 weeks of gallop training (only one out of two sessions per week was recorded) and at the CCI**** competition itself. Decrease in maximal heart rate attained halfway through the training period for a similar or slightly faster maximal speed was interestingly significant. It was also reassuring to see that the horse was not pressed beyond its physiologic training level in competition at the 4* level, even though it was run hard cross-country to finish 10 seconds inside the optimal time (see Table 23-3).

TABLE 23–3

Maximum Heart Rates (bpm) and Speeds (km/h) Attained during 6 Weeks of Training Prior to a CCI** Competition and during a CCI**** Competition**

	Maximal Heart Rate	Maximal Speed
Week 1	229	46.7
Week 2	227	46.1
Week 3	226	46.3
Week 4	209	48.0
Week 5	207	48.3
Week 6	200	47.0
CCI****	208	46.7

TEAM TRAINING FOR INTERNATIONAL COMPETITION

Team training camps are often run for 8 to 14 days prior to the horses leaving for a major international competition. This period allows the riders to focus solely on best preparing their horses for the upcoming championships without any distractions of business or family commitments. The team trainers and veterinarians keep a close eye on the horses' preparation and help develop strategic management plans for producing the best from each team horse. Although it is best that the training itself is not significantly different from how riders would normally prepare their horses for major competitions, some non-negotiable parts do exist, especially the gallop days during which the team veterinarian will ensure that the horses' fitness is well on track to cope with the demands of the upcoming championship cross-country phase, which, in recent times, has often been run in demanding environmental conditions.

PROLONGING THE CAREER OF THE INTERNATIONAL EVENT HORSE

Close teamwork between private and team veterinarians and allied therapists is essential to best maintain the health and soundness of the international event horse. Oral joint supplementation is also recommended to help prolong the career of these elite horses. All management factors previously discussed, such as excellent shoeing, only running the horse on good ground in both training and competition, having a high standard, consistent arena surface, and limiting competition outings to major competitions and important preparatory ones, are important in the training of the elite horse.

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CHAPTER

Dressage Tests, Movements, and Training: A Primer

24

DAVID R. HODGSON

Dressage is a term derived from the French term *dresser*, which means *to train*. Originating centuries ago in Europe, dressage became much more prevalent during the Renaissance period. It was later that progressive, strategic training regimens were developed by the great riding masters. This is referred to as *classical dressage*. In the twenty-first century, modern dressage is a competitive equestrian sport, with common training protocols that demonstrate changes from, but clear relationship to, those used in classical dressage (Chamberlain, 2006). Reference to the website of The Fédération Équestre Internationale (FEI), the governing body of international dressage competitions (http://www.fei.org/disciplines/dressage/about-dressage), indicates that dressage is considered one of the highest forms of horse training. The site goes on to state that at completion of training, the horse and rider are expected to perform, from memory, a series of predetermined movements. Dressage competitions are held worldwide, with the Olympic and World Equestrian Games being considered the pinnacles of the sport. The key purpose of the training discipline is to have the horse undertake a relatively standardized training protocol whereby the limits of a horse's natural athletic ability are approached. This should be associated with the horse's willingness to perform, with the outcome being achievement of its maximal potential as a riding horse. The objective is for the horse to respond without hesitation to the rider's commands (aids), with these aids being kept to a minimum and the horse appearing relaxed and free of apparent effort. Commentators at televised events such as the Olympic Games often refer to dressage as equine ballet. Although the discipline has ancient roots in Europe, with the Greeks laying claim to early dressage "type" training around 400 BC. Dressage was first recognized as an important equestrian pursuit during the Renaissance. The great European riding masters of that period developed a sequential training system that has changed little since then. Classical dressage is still considered the basis of modern dressage (Chamberlain, 2006).

Competitions in modern dressage involve undertaking a riding test, a process in which horse and rider are expected to undertake series of prescribed movements. The movements are graded in difficulty according to the training level of the horse and become progressively more difficult as the horse advances to higher levels of performance. A dressage test is conducted in a standardized arena, with judges assessing each of the movements. Judges use an objective standard for each movement and ascribe a score on a scale of 0 to 10: 0 if the movement is not executed and 10 being the perfect score, which is almost never awarded. As such 9 is considered an excellent very high score. As a general rule, horses and riders achieving consistent average scores of 6 or above are generally qualified to move up to the next level of competition (http://www.usef.org/_IFrames/breedsDisciplines/discipline/alldressage/about.aspx).

In addition to modern dressage, traditional or classical dressage is maintained in several regions of the world. The term *classical dressage* is used when dressage is executed as an art form as opposed to a competition form. This art form of the discipline maintains many of the traditions developed by the original dressage masters, particularly those from the Renaissance period (fourteenth to seventeenth centuries. Today, these traditions are maintained by the Spanish Riding School in Vienna, Austria, and the Cadre Noir in Saumur, France. This type of schooling is also used in the training of horses that participate in Portuguese and Spanish bullfighting.

BREEDS OF HORSES USED IN DRESSAGE

Generally, all riding horses could derive some benefit from the use of some form of dressage training. At the entry and middle levels of competition, many breeds are represented. However, when it comes to competition at the highest levels, Warmblood breeds are the most frequently used. Indeed, at the Olympic or World Equestrian Games, it is rare to find any horse that is not of Warmblood origin.

DRESSAGE ARENAS

The two accepted sizes of arena (dressage manège) are referred to as *small* and *standard*. The small arena is 20 m × 40 m (66 × 131 ft) and tends to be used for lower level competition. The standard arena is 20 m × 60m (66 × 197 ft) used for all upper-level national and international competitions (Figure 24-1).

Each size of arena has letters (often associated with cones) assigned to positions around the edges of the arena to specify where the requisite movements are to be performed. For the small arena, the letters are A-K-E-H-C-M-B-F with A located nearest the point of entry to the arena and counted off in a clockwise fashion. Additionally, a set of letters are placed down the center line of the arena: D-X-G, with X being at the center. The standard arena, used in dressage and eventing competitions, has the letters A-K-V-E-S-H-C-M-R-B-P-F around the periphery. The letters D-L-X-I-G are in the middle (short ends) of the arena, again with X being on the midline (see Figure 24-1). As such, dressage has a defined centerline (from A to C, going through X in the middle) as well as two quarter-lines (halfway between the centerline and the long sides of each arena) (see Figure 24-1).

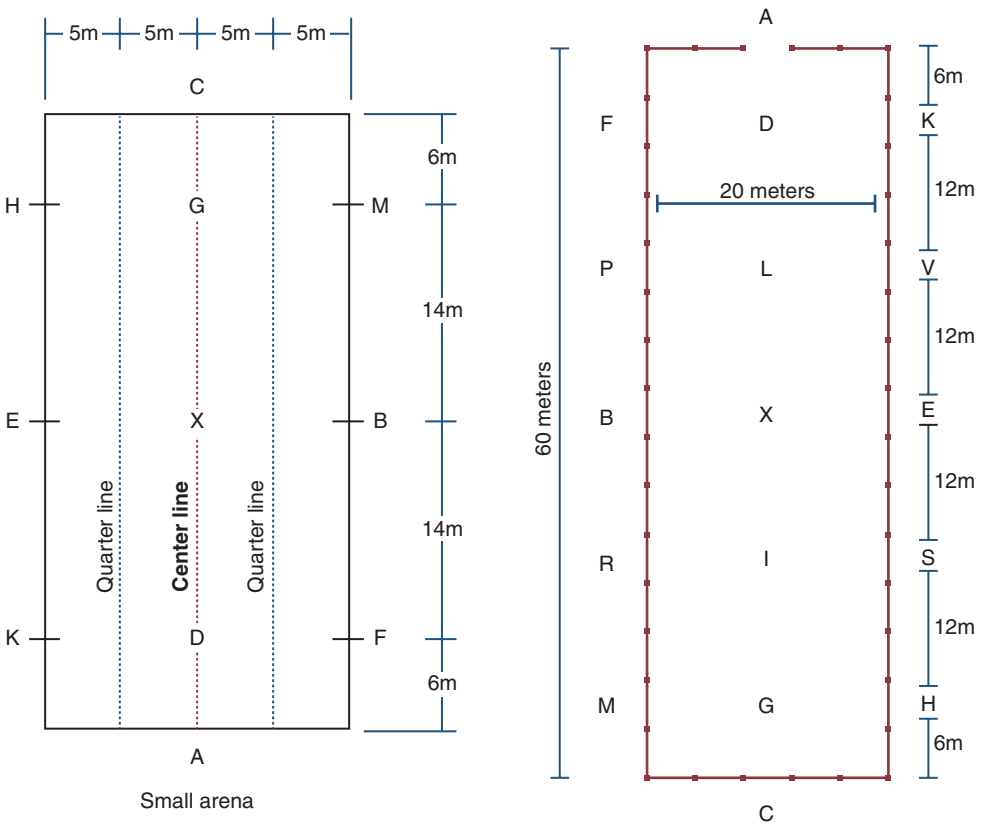

FIGURE 24-1 Small and standard dressage arenas with the accepted measurements (meters) and letter configurations.

Among the several theories explaining the origins of the markings of the arenas, the two probable explanations exist for the lettering surrounding the manège. Markings found on the walls of the Royal Manstall (Mews or Stables) of the Imperial German Court in Berlin (prior to 1918) suggest that they indicated where each courtier's or rider's horse was to stand awaiting its rider. The Manstall stabled 300 of the Kaiser's horses, as well as carriages and sledges. The "hof" (stable yard) was large enough for horses and their riders to parade for "morning exercise" or assemble for ceremonial parades. The length of the hof was three times greater than the width: 20 m × 60 m, hence the likely origin of the accepted size of the standard arena today. The markings on the walls of the Manstall are provided in Box 24-1. The letters D-L-X-I-G were added in 1932 for the Berlin Olympic Games. The origin or meaning of these extra letters is not well explained.

The German Cavalry is also credited with establishing the markings. The space between the stable blocks in many German Cavalry barracks also measured 20 m × 60 m. This space was also used for assembly and morning exercise, and it is not unreasonable to assume that the Cavalry would adopt similar markings as those used in the Kaiser's Manstall. Additionally, the German Cavalry Manual shows a diagram of the Reit Bahn (indoor school) as 40 m × 20 m. The markings A-B-C-D were in the four corners and E-F on the midline. These markings were to indicate school figures for the training of riders and not for competition and are considered one of the possible origins for the lettering used in the small arena (manège).

BOX 24–1

Markings on the Walls of the Manstall

A: Ausgang (Exit).
K: Kaiser (Emperor).
F: Fürst (Prince).
P: Pferknecht (Ostler or Groom).
V: Vassal (Servant/Squire/Equerry).
E: Edeling/Ehrengast (Chieftain or Honored Guest).
B: Bannertrager (Standard Bearer).
S: Schzkanzler (Chancellor of the Exchequer).
R: Ritter (Knight).
M: Meier (Steward).
H: Hofsmarshall (Lord Chancellor).

At the start of the test, the horse enters at A. A judge is always sitting at C, although for upper-level competition, up to five judges are present at different places around the arena—at C, E, B, M, and H—which allows the horse to be seen in each movement from all angles. This helps prevent certain faults from going unnoticed, as it may be difficult for a judge to see everything from only one area of the arena. For example, the horse's straightness going across the diagonal may be assessed by the judges at M and H.

COMPETITION

Dressage competitions may take place at any level, from the beginner level to the elite level. Often, community riding clubs will hold tests for introductory level classes, where riders may only require their horse to walk and trot. Generally, worldwide, horses and riders advance through a graduated series of nationally defined levels, with the difficulty of the tests increasing at each level. The most accomplished horse-and-rider combination competing at the elite level perform tests prescribed by the FEI. These tests are designed by an international committee drafted through the auspices of the FEI. The highest level of modern competition is referred to as *grand prix* and is the level set at prestigious competitions such as the World Equestrian and Olympic Games. Other levels governed by the rules of the FEI are Prix St. Georges, Intermediare I (Intermediate I), and Intermediare II (Intermediate II). Lower-level competitions, however, are regulated by individual nations where the competitions are held. Generally, the convention is to have four to six of these lower-level skill tests. Relative to the FEI-regulated tests, these lower-level competitions require the horse and the rider to demonstrate basic gaits, exercise in relatively large circles, and demonstrate lower levels of collection (defined as the horse carrying more weight on its hindlimbs than in its forelimbs, which differs from the natural tendency of the horse when it is ridden) compared with the international levels. Lateral movements are not required in the lower levels, with movements such as the leg yield, shoulder-in, or haunches-in being gradually introduced as the horse progresses.

DRESSAGE TESTS

Dressage tests for competition purposes are formulated to include a sequence of a requisite number of dressage movements (http://www.usef.org/_IFrames/breedsDisciplines/discipline/alldressage/about.aspx).

Although horse-and-rider pairs are competing against each other, these tests are completed by one horse–rider combination at a time, with the pair judged against a common standard, as opposed to the performance being evaluated relative to other competitors.

As stated earlier, upper-level tests, particularly those used in elite competitions such as the Olympics, are administered under the auspices of the FEI. At lower levels, each country's equestrian regulatory authority approves its own set of tests. For example, in the United States, these bodies are the United States Equestrian Federation and the United States Dressage Federation. Pony Clubs may also write basic tests that involve the walk and the trot.

SCORING DRESSAGE TESTS

The overall dressage score comprises individual movement scores as well as collective marks (Anon 2012a). Coefficients are also applied to add an element of importance to certain movements. Scores are calculated using a 0-to-10 scoring scale, which is broken down in Table 24-1.

A score of "0" on a given movement means that practically nothing of the required movement was performed. In addition to the scores shown above, difficult and infrequent movements are weighted by coefficients to underscore the importance of these movements.

TABLE 24–1

Scoring Scale

Score	Level of Execution	Score	Level of Execution
0	Not Executed	6	Satisfactory
1	Very Bad	7	Fairly Good
2	Bad	8	Good
3	Fairly Bad	9	Very Good
4	Insufficient	10	Excellent
5	Sufficient		

Collective marks are given after the rider–horse pair has completed the test. These marks are also based on the 0-to-10 scoring scale and are based on gaits, impulsion, submission, and the rider's position and seat. These marks are also given a coefficient (usually a factor of two, thereby doubling the marks given for that segment), since these areas represent the natural and training ability of the horse-and-rider combination and are considered critically important in the evaluation of the horse–rider team. The following describes briefly each of the elements (Anon 2012a):

- *Gaits:* the freedom and regularity of the horse's movement.
- *Impulsion:* the horse's desire to move forward, elasticity of steps, roundness of back and body.
- *Submission:* the horse's attention and confidence; harmony with the rider, apparent lightness of movements and acceptance of the bridle/bit.
- *Rider's seat and position:* correctness and effect of aids.

Tests at the Olympic Games are scored by a panel of seven invited, highly qualified international judges. Each movement in each test receives a numeric score, as described above, with the resulting final score converted to a percentage carried to three decimal points. Thus, the higher the percentage, the higher is the score that the horse and the rider achieve (Anon 2012a).

SCRIBING

An assistant to the judge undertakes "scribing" (also known as *penciling*), which is a recording of the scores and comments of the judge, so that the judge is able to concentrate on the performance. In addition, at many competitions, the assistant may be required to check the identity of each competitor and ensure that the test papers are completed and signed by the judge before they are handed to the scorers. The scribes are expected to have knowledge of dressage terminology (Anon 2012b).

MOVEMENTS REQUIRED IN DRESSAGE TESTS

When tests are formulated for the elite or Grand Prix level, the following movements are expected to be performed by the horse:

- *Piaffe:* This gait is a calm, composed, elevated trot in place (although some minimal movement forward).
- *Passage:* This gait is a very collected trot, in which the horse has great elevation of stride and appears to pause between each step (i.e., the horse demonstrates a great amount of suspension in the stride). Some observers

compare this gait "to trotting under water." Ideally, in piaffe and passage, riding or gait tempo should be the same as that in the trot.

- *Extended gaits:* Performed at the trot and the canter, the horse is required to lengthen its stride to the point where it approaches maximum stride length. Equally important is the extended walk, which shows that the horse is able to easily relax between periods when the more collected movements are being evaluated.
- *Collected gaits (trot and canter):* Collection, as mentioned above, is reflected by a shortening of stride, where the horse brings its hindquarters more underneath the body and, by definition, carries more weight on the hind end or hindlimbs. When effectively performed, the gait tempo should not change, whereas the horse shortens and elevates the stride.
- *Flying changes in sequence:* In the gait referred to as tempi, the horse is required to change leads in the canter at every stride. It is referred to as "one tempi" for one stride, "two tempi" for two strides, and "three tempi" for a change every three strides.
- *Pirouette:* This gait involves 360-degree or 180-degree turn in place (the higher the level, the more substantial is the turn) generally expected to be performed at the canter. In a Freestyle test with accompanying music (Kür), a turn of up to 720 degrees is allowed.
- *Half-pass:* In this gait, the horse is required to move on the diagonal, moving sideways and forward at the same time, being bent only slightly in the direction of movement.

BASICS OF TRAINING THE HORSE FOR DRESSAGE: THE TRAINING SCALE

The dressage training scale is often arranged in a pyramidal or sequential fashion, with "rhythm and regularity"; the easiest to master at the base of the pyramid and "collection," and a much more difficult accoutrement occurs at the apex (Figure 24-2) (Barretto de Souza 1922; 1925; 1926). Many trainers refer to the training scale as they find it an effective and helpful guide for the training of any horse (Barretto de Souza, 1925; McNeil, 2011). However, the "pyramid" has been most frequently referred to in discussions of training of dressage horses. As with any plan, the pyramid serves as a guide and is not considered a part of a rigid, inflexible format. The general concept, translated here somewhat loosely from the original German, is that each step in the pyramid represents the level of training achieved. Thus, as the horse progresses through the different phases or levels of training, it climbs up the levels of the pyramid. Thus, even riders and trainers of elite horses will work on ensuring mastery of each level of the pyramid instead of focusing on just those activities prescribed for the upper parts of the pyramid. Not surprisingly, trainers regard the various levels of the pyramid as being interconnected.

Some broad interpretations of what each level of the pyramid refers to and requires of the horse-and-rider combination are discussed below (see Figure 24-2). Again, the terms are a loose translation from the original German terms used to describe the steps in the pyramid.

Rhythm and Regularity (Takt)

Rhythm, gait, tempo, and regularity should be the same on straight as well as bending lines of locomotion, through lateral movements, and through transitions in gait. *Rhythm* refers to the sequence of footfalls, which, in this context, refers only to the walk, trot, and canter. The *regularity* (sometimes referred to as the *purity*) of the gait includes the evenness and levelness of the stride. Once a rider can achieve pure or consistent gaits from the horse, thereby avoiding irregularity, the pair are likely prepared to undertake more complex exercises. Riders and trainers strive to achieve regularity in the most difficult of maneuvers, for example, the piaffe, where the horse is expected to achieve regularity even when trotting in one place.

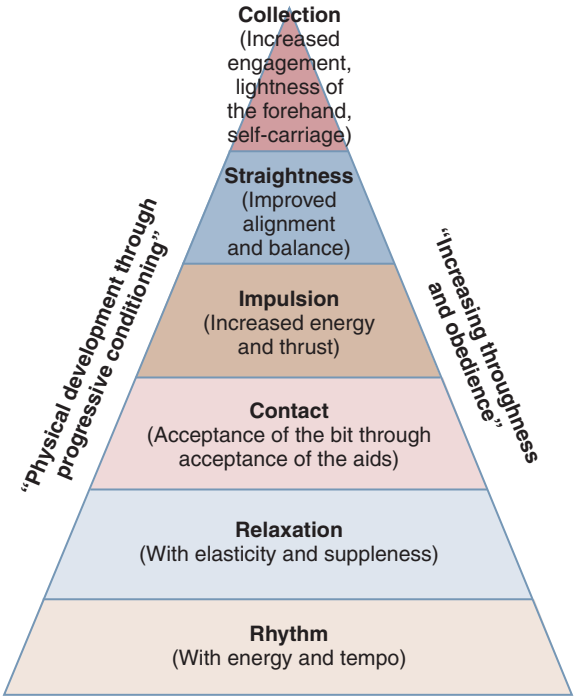

FIGURE 24-2 The pyramid of dressage training. Horses at the start of their training begin learning the skills outlined at the base of the pyramid and progress toward the apex through the levels of training. Elite-level horses that have mastered all the basic skills tend to focus on those skills described at the upper or apex levels of the pyramid. *(Adapted from Barretto de Souza J:* Advanced equitation, *New York, 1926, EP Dutton & Co.; Barretto de Souza J:* Elementary equitation, *New York, 1922, EP Dutton & Co.; Barretto de Souza J:* Principles of equitation, *New York, 1925, EP Dutton & Co. www.books.google.com/books/about/Principles_of_equitation.html?id51RZDAAAAIAAJ.)*

Relaxation (Losgelassenheit)

The second level of the pyramid is relaxation or looseness. Signs of looseness or relaxation can be noted when the horse has even stride that swings through the back of the animal, resulting in the tail swinging rhythmically like a pendulum. The horse also appears loose and relaxed at the poll. Also, the horse is noted to make smooth transitions between gaits. Relaxed or loose horses also show the propensity to reach down with their heads to make light contact with the bit in the corners of the mouth in response to the rider lengthening the reins.

Contact (Anlehnung)

Contact is the third level of the pyramid and is achieved partly as a result of the horse's "pushing power" (see Impulsion

below). During this movement, hopefully with the horse's cooperation, the horse is trained gently but effectively, to stretch and lift the neck such that the horse maintains contact with the bit. Contact, with equal tension on both reins, is to occur as a result of the willingness of the horse to flex the neck and not as a result of the rider forcefully withdrawing the hands (i.e., pulling on the reins). Dressage aficionados may use the term "soft hands" in relation to the ease with which a horse achieves contact.

Impulsion (Schwung)

Level four on the pyramid is the thrust or "pushing power" of the horse, referred to as impulsion. Impulsion is achieved by the horse when it brings its hindlimbs up under the body such that these limbs contribute a greater proportion of the energy of locomotion than would normally be the case. Horses with good impulsion may be referred to as being "engaged," that is, an appropriate engagement between the forelimbs and hindlimbs during the prescribed movement. As with all the gaits and movements described here, impulsion can only be achieved if the rider utilizes the appropriate aids (sometimes referred to as "driving aids") and the horse remains relaxed.

Throughness (Durchlässigkeit)

Throughness refers to what is perceived as the flow of locomotor energy through the horse from front to back, and vice versa. The overall appearance to the trained observer is that the musculature of the animal appears intimately connected and coordinated and yet is supple and elastic with no obvious impediments. This can be achieved only if the horse responds to the driving aids from the rider willingly and without obvious resistance or untoward evidence of their application.

The better the horse becomes trained, the more is expected in terms of impulsion. Impulsion can be expected to occur at the walk, trot, and canter. The expectation is that appropriate impulsion should first be achieved at the walk, as this will allow development of appropriate transitions to "forward" trot and canter, that is, gaits with desired degrees of impulsion.

Straightness (Geraderichtung)

Straightness, which is level six of the pyramid, refers to the concept of a horse's hindlimbs following the line or path of the forelimbs. This should occur when the animal is moving in both straight and bending lines. In the latter case, the horse's body is expected to follow the line of movement. Straightness is considered an essential skill, as it allows the horse to direct impulsion directly toward the animal's center of balance.

Collection (Versammlung)

Collection is a natural movement for horses and can be recognized when horses play outside in the field. It is a form of locomotion where the horse moves its center of gravity toward the hind end. As shown in Figure 24-2, collection is the skill set at the apex of the training scale. It may refer to collected gaits, which can be used occasionally to supplement less vigorous work. It involves difficult movements (such as flying changes) in more advanced horses. Collection requires greater muscular strength, so the horse must be trained to consistently and accurately achieve it, and this takes time and persistence. Horses performing at collected gaits will demonstrate an apparent decrease in stride length and an increase in flexion of the joints of the hindlimbs, thus allowing the hindlimbs to move up under the body, but with no obvious increase in energy expenditure.

MOVEMENTS IN CLASSICAL DRESSAGE: "AIRS ABOVE THE GROUND"

A series of classical dressage movements (Barretto de Souza, 1926; readers are also referred to the DVD series by Phippe Karl: *Classical Dressage the Philosophy of Ease*) have been preserved to the present as classical equestrian art, however, none is used in modern dressage. Originally, these movements were used in training to increase the flexibility and agility of horses, not for purposes of warfare, as suggested frequently. Generally, such movements require considerable strength and athletic ability. "Airs above the ground" can be divided into a variety of movements. Some of these include the following:

- *Pesade:* Pesade is a maneuver in which the horse is trained to rear, keeping the hindlimbs stationary and forelimbs drawn into the body. http://www.lipizzaner.com/airs.asp
- *Mezair:* Mezair represents a series of successive levades, in which the horse lowers its forefeet to the ground before rising again on hindquarters, achieving forward motion. http://www.lipizzaner.com/airs.asp
- *Courbette:* The horse balances on its hindlimbs and then jumps, keeping the hindlimbs together and the forelimbs off the ground. http://www.lipizzaner.com/airs.asp
- *Levade:* The horse must maintain a hunched position at a 45-degree angle to the ground, which requires muscle control and perfection of balance that is quite difficult. http://www.lipizzaner.com/airs.asp
- *Capriole:* The horse leaps into the air, drawing its forelimbs under its chest at the height of elevation, and kicks out violently with its hindlimbs. Many years of training may be required to master the capriole.
- *Croupade:* In this movement, the horse jumps up from a pesade with all four legs drawn up under its body. The horse then lands in the same place from where it launched its jump.

In the twenty-first century, "airs above the ground" are generally performed by horses from various riding academies, including the Spanish Riding School in Vienna and the Cadre Noir in Saumur, France. Baroque horse breeds such as the Andalusian, Lusitano, and Lipizzan are most often trained to perform the "airs" today.

CONCLUSION

Dressage is often considered the equine equivalent of ballet. Horse and rider perform a "test" consisting of difficult moves and gait changes. Considerable training is required to master even the simplest of movements required during a dressage test. Riders cannot use voice commands and the horse–rider pair is scored on how well it performs the test. Many levels exist in dressage. In the United States, the generally accepted levels are introductory, training, first, second, third, and fourth. The International levels are Prix St. George, Intermediate I, Intermediate II, and Grand Prix. Dressage originated in ancient Greece and was refined to classical dressage in the Renaissance period. Today, modern dressage is considered the mainstay of competitive dressage.

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CHAPTER

Training Show Jumpers

25

RENE VAN WEEREN*

The first officially organized jumping competitions were included in the program for the Royal Dublin Society's show in 1864. There were three competitions: The first was a high jump of gorse and three rails, the second was a wide jump of hurdles, and the third was a stone wall. The dimensions of the jumps are no longer known, but a wooden fence used in a qualifying competition was 4½ feet (1.35 m) high. In the course of the following years, show-jumping competitions rapidly gained popularity in England and continental Europe, and the first international jumping trials were included in the World Exhibition in Paris in 1900.

In the early years of the twentieth century, Italians dominated the international jumping scene, largely because of the outstanding performances of Federico Caprilli, the first proponent of the forward seat, which was demonstrated at the International Horse Show in Turin in 1901. Despite some initial opposition, the forward seat was later adopted by all cavalry schools. Riding a horse called Melopo, Caprilli established a high jump record of 2.08 m and a long jump record of 7.40 m in 1902. In the same year, the German riders performed so abysmally that the emperor forbade their participation in international competitions.

When the Olympic Games were revived in 1896 in Athens, the intent was to include equestrian events. However, the logistics of transportation and organization proved insurmountable, and it was not until the 1912 Stockholm Olympics that dressage, show jumping, and eventing were included. Since 1956, women have been accepted as team members, and show jumping is now one of the few sports in which men and women compete as equals.

The first Olympic show-jumping course consisted of 15 fences, some of which were combinations and some jumped twice for a total of 29 jumping efforts. The maximum dimensions were 1.40 m high and 4 m wide. Today, the maximum dimensions under the rules of the *Fédération Equestre Internationale* (FEI, International Equestrian Federation) are 1.70 m high (except in puissance, power, and skill or a high jump record) 2 m wide for a spread fence (except a triple bar, for which the limit is 2.20 m), and 4.50 m wide for the water jump (except when trying to establish a record) (Anon, 2011) (Figure 25-1).

CONFORMATION AND SHOW JUMPING

A horse's conformation undoubtedly affects its jumping ability, but data correlating specific conformational attributes to success in jumping competitions are limited. Typically, good jumpers are taller at the withers (Fabiani, 1973; Langlois et al., 1978; Neisser, 1976), compared with dressage horses (Muller and Schwark, 1979). The shoulder is preferably sloping and the forepastern has been shown to be significantly larger in elite jumping (and dressage) horses compared with other riding horses (Holmstrom et al., 1990). The ratio between chest girth and wither height tends to be relatively low in good show jumpers, as does the ratio between cannon circumference and wither height (Fabiani, 1973). A long neck is thought to be an advantage for jumping horses, possibly because this feature makes it easier for the horse to maintain balance over the fence (Holmstrom, 2001). Good width through the hip and gaskin, as viewed from behind, is desirable. The length of the pelvis has been reported to have a positive correlation with jumping ability and a forwardly sloping femur was also found to correlate to good jumping performance in the same study (Langlois et al., 1978). The latter feature has, by the way, been shown to have the strongest correlation to overall quality gait of all studied conformational variables (Holmstrom and Philipsson, 1993). Show jumpers have shorter backs compared with dressage horses, which is possibly related to the required suppleness in the dressage horse (Johnston et al., 2004).

BIOMECHANICS OF SHOW JUMPING

Biomechanics applies the laws of mechanics to living systems. In jumping horses, mechanical laws govern the trajectory of the center of gravity and the rotation of the horse's body during the airborne phase. Mechanical considerations are also involved in the stride adjustments that precede the take-off and follow the landing.

In a canter, horses usually approach and move away from a fence. In this gait, the sequence of limb placements in each stride is trailing hindlimb (TrH), followed by the leading hindlimb (LdH) and trailing front limb (TrF), which makes impact almost simultaneously, and finally, the leading front limb (LdF). After the LdF leaves the ground, usually a short suspension occurs before the next impact of the TrH. When a horse is cantering on the left lead, the TrH is the right hindlimb, the LdH is the left hindlimb, the TrF is the right front limb, and the LdF is the left front limb. On the right lead, the sequence is reversed.

The stride in which the horse jumps the fence is called the *jump stride* (JS). It is characterized by a long suspension between liftoff of the LdH and impact of the TrF, during which the horse is airborne over the fence. The airborne phase is

*The authors acknowledge the work of H.M. Clayton on this chapter in the previous edition.

FIGURE 25-1 Henrik von Eckermann on Quibell (Quintero x Cento), Rome, 2012. *(Photo courtesy Jacob Melissen.)*

called the *jump suspension;* the hindlimb stance phases preceding the jump suspension constitute the *take-off,* and the front limb stance phases following the jump suspension are referred to as the *landing.* The strides preceding *(approach strides)* and following *(move-off strides)* the jump stride are named from the jump outward (see Figure 25-l). Starting three full strides before the jump, they are known as approach stride 3 (A3), approach stride 2 (A2), approach stride 1 (A1), and jump stride (JS), move-off stride 1 (M1), move-off stride 2 (M2), and move-off stride 3 (M3) (Clayton, 1989). The strides A1 and M1 are highly modified canter strides; Al initiates the upward movement of the forehand prior to takeoff, whereas M1 re-establishes the horse's balance after landing. The horizontal velocity, stride length, and stride rate for strides A2, A1, JS, and M1 are shown in Table 25-1.

APPROACH

The horse approaches the fence in a canter at a suitable speed in accordance with its strength and level of technical skill. Generally, a more experienced jumper can cope with a faster approach through being stronger and having better control of its motor skills. If the take-off speed is too fast, the horse may not be able to generate enough force sufficiently rapidly to clear the fence in good form. Consequently, the horse either hits the fence or clears it by making a compensatory action such as rolling a shoulder or twisting in the air.

The stride length is adjusted during the approach so that the take-off occurs at an appropriate distance from the base of the fence. In a study of four Grand Prix horses jumping vertical and square oxer fences with a height and width of 1.10 m to 1.40 m, no significant difference was found for the limb displacements from the base of the fence in strides A2, Al, JS, or MI (Clayton and Barlow, 1989). Therefore, the take-off distance was the same regardless of fence height or width, within the range tested, and any adjustments in stride were made prior to stride A2. The limb placed closest to the fence on the take-off side is usually the LdF in stride A1 (Clayton and Barlow, 1989). One study found a correlation between fewer penalties during a competitive round and the tendency to place the LdF closest to the base of the fence on the take-off side (Deuel and Park, 1991).

During approach strides up to and including A2, the horse canters in a collected frame. The head and neck are elevated, the nose is ahead of the poll (Figure 25-2), and the TrH and LdF make impact almost synchronously.

Compared with the preceding strides, Al is a short, quick stride (see Table 25-1), in which the horse's motion is often described as "patting the ground." Stride Al has a four-beat rhythm due to dissociation of the diagonal limb pair, with the LdH making impact before the TrF. The head and neck stretch forward and down as the horse lowers its center of gravity prior to initiation of the upward movement (see Figure 25-2). The front limbs are thrust forward in a strutting action, hitting the ground at a more acute angle than usual (Clayton and Barlow, 1989), which allows them to decelerate the forward movement of the body (Merkens et al., 1991). Consequently, the reduction in horizontal velocity during stride Al (see Table 25-1) is quite significant. The front limbs also provide a large vertical force that starts the upward movement of the forehand. Since the TrF exerts a higher peak vertical force (Merkens et al., 1991) and has a longer stance duration than the LdF (Clayton and Barlow, 1989), it makes a greater contribution to the upward impulse. A short suspension follows liftoff of the LdF (Clayton and Barlow, 1989; Merkens et al., 1991) and then the hindlimbs hit the ground to initiate the take-off.

TABLE 25–1

Mean Values for Velocity, Stride Length, and Stride Rate in Four Horses Jumping a Vertical Fence 1.55 m High

	Approach stride 2	Approach stride 1	Jump stride	Move-off stride 1
Velocity (m/s)	7.3	6.3	5.9	6.5
Stride length (m)	4.1	2.4	4.9	3.3
Stride rate (strides per second)	1.8	2.6	1.2	1.9

TAKE-OFF

During the take-off, impact of the TrH may precede that of the LdH, but often the two hindlimbs are placed almost synchronously (Clayton and Barlow, 1989; Leach et al., 1984). Initially, the hindlimbs decelerate the forward movement, but then a large horizontal accelerative force is applied in the later part of the take-off (Merkens et al., 1991). The hindlimb stance duration is longer during take-off than in a normal stride, which allows the generation of a large impulse (Clayton and Barlow, 1989). Since the center of gravity lies ahead of the line of action of the force exerted by the hindlimbs against the ground, this force imparts angular momentum that causes the horse's body to rotate forward around the center of gravity during the jump suspension. As a consequence of the angular momentum, the horse takes off from the hindlimbs and lands on the forelimbs.

Some important mechanical characteristics of the jump, including the path of the center of gravity and the angular momentum, are established during the take-off and cannot be changed until the horse makes contact with the ground or some other object. Sometimes, horses bank a fence (push off the top of a solid fence) or make adjustments such as dropping the hindlimbs between the rails of an oxer in an attempt to compensate for a take-off that is mechanically inappropriate.

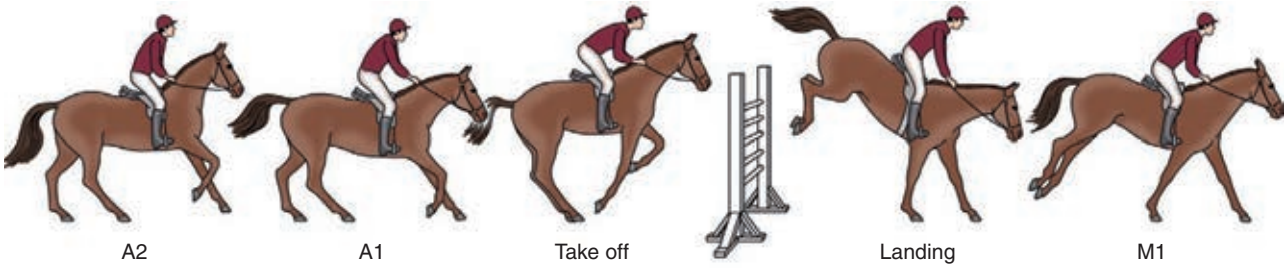

FIGURE 25-2 Outline drawing of horse and rider during the approach, jump, and move-off (A2, approach stride 2; Al, approach stride 1; JS, jump stride; Ml, move-off stride 1).

JUMP SUSPENSION

During the jump suspension, horses use different styles or techniques. Jumping ability is optimized by adopting a technique that minimizes the height discrepancy between the top of the fence and the horse's center of gravity. When the horse bascules (flexes the vertebral column), it lowers the position of the center of gravity within its trunk. Elevation of the limbs as they pass over the fence minimizes the height to which the center of gravity must be raised for all the body parts to clear the fence. In the case of the forelimbs, elevation of the lower limb is accomplished by swinging the point of the shoulder forward and upward, which pulls the elbow forward. With the upper limbs in this position, the horse is able to flex the elbow and raise its knees (carpi). As the hindquarters pass over the fence, the lumbosacral joint extends to elevate the hindquarters, while extension of the hip joint raises the lower limbs.

LANDING

The TrF makes impact first with an almost vertical orientation, quickly followed by the LdF, which has a more acute angulation to the ground (Clayton and Barlow, 1989). Both forelimbs experience high peak vertical forces as they absorb the concussion of landing. The TrF, which has a very short stance duration at landing, provides some horizontal propulsion to assist in moving the horse away from the jump, whereas the LdF reverses the direction of rotation of the trunk around the center of gravity. The trunk continues its downward trajectory as it passes over the front limbs.

MOVE-OFF

In the short suspension that follows landing, the hindquarters swing underneath the trunk. The TrH is placed further from the fence than the TrF but closer than the LdF (Deuel and Park 1991; Clayton and Barlow, 1989). In contrast to the fairly predictable position of the TrH, that of the LdH is quite variable. Sometimes, it lands adjacent to the TrH; other times the two hindlimbs are widely separated. The LdH placement seems to depend on the horse's balance during landing and move-off. Stride M1 covers a short distance, but the stride rate is approximately the same as that of stride A2 (see Table 25-1). It has a distinct four-beat rhythm due to dissociation of the diagonal limbs with the LdH preceding the TrF (Clayton and Barlow, 1989). In the subsequent move-off strides, the horse re-establishes a normal stride pattern.

PHYSIOLOGIC DEMANDS OF SHOW JUMPING

The majority of show-jumping competitions require a minimum speed in the range of 325 to 400 meters per minute (m/min), although the average speed during a round may be considerably faster when time is a deciding factor. Heart rate recordings have shown that the exercise intensity is greater than would be expected from the average speed because large amounts of energy are expended in overcoming the body's inertia during take-off and landing.

The warmup for a show jumping competition involves a moderate exercise intensity at an average heart rate of 96 beats per minute (beats/min) (Lekeux et al., 1991), but with peak values as high as 173 beats/min (Barrey and Valette, 1993). The heart rate peaks each time the horse jumps a practice fence, and a direct correlation seems to exist between heart rate and fence height, which may be related to a faster speed of approach to the larger fences (Barrey and Valette, 1993). When the horse waits at the in-gate, the heart rate is in the range of 71 to 93 beats/min. During a competitive round, the heart rate rises steadily as the horse progresses around the course (Art et al., 1990; Lekeux et al., 1991), as shown in Figure 25-3, reaching peak values as high as 205 beats/min. The fact that the heart rates are so high confirms that show jumping is a strenuous sport despite the relatively slow average speed. It has been suggested that the energy expended by a show jumper is equivalent to that expended during galloping the same distance at a speed of 600 m/min (22 miles per hour [mph]) (Lekeux et al., 1991).

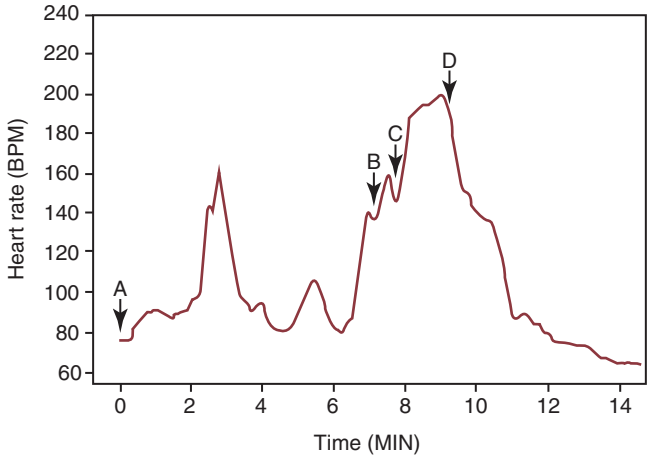

FIGURE 25-3 Heart rate of a horse from the start of the warmup until after the warmdown following a competitive jumping round. **A,** Start of warmup. **B,** Horse enters course. **C,** Start of the round. **D,** End of the round and start of recovery. *(From Art T, Amory H, Desmecht D, et al: Effect of show jumping on heart rate, blood lactate and other biochemical values,* Equine Vet J. *9(Suppl):78, 1990, with permission.)*

The onset of blood lactate accumulation occurs at heart rates in excess of 150 to 160 beats/min (Persson, 1983), so it is not surprising that show jumping is associated with a marked elevation in blood lactate (Art et al., 1990; Covalesky et al., 1992; Lekeux et al., 1991). Significant alterations also have been recorded in the packed cell volume, which rises to as high as 60% from a resting value of around 35%, and the red blood cell count, which has been shown to increase to 9.49×10^6 from a resting value of 5.8×10^6 because of the ejection of red blood cells from the spleen (Barrey and Valette, 1993).

A rise in plasma cortisol from a resting value of 67.6 millimoles per liter (mmol/L) to 118.7 mmol/L has been found after a show-jumping competition (Lekeux et al., 1991). Cortisol levels reflect adrenal activity and physiologic stress, and during exercise, the extent of the rise is related to the duration of the workout, the intensity of the workout, or both. The cortisol increase in show jumpers is less than in Thoroughbred racing (higher intensity exercise) or Endurance racing (longer duration exercise). During competitions, experienced jumpers have significantly smaller increases in plasma cortisol than inexperienced jumpers, suggesting that horses become conditioned to the psychological stress of the show environment (Clayton, 1989).

Show jumping is associated with reductions in plasma calcium (Ca^{2+}), chloride (Cl^-), and bicarbonate (HCO_3–), an increase in sodium (Na^+), and no change in potassium (K^+) concentrations (Art et al., 1990; Clayton, 1989). Total plasma protein increases, and this finding, together with the increased plasma Na^+, is indicative of a reduction in plasma volume because of a net movement of fluid out of the extracellular compartment. Metabolic acidosis occurs because of the increased HCO_3–, and this is associated with the substantial increase in lactic acid. Marked increases in the activities of lactate dehydrogenase, creatinine kinase, and aspartate aminotransferase have been found, but not in gamma glutamyl transferase following jumping competition (Art et al., 1990; Clayton, 1989).

Overall, the physiologic response to show jumping suggests that the relatively slow average speed belies the intense effort required to jump fences every 5 seconds or so. Anaerobic metabolism makes a significant contribution to the energy supply, as shown by the accumulation of lactate.

PREDICTABILITY OF JUMPING PERFORMANCE AND THE EFFECT OF EARLY TRAINING ON JUMPING ABILITY

In show jumping (and dressage), horses reach their peak performance at approximately 10 to 16 years of age. This means that unlike in racing, the time lapse between birth and the final evaluation of the athletic capacity of the animal is very long and that substantial investment has to be made before return on it, if any, can be expected. In a long-term (5 year) study, Santamaría and coworkers (2002; 2004a; 2004b) investigated two possible cost-effective ways to raise show jumpers. The first question they asked was whether good predictors for future jumping ability could possibly be found through the analysis of jumping technique at foal age, which would make earlier selection possible. The second question was whether jumping performance could be improved by starting training much earlier than the usual age of 3 years, thus taking better profit from the time before the horse is broken.

To answer these questions a cohort of 40 Warmblood foals was selected after weaning at age 6 months and divided into two groups. One group was raised in the classic way (free paddock exercise or group housing in open front stalls, depending on the season) until they were broken at age 3 years; the other group was subjected to a training program consisting of specific jumping training two times a week combined with a more general exercise regimen to ensure physical fitness for the jump training. The foals in this group started training at age 6 months and continued until age 3 years when they were joined with the other group to be broken and to receive a common exercise program to prepare for work under the saddle. After a 6-month rest period, 30 of the now 4-year-olds (15 from each group, the 5 worst horses from each group had been culled because of constraints of economy) were trained in a single group as show jumpers for an entire year. Kinematic examinations using an infra-red–based automated gait analysis system (ProReflex®, Qualisys AB, Gothenburg, Sweden) were performed at ages 6 months (Figure 25-4), 4 years, and 5 years while free jumping and at 5 years also when ridden. As an outcome parameter for jumping performance, a puissance competition was held at the end of the experiment during which horses had to jump fences up to 1.50 m; in the preceding year, they had been trained using obstacles with a maximum height of 1.20 m. At the end of the puissance competition, performance was characterized as good (faultless), moderate (able to finish the contest but with faults, refusals, or both) and bad (unable to finish the competition) (Figure 25-5).

The jumping technique appeared to be quite consistent from foal to adult horse (Santamaría et al., 2002; 2004a). When relating the kinematic variables that were consistent over time to the outcome of the puissance competition as the main parameter for performance, it appeared that elbow flexion, retraction angle of the hind limbs, and inclination of the trunk with respect to the horizontal were among the variables that were related to performance at age 5 years and could be identified as such already in the 6-month-old foals (Santamaría et al., 2002; 2004a; 2004b). It is, therefore, possible to use some of the characteristics of the jumping technique exhibited by foals when they are free jumping as predictors for future

FIGURE 25-4 Six-month-old foal equipped with reflective markers jumping a fence.

FIGURE 25-5 Research has shown that what is common practice nowadays at certain foal actions, judging the animals free jumping, indeed, makes sense. Although it is improbable that future top performers can be scouted this way, certain aspects of jumping technique seem to be innate and hence can be used to give an indication of the animal's jumping potential at adult age. *(Photo courtesy Jacob Melissen.)*

athletic performance. This being said, it should be stressed that many more factors determine the eventual success of a show jumper than jumping technique alone. The kinematic characteristics that were identified may, therefore, be of more help in developing an effective culling strategy than in picking out the future top performer.

Early training resulted in a less variable and, above all, more efficient jumping technique in 4-year-olds (Santamaría et al., 2004b, 2005). However, when reassessing the effects after a year of common and similar training, no significant difference remained between the two former training groups. Also, there was no relation of early training with performance, as measured by the outcome of the puissance competition. It was concluded that specific training for jumping at foal age, at least when carried out according to the relatively mild protocol as used in this study, had no measurable effect in the long term. It was realized, however, that early training had a temporary effect on naïve horses, which made them jump more efficiently and, thus, seemingly better compared with untrained horses. Early jump training might, therefore, cause bias in the selection events of many studbooks that nowadays include free jumping sessions for would-be breeding stock at ages 3 or 4 years (Santamaría et al., 2006). Further, the lack of effect of specific training for jumping does not take away the well-documented beneficial effect of early exercise on the general development of the equine musculoskeletal system (van Weeren 2007; van Weeren et al., 2000).

TRAINING ON THE FLAT

Course designers rely heavily on technical challenges to sort out a field of high-class show jumpers, rather than depending on the size of the fences alone. Consequently, show jumpers must be well schooled on the flat surface so that the horse is rideable and obedient between fences and is able to cope with the adjustments in stride length and the tight turns that are required in competition. The well-trained show jumper turns, moves laterally, and adjusts the stride length immediately in response to the rider's aids. Practice of these skills improves the horse's motor control and teaches it to move efficiently and economically, thus saving energy and delaying the onset of fatigue from lactate accumulation.

Many systems of training exist. Through knowledge of and familiarity with these different systems, the versatile trainer is able to select the appropriate exercises for training a particular horse-and-rider combination, taking into account the physical and mental talents and deficiencies of both individuals. A detailed description of one training system would be inappropriate in a book of this nature; rather, the goals and objectives of the training program will be considered, leaving the specific methods of accomplishing the objectives to the discretion of the trainer.

In classic dressage training, the horse works through the six steps of *rhythm*, *suppleness*, *contact*, *straightness*, *impulsion*, and *collection*, and this progression is also applicable to flat surface training for show jumpers. The horse should move in a round frame supple in the jaw with direct flexion at the poll and the vertebral column flexed. When the horse works in this frame, it develops the muscles along the top line, allows engagement of the hindquarters, and facilitates the development of collection. Lateral bending of the vertebral column and lateral flexion at the poll are equally important. The horse is taught to yield laterally both to the rein (shoulder yielding) and to the leg (leg yielding), which improves rideability and adjustability between fences. The more classic exercises of shoulder-in, shoulder-out, haunches-in (*travers*), and haunches-out (*renvers*) are particularly beneficial for lateral suppleness. Turns and circles are performed in true flexion and in counterflexion to improve lateral suppleness and control.

From the start of the training program, the horse must learn to respond promptly and calmly to the aids to go forward, come back, and move laterally; instant obedience becomes increasingly important as the horse progresses through the levels of competition, and it is best to establish this type of discipline at an early stage. Frequent transitions help keep the horse's attention focused, and when they are performed correctly, transitions between and within the gaits engage the hindquarters. Consequently, the forehand becomes lighter, and this facilitates the rider's control.

The importance of being able to adjust the horse's stride length cannot be overemphasized; many of the problems on a course involve related distances between fences that require the ability to shorten or lengthen the stride to arrive at an appropriate take-off distance. Furthermore, because horses breathe in rhythm with the stride when cantering and galloping, the most energy-efficient way of changing speed is to lengthen and shorten the stride while maintaining a constant rhythm or stride rate. Each time the rhythm changes, extra energy is expended in breathing until the new rhythm is established.

The ability to turn quickly becomes increasingly important at the higher levels of competition. Turning skills are enhanced by teaching the horse to sit on the hindquarters during tight turns.

It takes a long time to develop the technical skills required to be competitive in high-level show jumping. The best results are achieved through a combination of schooling exercises, together with appropriate strength training and suppling to reduce the risk of breakdown. Adaptations in the ligaments and tendons occur very slowly, and the technical training should not be rushed lest musculoskeletal injuries are caused by inadequate conditioning.

TRAINING OVER FENCES

As in training on the flat surface, different systems of training over fences also exist. This section will give only an outline of the objectives and a reasonable order of progression, leaving the methods of achieving these objectives to the discretion of the experienced trainer. The best results are always obtained by modifying the exercises and the rate of progression in accordance with the individual horse's talents and deficiencies. Schooling over fences proceeds hand-in-glove with schooling on the flat surface, and the exercises used to improve jumping skills at any stage of training are limited by the horse's flatwork ability. Strength training exercises should be integrated into the program to strengthen appropriate muscle groups and reduce the risk of injury.

Training over fences aims to improve the horse's technical jumping skills, but the horse's inherent ability also plays an important role. In one study, a group of horses was classified as poor, intermediate, or good jumpers at the start of a 9-month training program. When the same horses were re-evaluated after completing the training program over fences, it was found that the classifications had not changed (Fabiani, 1973). In other words, inherent ability had more influence on the horse's final skill compared with training. This is in agreement with the outcome of the study on early training reported previously, where the effect of conditioning for jumping during the juvenile period was found to be temporary and not related to true inherent ability, as became apparent after all horses, conditioned and unconditioned, had undergone a similar training program (Santamaría et al., 2005; 2006).

The work over fences may include jumping on the lunge, free jumping in a jumping lane, and ridden exercises. At an early stage in the training program, the horse should learn to walk, trot, and canter over rails on the ground to improve coordination and develop a greater awareness of limb position. When the horse is able to trot rhythmically over five rails (1.2 to 1.5m [4 to 5ft] apart), it is an easy step to include a small cross-pole after the trot rails.

The progressive development of jumping skills involves negotiating single fences at a trot and later at a canter, jumping two fences at an easy related distance, and jumping small grids that incorporate bounce, one, two, and three stride distances. Ground lines may be used to encourage the horse to take off at a suitable distance from the base of the fence so that the horse has enough room to elevate the forelegs. Placing poles on the take-off and landing sides helps to ensure that the peak of the jumping arc is centered on the middle of the fence. The position of the placing poles relative to the base of the fence depends on the way the horse jumps and the problems that need to be corrected.

Gymnastic jumping grids are invaluable for teaching the horse to jump in good form and for correcting faults in technique. The profile of the fences (vertical, ramped oxer, square oxer, etc.) and the distances between them are adjusted in accordance with the training objectives. Ideally, the horse learns to adjust its stride length and to take off at an appropriate trajectory to clear the fence without overjumping it. After a lesson has been mastered technically, multiple repetitions can be used to build muscular strength in a highly sport-specific manner.

Fences set at related distances are used to teach the horse to adjust stride length. In the early stages, the horse is asked to add a stride in a line by shortening the stride length. Later, the horse is asked to take out a stride by increasing the stride length, taking care that the horse does not flatten the arc when jumping from a longer stride.

In the early stages of training, horses should jump frequently to facilitate the learning of technical skills and to promote musculoskeletal adaptations in response to the unique orientation of the stresses on limbs. Since the tissues adapt slowly over a period of many months, it is wise to restrict the jumping activities to small fences for at least 3 to 6 months. During this time, a variety of fence types can be introduced, including verticals and oxers, solid fences, narrow fences, and small ditches. The objective is for the horse to approach the fences straight, in a good rhythm, and with a confident but relaxed attitude. Over the fence, the horse should lower its head and neck, round its back, and raise the lower limbs as high as necessary to clear the jump in good form but without overjumping. The horse should move away from the fence with a relaxed, rhythmic stride, prepared to shorten or lengthen, as necessary.

As the training over fences progresses, new skills are introduced, including bending lines, jumping on an angle, and coping with difficult distances in combinations. The horse should master normal, short, and long strides in a two-stride distance and then in a one-stride distance. By maintaining the same approach speed, the horse is encouraged to adjust between the fences rather than changing the take-off or landing distances. Gradually, the size of the fences is increased, but progress is slow in accordance with the rate of musculoskeletal adaptation.

CONDITIONING THE SHOW JUMPER

The successful show jumper combines a unique blend of power, precision, and speed. The airborne time over each fence is only about half a second, and most of the time in the ring is spent cantering or galloping between fences. However, each jump involves brief periods of rapid energy expenditure at take-off and landing, which calls for powerful contractions in specific muscle groups. It has been calculated in 600-kg Warmblood horses jumping a 1.15 m fence that total generated energy was 2.5 joules per kilogram (J/kg) with a power output of 71 watts per kilogram (W/kg), or over 40,000 W for the entire horse (Bobbert and Santamaría, 2005). These intense muscular efforts are repeated every 5 seconds or so.

In designing a conditioning program for show jumpers, three areas are addressed: (1) cardiovascular fitness, (2) muscular strength, and (3) suppleness. Cardiovascular conditioning improves the aerobic capacity while maintaining sufficient anaerobic power for the energy-intensive aspects of the competition, strength training enhances muscular power in the appropriate muscle groups, and suppling exercises improve the horse's athleticism and reduce the risk of injury.

CARDIOVASCULAR CONDITIONING

Cardiovascular conditioning improves the ability of the cardiovascular, respiratory, and muscular systems to produce energy by the appropriate metabolic pathways for the sport. In show jumping, the proportions of the total energy requirement supplied by aerobic metabolism and anaerobic metabolism depend on the fitness of the horse, the size and number of fences, the length of the track, and the horse's speed on

course. A relatively long Derby course relies more heavily on aerobic metabolism compared with a speed competition over a short, twisting course, when the faster speed and shorter recovery time between fences place a greater demand on anaerobic metabolism.

In a horse that has recently started work under saddle, cardiovascular conditioning begins with a period of slow, long-distance work in which the frequency of exercise is every second day, the duration is short (10 to 15 minutes), and the exercise intensity is low (walking, trotting). An incremental increase in either the duration or intensity (speed) of the work is applied on a weekly basis. Gentle gradients are introduced in the later stages of the slow, long-distance program, with the horse working up, down, and across the slope.

The objective of the slow, long-distance phase is to get the horse fit enough to exercise for about 50 minutes average speed of 6 to 8 kilometers per hour (km/hr; 4 to 5 miles/hr), including 2- to 3-minute periods of cantering. It takes 6 to 12 months to complete the slow, long-distance phase, depending on the age, breed, and history of the horse. As a general rule, the younger the horse, the slower is the rate of progression. On completion of the slow, long-distance phase, there is a gradual transition to an interval training format, in which cardiovascular workouts are performed three times a week and progressively more of the conditioning is done at a canter because this is the gait used in competition.

Initially, two 2-minute canters are performed at a speed of 350 meters per minute (m/min), separated by a 2-minute rest at a walk. Progressive loading involves increasing the number of repetitions to three, after which the duration is raised to 3 minutes and then 4 minutes. An equal increase in the rest interval occurs to maintain a 1:1 work–rest ratio. The next step is to raise the speed of the canters to 375 m/min and then 400 m/min. At this stage, a workout consists of three 1-minute canters at 400 m/min separated by 4 minutes of walking. The heart rate during the workouts is in the range of 130 to 160 beats/min, and it should fall below 100 beats/min in the rest intervals.

Speed play is introduced into the periods of cantering at a fairly early stage to recruit the fast-twitch muscle fibers; the horse accelerates over a distance of 50 to 100 m and then decelerates to the previous cantering speed. Initially, two accelerations are included in a workout, and the number is increased by two per week until 10 short sprints are performed in each workout. During the sprints, the heart rate reaches 160 to 170 beats/min. The next step is to increase the distance of some of the sprints to 100 to 200 m. When the horse reaches this stage, it is ready to compete in novice competitions.

For horses moving up the competitive levels, the intensity of the speed play is increased by accelerating and decelerating more sharply, sprinting at faster speeds, or using a slight uphill gradient. The heart rate is maintained at 170 to 200 beats/min for periods of 20 to 60 seconds.

Show jumpers use a considerable amount of energy in overcoming inertia, and this is a major contributor to the anaerobic nature of the sport. The effects of inertia are felt every time the horse accelerates, decelerates, or turns. Taking off and landing over a jump require an intense muscular effort and are particularly expensive energetically. Therefore, an important component of the cardiovascular conditioning program is inertial drills, which include acceleration sprints and turning drills (Clayton, 1991).

In an acceleration sprint, the horse accelerates from a standing start, maintains the speed over a short distance, and then slows to a walk. A work–rest ratio of 1:6 is used due to the high intensity of the workouts. In the rest intervals, the horse performs suppling exercises at a relaxed trot or a walk. Progressive loading is applied by increasing first the number, then the duration, and finally, the speed (intensity) of the acceleration sprints. The use of an uphill gradient is an effective method of increasing the intensity. Some horses get very excited by acceleration sprints, and if this is the case, it is preferable to use high-intensity speed play instead.

An example of an inertial drill for show jumpers is to have the horse accelerate over a distance of 50 m, decelerate, turn through 180 degrees, and then accelerate again. Using an IT (interval training) format, two to four accelerations, decelerations, and turns constitute one work, and this is followed by a rest interval in which the horse performs suppling exercises at a relaxed trot. A work–rest ratio of 1:6 is appropriate for this type of exercise. Gymnastic jumping also can be regarded as a sport-specific inertial drill because of the large energy expenditure in overcoming inertia at each take-off and landing.

STRENGTH TRAINING

Jumping is a highly specialized activity. Strength training for show jumpers is aimed at developing explosive power in the muscles that provide the force needed to elevate the horse's body mass into the air at take-off. Elevation of the center of gravity over the fences is minimal over fences less than 1 m high, but muscular strength becomes progressively important over larger fences. Strength training should mimic the range and speed of joint motion used in the sport. Each time the horse takes off over a fence, the stifle and hock joints flex deeply and then extend powerfully to project the horse's body into the air. Strength training exercises that use a similar motion pattern include gymnastic jumping and bounding up steep gradients.

Strength training is introduced after completion of the slow, long-distance phase and is performed two or three times a week, which balances the need for sufficient muscular stimulation with enough rest. A strength-training workout causes some minor (microscopic) tissue damage, which is repaired on the days between workouts. If the exercises are repeated too frequently, damage accumulates, predisposing to injury or breakdown. In the annual conditioning cycle, the ideal time to improve strength is during the off-season from competition.

Over a period of 6 months, it is possible to increase the strength in specific muscle groups by as much as 50% to 100%, which will produce a measurable improvement in performance. During the competitive season, if the horse has a heavy competition schedule, the gains in strength will be maintained without the need for specific strength-training workouts. In horses that are competing lightly, a single strength-training workout each week is sufficient for maintenance.

It is important to ensure that the horse works in good form throughout the strength-training exercises so that the appropriate muscle groups are trained. As fatigue develops, the horse compensates by using different muscles, and the exercise

is then counterproductive because the wrong muscles are strengthened. The trainer must be alert to this possibility, and either correct the horse's technique or stop the exercise as soon as compensatory movements occur. In contrast to the situation in human athletes, strength training is not continued to the point of exhaustion in horses because of the risk of injury. Instead, the workout is terminated when signs of muscular fatigue are evident.

Gradients

Bounding up a steep gradient is useful for strength training in jumpers; the two hindlimbs are pulled forward beneath the trunk and then are extended forcefully as they push off against the ground in a movement that mimics take-off. Since the joints of the hindlimbs move through a wide range of motion, this exercise also benefits suppleness. The several bounds taken by the horse in succession prepare its muscles for the repeated take-offs in a combination. The IT format, in which bounding up the gradient is the work and descending at a walk is the rest interval, is used. A work–rest ratio of 1:6 is appropriate.

Gymnastic Jumping

Gymnastic jumping is a highly sport-specific strength-training method for show jumpers. By adjusting the height and width of the fences and the distances between them, the trainer can improve the horse's mental as well as physical agility and its muscular strength. Gymnastic jumping strengthens the muscles responsible for snapping up the forelegs after take-off, enabling the horse to raise its knees higher and faster.

The key criteria for using gymnastic jumping as a strength-training tool are: (1) the horse be familiar with the technical skills; and (2) sufficient repetitions must be performed to stimulate muscular adaptation. For strength training, the IT format, with a work/rest ratio of 1:6, is used. Jumping through the grid is the work, and returning to the start of the grid at a trot or walk is the rest interval. Suppling exercises are performed in the rest intervals. Progressive loading is accomplished by a weekly increase in the size or number of fences or in the number of repetitions performed. The use of a series of small fences (60 to 90 cm high) leading to two or three large fences at the end of the grid is effective for the preparation of muscles for jumping through combinations, and it emphasizes the development of explosive muscular power in the hindquarters.

When the horse jumps regularly, its bones, ligaments, and tendons are strengthened in a highly sport-specific manner, which cannot be achieved by other types of exercise. Even experienced jumpers should be schooled over fences at least once a week to maintain the strength of these tissues.

SUPPLING EXERCISES

Suppleness is important for enabling the horse to jump in good form, for maximizing the horse's athletic ability, and for minimizing the risk of injury. A greater range of joint motion benefits the equine athlete by providing more shock absorption when the leg is on the ground, thereby reducing the incidence of injuries. It also allows the horse to apply forces against the ground over longer periods, producing increased velocities and accelerations. A limited range of motion is associated with an inferior ability to generate momentum and absorb the impact forces.

Some aspects of suppleness are specific to jumping sports. When the horse bascules over a jump by rounding (flexing) the neck and back, it lowers the position of its center of gravity within the trunk, and this reduces the muscular effort required to clear the fence. Movements originating at the base of the neck are important in jumpers because the horse's head and neck are used to adjust its balance and to change the location of the center of gravity. Therefore, suppleness of the neck and back should receive continual attention throughout the horse's career. Other parts of the body in which suppleness is particularly important in show jumpers include the shoulder region, which determines the ability to elevate the lower limb. In the hindlimbs, the lumbosacral and hip joints are important because they are responsible for elevating the hindquarters over the apex of the fence.

Suppling exercises are performed daily. They increase the range of joint motion by reducing tension and resistance in muscles or connective tissues (tendons, ligaments, joint capsules), and regular suppling progressively increases the range of motion of a joint or set of joints. A period of 3 months produces significant improvements in flexibility, but suppling exercises should continue to be a part of the daily routine throughout the horse's athletic career. The amount of tissue lengthening that persists after cessation of the suppling exercise depends on the force used to stretch the tissues and the duration over which the force is applied (Stubbs and Clayton, 2008). High-force, short-duration stretching at low temperatures favors elastic deformation. This is a temporary change that is reversed when the force is removed. Permanent lengthening of the ligaments and tendons is maximized when a low force is applied for a longer duration to tissues that are warm. Cold tissues are also more brittle and susceptible to tearing, so the horse should be thoroughly warmed up by a period of active forward movement before starting the suppling exercises.

Suppling exercises are classified according to whether the stretching force is passive or dynamic in nature, and both types are useful and beneficial in jumping horses. Integration of the suppling exercises into the daily routine is accomplished by starting the workout with a forward-moving warmup to increase the temperature of the tissues, after which dynamic suppling exercises become an integral part of the warmup, the workout, and the warmdown. Passive suppling is performed after exercise each day while the horse's tissues are still warm.

Dynamic Suppling

Dynamic suppling involves rapid rotation of a joint through its range of motion resulting from muscular contraction or weight bearing. Examples of dynamic suppling exercises that are a part of the normal schooling routine include turns, circles, voltes, and lateral movements (leg yielding, shoulder-in or shoulder-out, haunches-in or haunches-out, half-pass). The beneficial effects of this type of exercise arise from the scapulae sliding across the chest wall, the hindlimbs swinging through a wide arc of motion, and the vertebral column undergoing flexion, bending, and rotation. Other exercises that have a dynamic suppling effect include walking and trotting over raised rails, gymnastic jumping, and bounding up steep gradients. All these exercises are associated with

active flexion and extension of the joints through a wide range of motion.

Since the shoulder movements responsible for snapping up the horse's knees at take-off are brought about by active muscular contractions, gymnastic jumping has a highly sport-specific effect in terms of both dynamic suppling and strength training. For the hindlimbs, gymnastic jumping and bounding up steep gradients fulfill the dual objectives of improving strength while enhancing suppleness in the lumbosacral and hip joints. The suppling effect comes from working the joints through a wide range of motion; the hindlimbs are pulled forward beneath the body at impact, which flexes the lumbosacral and hip joints, and then are extended fully as the horse pushes off against the ground.

Passive Suppling

Passive suppling involves a slow, controlled movement of a joint to the limit of its range of motion through the application of an external force. Because the force is applied slowly, stimulation of a reflex muscular contraction that would oppose the stretch is avoided. When the limit of movement in a particular direction is reached, the stretched position is held for 20 seconds to enhance permanent elongation of the ligaments, tendons, and joint capsules. Passive suppling is used to bring about long-term increases in the range of motion in the neck, shoulders, and hips, to promote relaxation, and to reduce postexercise muscular soreness. As with all types of suppling exercises, the tissues should be warmed up before passive suppling is performed. This is best accomplished by a period of exercise under saddle or on the lunge, which means that it is more appropriate to perform passive suppling during or after, rather than before, a workout.

In show jumpers, the ranges of motion in the shoulder region and the hip joints are maximized by stretching them in all directions using a series of passive suppling exercises which have been described in detail elsewhere (Stubbs and Clayton, 2008). The horse should be in a quiet environment and standing squarely before starting. It is safer if the horse is held by an assistant rather than being tied up, and the person performing the stretches should have plenty of room to maneuver around all sides of the horse.

The front leg is pulled forward and upward to stretch the elbow and shoulder, keeping the knee slightly bent to relieve tension in the flexor tendons and suspensory ligament. In turn, the leg is moved backward, medially, and laterally, with one hand applying pressure above the horse's knee. Each stretched position is held for 20 seconds.

In the hindlimb, passive suppling concentrates on the hip joint. The hindlimb is pulled forward with the stifle and hock flexed to about 90 degrees, the tibia vertical, and the cannon bone horizontal. By applying upward pressure from below the hock, the tibia and the stifle are raised, and the hip joint is flexed. When the leg is stretched backward, the hock is flexed to 90 degrees, with the tibia horizontal and the cannon bone vertical. Gentle pressure is applied to the front of the stifle, pulling the femur back to extend the hip joint. In the medial stretch, the stifle and the hock are moderately extended so that the hock of the stretched leg moves across in front of and slightly above the opposite hock. For the lateral stretches, the whole leg is moved laterally.

Other suppling exercises that are not strictly passive in nature because the horse is responsible for the movement involve feeding a tidbit in different positions such as between the front legs to flex the neck and at the flank to bend the neck laterally.

OTHER CONSIDERATIONS

Show jumpers that are not completely sound pose a conditioning problem. Usually, these are older horses with chronic injuries to the flexor tendons or suspensory or check ligaments or mild osteoarthritis. These horses have sufficient experience that they do not need to jump frequently to improve their technical skills, but unless they jump at least once a week, the strength of the musculoskeletal tissues is compromised. If the horse is saved for big competitions and then rested between shows, the tendons and ligaments lose strength and become vulnerable to injury. The best insurance against injury is to perform adequate strengthening exercises early in the horse's career and to maintain the strength by jumping small fences once or twice a week during the off-season. Shortcuts in strength training for jumpers may lead to strains of the suspensory ligament, check ligament, or superficial flexor tendon. If a layoff is unavoidable, adequate time must be allowed during the reconditioning period for the support tissues to regain strength. These tissues adapt very slowly, but unless sufficient time is allowed, the risk of breakdown when full work is resumed is increased.

The food intake of show jumpers should be restricted to a maximum of 1.5% of body weight daily to reduce the weight of water retained in the intestine by large amounts of fibrous food (Meyer, 1987). Concentrates are fed as needed to maintain condition and performance. When horses sweat copiously or frequently, electrolytes are added to the feed or water to ensure adequate replenishment. Plain water must be freely available so that excess electrolytes can be excreted in urine. A good electrolyte mixture is three parts sodium chloride (common salt) to one part potassium chloride. For show jumpers, this mixture is fed at a rate of 1 to 2 tablespoons daily.

When the horse is at a show, the stable routine is adjusted as necessary to ensure that the horse is produced in top form. Specific considerations include ensuring adequate rest, providing water throughout the day, and adjusting the diet to allow for traveling and different levels of exercise. When the horse is out of the stall (warming up, waiting to compete, competing), stress is reduced by having drinking water available as required and by taking measures to warm or cool the horse according to the weather. When the weather is cool, blankets are used to conserve body heat, and the horse is kept moving to prevent chilling. When it is hot, heat buildup is reduced by standing the horse in the shade and using cool water to sponge the horse down between rounds. Since show jumpers accumulate fairly large amounts of lactate during a round, they should be warmed down with a few minutes of easy exercise after leaving the ring. This hastens removal of lactate from muscles, allows a gradual redistribution of blood flow away from the working muscles, and reduces postexercise muscle soreness. Massage and passive stretching are also beneficial for reducing muscle soreness after strenuous exercise.

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CHAPTER

Training Working Horses

26

BRIAN HAMPSON

PROFILE OF THE WORKING HORSE

For the context of this chapter, the working horse will be considered the performance horse bred and trained for the specific events of reining and cutting. *Reining* involves the horse and rider performing a specific pattern of movements, including galloping in small circles, flying lead changes, rapid spins of the forelimbs around a stationary hind limb, rapid accelerations followed by equally rapid decelerations, and sliding stops. *Cutting* requires that the horse, with little assistance from the rider, separates a steer from a herd and attempts to prevent the steer from returning to the herd. This requires a series of high-speed pursuits in time with the steer's movements, with abrupt stops and turns, as well as an ability of the horse to predict stock movement and behavior (Figure 26-1).

The principles of training, biomechanics, and injury prevention discussed in this chapter may also apply to other disciplines requiring the equine athlete to possess the skills and abilities for quick acceleration and fast stopping, as well as extreme agility. Such disciplines include rodeo and other Western events, Australian Stockhorse competition, some pony club activities, and campdrafting, to name a few. Equine athletes competing in such disciplines are required to reach peak speed from a standing start within 10 meters (m), stop rapidly from peak speed, turn 180 degrees, and gallop in the other direction within a few seconds. Performance of these demanding tasks is akin to the combined requirements of the human gymnast and sprinter and demand strength, speed, fast reaction time, and a high level of motor skill acquisition from the equine athlete.

As for the majority of equine athletic disciplines, the working horse is specifically bred over many generations to refine the ideal genetic characteristics suitable for the performance of tasks. The American Quarterhorse and, more recently, the Australian Stockhorse, are the main contributors to the specific genetic pool from which the working horse is drawn. The most successful working horses are typically of medium height (1450–1550 cm at the wither) (Scott, 2008). Quarterhorses possesses a high percentage (51%) of fast-twitch muscle fibers (Snow and Guy, 1980), suggesting that they have been selected for speed and power over a short distance. Conformation, particularly with respect to the spine, pelvis, hip, and tarsus, is an important selection factor, as the musculoskeletal injury rates in these regions are high (see summary of the working horse profile Table 26-1).

SPECIFIC PHYSIOLOGICAL REQUIREMENTS OF TRAINING AND COMPETITION

The most prestigious working horse events are the *futurities*, for horses age 3 to 4 years. Working horses are selected for early maturation, trainability, and a workable nature so that they are able to begin training under saddle at the age of 18 to 24 months. This allows the reining horse age 2 to 2.5 years to develop the required skills and maneuvers of the reining pattern, and the cutting horse has this time to build athleticism and learn to anticipate and control movements of cattle with little aid from the rider.

The average duration of the working horse event is 2.5 to 3 minutes. Working horse competition represents intense, near maximal workload. The competition phase in combination with the preceding warmup results in mild lactate accumulation (mean plasma lactate levels 5.1 ± 1.9 millimoles per liter [mmol/L]) (Kastner et al., 1999), indicating that energy from anaerobic sources is being utilized. The reining competition covers a distance of approximately 680 m (mean average velocity 3.9 meters per second [m/s]) (Figure 26-2), and athletes reach heart rates of 181 ± 13 beats per minute (beats/min) (Kastner et al., 1999). At the end of the competition phase, horses are normally sweating and tachypnoeic, but recovery is rapid.

The training regime of the working horse is designed to stimulate aerobic and anaerobic energy pathway adaptations in preparation for the anaerobic nature of the competition's requirements. Fast power training, with some emphasis on aerobic conditioning in addition to the learning and practice of motor skills is indicated. Training periods of 30 minutes duration, 6 days per week, are typical throughout the industry. The author of this chapter has performed monitoring of professional working horse training sessions by using the global positioning system (GPS) and has shown a mean training distance per session of 2.06 km (range 1.8–2.3 kilometers [km]). This training distance appears to be below expectations. This confirms the low significance placed on the aerobic requirement for the activities of reining and cutting but possibly also reflects the professional nature of the sport. Professional working horse trainers work with a high throughput of horses and typically train 10 to 15 horses per day. The neglect of a specific aerobic conditioning program within the training program may also represent training error; however, currently no research supporting the need for increased aerobic specific training for working horses exists. Training sessions are intense in terms of anaerobic contribution but also require the total commitment or concentration of the horse to perform difficult motor tasks that are often not in the usual repertoire of their natural motor skills.

MUSCULOSKELETAL REQUIREMENTS

Although the physiologic requirements of training and competition are only moderately intense in comparison with

FIGURE 26-1 The cutting horse in action. *(Copyright One Stylish Pepto Syndicate. Photo by Glenn Mandl.)*

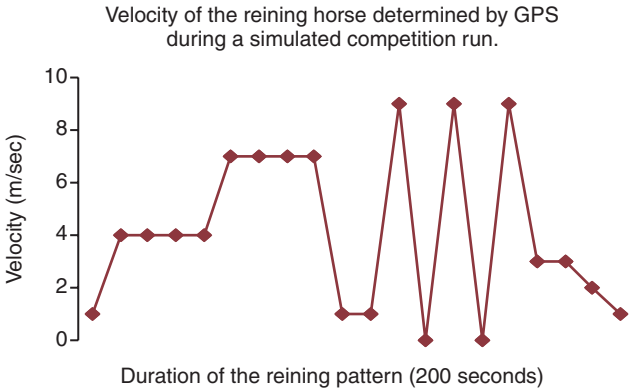

FIGURE 26-2 Velocity profile of the reining horse competition pattern derived from global positioning system (GPS) monitoring at 10-second intervals. An initial walk into the competition arena is followed by a trot phase, a series of circles with flying lead changes at the canter, and a stop and pause in the center of the pattern. This is followed by a series of short gallops to sliding stops and a short trot and walk off on completion.

TABLE 26–1
Summary of the Working Horse Profile

Breed	Quarterhorse/Australian Stockhorse
Age at starting training	18–24 months
Age at commencement of competition	3 years, rising 4 years
Muscle fiber type	51% fast twitch (Snow and Guy, 1980)
Conformation requirements	1450–1550 centimeters (cm) tall, sound, robust
Type of event	Near maximal, anaerobic (Kastner et al., 1999)
Duration of event	2.5–3 minutes
Distance of event	680 meters (Kastner et al., 1999)
Average velocity of event	3.9 meters per second (m/s) (Kastner et al., 1999)
Max heart rate of competition	181 beats per minute (beats/min) (Kastner et al., 1999)
Cardiovascular load	Moderate
Musculoskeletal load	High to extreme
Motor skill acquisition load	Extreme
Lateral preference (left/right handedness)	Bilateral, no preference
Injury rate/wastage	High
Industry standard training program	30 minutes, 6 times per week
Distance travelled in industry standard training session	2.06 kilometers (km)

some other disciplines, the load on the musculoskeletal system is excessive. This is particularly so with regard to the immature age of the equine athletes subjected to such a rigorous training schedule. Because of limited preparation time and the necessity of the young horses to perform such complex motor patterns, the training schedule has limited allowance for recovery and physiologic adaptation. The working horse trains 6 days per week on most weeks from ages 18 to 24 months to competition at age 3 years. Figure 26-1 shows the extremely demanding musculoskeletal requirements of the cutting horse event. It is helpful at this stage to briefly analyze two of the important motor tasks performed by the working horse to understand the specific injury profile of the horse and the training requirements for good performance.

Stop and Roll Back

The position depicted in Figure 26-1 is known as "getting into the ground" and is the pinnacle sign of the well-bred and well-trained cutting horse. The horse has just made a 10-m sprint across the deep sand arena shadowing the movements of a steer. The horse has rapidly come to a complete stop and is about to roll back to the left over its hocks in a 180-degree turn to sprint off in the opposite direction. The left hindlimb is the pivot limb for this turn and will support the majority of the horse's (and rider's) weight. Once the turn is initiated, the horse's forelimbs will be totally unloaded and its front end will sweep around the planted left hindlimb and will not recontact the ground again until the 180-degree turn is completed.

It is worthwhile to look at this position in general terms. This horse is 1475 centimeters (cm) tall (14'3 hands[hh]) but has its thorax and chest inches from the ground. The rider's boot heel is at ground level. The left hind foot is more cranial to the stirrup, and both hips are abducted, allowing a very wide crouched stance. The hocks are just above ground level. Both forelimbs, particularly the left forelimb, are protracted, and the left forelimb is moving into abduction. The hips are maximally flexed, as is the lumbosacral spine.

As the horse transfers its weight caudally over the hindlimbs, the weight of the horse and the weight of the rider are borne by the gluteal, tensor fascia latae, biceps femoris, sacrocaudalis dorsalis, sartorius, and adductor muscles of the horse, as the muscles stabilize the pelvis and hip, with the

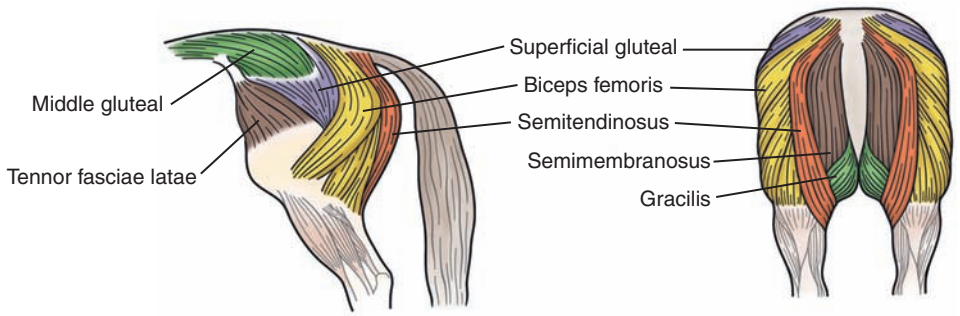

FIGURE 26-3 Pelvic stabilizer muscles of the horse. *(Reprinted with permission, Narelle CS, Hilary C: Activate your horse's core: unmounted exercises for dynamic mobility, strength and balance,* Sport Horse Publications *2008.)*

powerful biceps femoris, semitendinosus, and semimembranosus (hamstrings) muscles stabilizing the hip, stifle, and hock (see Figure 26-1; Figure 26-3). The gracilis is a large muscle in the working horse and is particularly important in breaking and exerting power in the crouched abducted position of the hips. The progression from eccentric (lengthening) to concentric (shortening) muscle contraction during this maneuver is very rapid and is the danger period for muscle tearing to occur. The hamstring muscles are particularly vulnerable during this period and are commonly injured in the working horse (Green et al., 2008). Once the weight of horse is accepted by the hindlimbs, the unloaded cranial skeleton begins to laterally flex, and the horse's front end sweeps through a 180-degree rollback. Once the center of mass passes the line of the hip during the rollback, the pelvic and caudal limb prime mover muscles, in particular the gluteal and hamstring muscles, shift from an eccentric stabilizing role to a propulsive function, as the horse begins to accelerate out of the stop and turn in the opposite direction. This is a very demanding maneuver requiring great strength as well as agility. The role of the core stabilizing muscles to enhance dynamic stability, particularly in the lumbosacral spine and pelvis, during this type of maneuver should not be underestimated (see Figure 26-3) (Stubbs and Clayton, 2008).

The classic sliding stop of the reining horse is depicted in Figure 26-4. One major difference between this stop and the stop executed by the cutting horse (see Figure 26-1) is that with the reining horse, the stop is prolonged and, due to the application of sliding plate horseshoes (Figure 26-5), becomes a slide over the surface progressing to a stop. This stop is an isolated maneuver, and the horse is allowed to recover to a more neutral anatomic position before commencing the next maneuver. In contrast, the cutting horse is forced to stop rapidly and turn 180 degrees immediately in time with the steer it is attempting to block. Musculoskeletal tissues of the cutting horse are, therefore, forced to accept high loads over a shorter period, increasing the impulse of the potentially injurious loads. The reining sliding stop is a caudal limb–loading maneuver. The hips are flexed and abducted so that the hindfeet are planted wide to the forefeet, allowing the unloaded forelimbs to continue to cycle as the slide progresses for well over 10 m. Spinal flexion is a feature of the reining stop with a high magnitude of flexion at the cervical spine and the caudal thoracic and lumbar spine and a large emphasis on flexion at the lumbosacral junction (Goff and Stubbs, 2007; Rumens et al., 2007). High loading over an extended period (as the slide is prolonged) is potentially injurious to the lumbosacral region as well as the sacroiliac joint in the immature horse (Rumens et al., 2007). Morphologic variations such as fused transverse or dorsal spinous processes of vertebrae or sacralized sixth lumbar vertebrae (Stubbs et al. 2006) will limit range of motion and may exaggerate forces transmitted to the lumbosacral and sacroiliac regions under these conditions, wherein the spine is being stressed to its physiologic limit.

FIGURE 26-4 The sliding stop of a reining horse.

Motor Skill Acquisition

Many of the maneuvers performed by working horses are extreme in comparison with the normal movement patterns of the horse. The working horse has been selectively bred and trained to perform such movements as the exaggerated spinal flexion and hind limb protraction seen in the sliding stop (see Figure 26-4) and the crouched "catlike" position of the cutting horse (see Figure 26-1) with its low center of gravity and unloaded forelimbs. The forelimbs of the working horse must necessarily be lightly weighted and highly mobile and assume a weight-bearing role in positions of extreme protraction and abduction not normally attributed to the abilities of the horse. Horses, in general, are designed to locomote in the sagittal plane (Payne et al., 2005), but the working horse has adapted, through selection and training, to use the small but significant

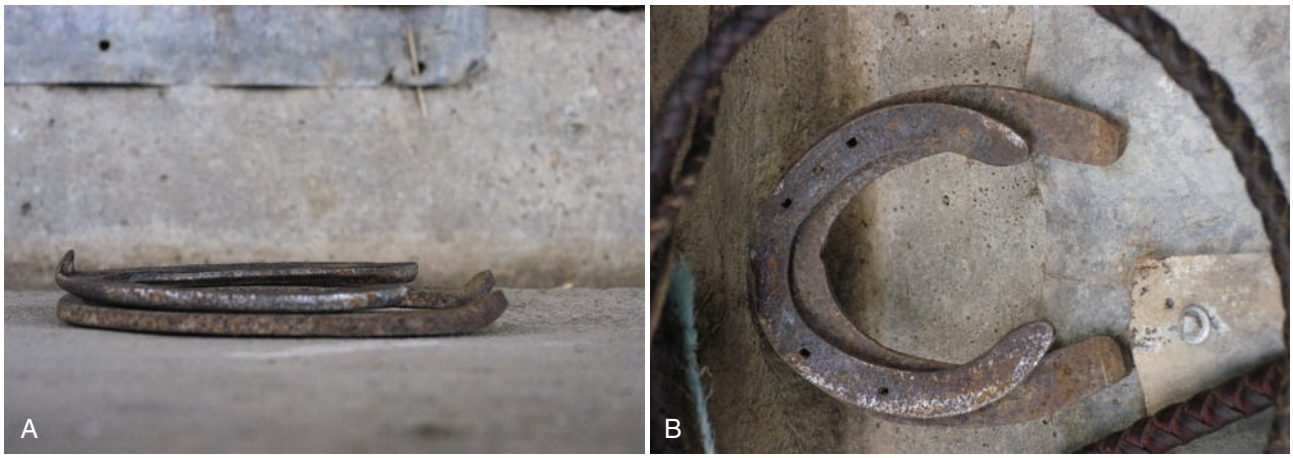

FIGURE 26-5 Lateral (*A*) and dorsal (*B*) views of the sliding plate of a reining horse designed to hold the back feet on top of the surface to prolong the stop and help the horse slide across the ground. The sliding shoes are wider and have caudal extensions as shown. The sliding shoes are fitted to the hindlimbs. The shoe for the forelimb is positioned on top of the sliding shoe in the images for comparison purposes.

degrees of freedom it has available in the frontal and transverse planes, which allows it to work effectively within a very small area. By using the movements of forelimb abduction and external rotation, for instance, the working horse is able to rapidly spin 360 degrees around a fixed-pivot hindlimb. Quarterhorses were found to be bilateral in forelimb preference compared with Thoroughbreds and Standardbreds, both of which have a lateral (left or right) preference (McGreevy and Thomson, 2006). This observation supports the effect of either breeding selection or training on the motor skill ability of the Quarterhorse. From age 18 months, the working horse is taught new skills and motor patterns that it would not have experienced in paddock life or in flat racing. These motor patterns are repeated several thousand times over the next 24 months until they are solidly entrenched in the horse's normal repertoire.

INJURY PROFILE OF THE WORKING HORSE

Quarterhorses, like Thoroughbreds and Standardbreds, are often selected at sale as yearlings and are made to compete at a high performance level as 3-year-olds. Horse breeders aim to present well-grown yearlings for sale and have been criticized for overfeeding young horses to meet the needs of the market (Castle et al., 2008). In a study of 1600 Thoroughbred yearlings (Castle et al., 2008), 26% had radiographic signs of osteochondrosis (OCD) in the limbs. Overfeeding has been implicated in this disease process (Castle et al., 2008; Foote et al., 2008). Scott (2008) found a high percentage of developmental orthopedic diseases in working Quarterhorses and implicated early maturation; selection for smaller, more agile (thus fragile) body types; and constant hard training from an early age (18 months) for the high wastage in the discipline. Because of the high musculoskeletal demands placed on the working horse at a young age, selection for conformation and musculoskeletal soundness (including past medical history) is imperative.

Two large studies (Dabareiner et al., 2005; Scott, 2008) reported the same list of common musculoskeletal injuries in working horses. These included palmar foot pain and osteoarthritis of the proximal interphalangeal joints, tarsal joints, and stifle, as well as pastern fracture, suspensory ligament desmitis, and back injuries. A third study (Fonseca et al., 2006), using diagnostic radiography, refined the diagnoses of back pain in cutting and reining horses as being predominantly midlumbar and lumbosacral supraspinous desmitis and myositis. It has been suggested (O'Grady, 2006; Scott, 2008) that incorrect trimming, shoeing, or a combination of both is often at fault when palmar foot pain is present, and the long toe–low heel foot conformation is common. The remaining plethora of injuries can be attributed to training faults such as excessive working in deep sand (common for working horse arenas) (Scott, 2008), overuse of immature soft and bony tissues, and lack of sufficient recovery time for tissue repair and adaptation. Strategies to address these issues will be discussed later in this chapter.

SPECIFIC TRAINING REGIME

A high attrition rate exists during the training of working horses. Part of this attrition is caused by exclusion of those horses deemed not good enough to make the futurity (selection), but many are lost to training because of injury. Training a working horse from age 18 months to go on and perform at an elite level at the end of its third year is both physically and mentally demanding. Horses that do make it are necessarily the most robust. It follows that the major goal of the training program is not just to "make" a performance horse but to assist the horse in maintaining musculoskeletal soundness during the rigors of the training program. This section is not concerned with the techniques used by trainers to train working horses. It will cover the major musculoskeletal training principles required to assist the horse in maintaining musculoskeletal soundness during the training period and becoming a sound competitor. As previously discussed, the cardiovascular load on the working horse during training and performance is not high, so a specific aerobic training program may not be critical. The anaerobic component of performance is demanding but is not a performance-limiting factor, and it is covered well in standard training regimes. Figure 26-6 shows a movement comparison of the reining horse in competition and

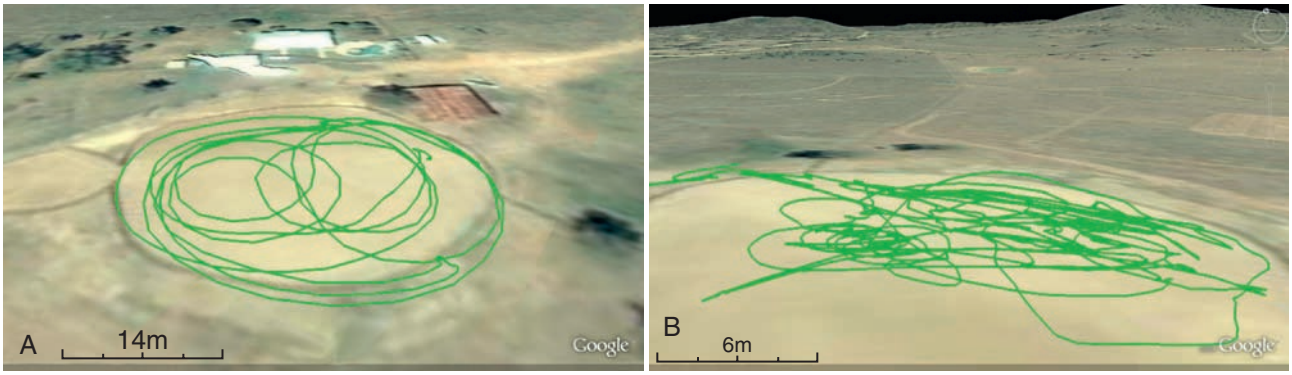

FIGURE 26-6 **A,** Global positioning system (GPS)–derived view of working horse movement during a practice reining pattern. Total distance 815 meters (m). The GPS logged data at 1-second intervals. The GPS track is displayed by integration with Google Earth Plus and displays the fluent nature of the reining pattern. **B,** Reining horse training pattern derived by GPS monitoring at 1-second intervals. Total distance is 2.3 kilometers (km). The training pattern displays the random nature of the work which is typically a series of small maneuvers linked by periods of trotting, cantering, or rest.

training. Although the competition phase depicts a smooth flowing series of mostly circular movements, the typical training period involves a combination of many varied movement patterns, with several periods of rest and relief for the horse.

Fortunately, the opportunity to do harm to the young working horse is reduced by the horse's inability to perform the types of maneuvers that make it most vulnerable. The young Thoroughbred, however, may be taken out on its first ride and galloped at maximal or near-maximal speed to fatigue, thus risking bone and soft tissue injury. The working horse is incapable of stopping hard, "getting in the ground," spinning, and rolling back at high speed, as it is incapable of performing these complex motor tasks. The opportunity, then, exists to prepare the musculoskeletal system of the young horse in parallel with its skill level to allow soft tissue and bone to develop at a rate which is acceptable in the context of the added stress and strain that the higher skill level causes.

The initial goal of the horse trainer is to get control of the front end of the horse. Likewise, the first goal of the trainer of the horse's musculoskeletal system is to get the horse to practice unaccustomed movements such as forelimb adduction and abduction (stepping across the inside limb and stepping out to the side) to begin to condition the prime mover muscles and gradually increase the range of motion of these movements. When the front end of the horse is being moved about and "freed up," the horse is necessarily transferring its weight to the lumbosacral spine and the hindlimbs. This weight shift is unusual for the horse, as it has spent the majority of its movement experience more heavily weighted on its front end. Thus, the caudal structures of the horse are progressively conditioned, while the front end of the horse is progressively trained to lighten up and move independently of the back end of the horse.

The epaxial muscles—namely, the multifidus, longissimus, and iliocostalis—that lie just above the transverse processes of the lumbar vertebrae are important in stabilizing and correctly aligning the spine (Stubbs, 2008), particularly in the hind end–loaded movements such as stopping, backing, rolling back, and lead departures. These muscles are susceptible to fatigue, leaving the spine unsupported and incorrectly coordinated with the propulsive forces of the limbs. Along with the abdominal and sublumbar muscles, the epaxial muscles must be progressively trained and conditioned to maintain their role throughout the full routine of training and performance. Figure 26-7 shows four simple exercises used to condition the weight-bearing structures of the hindlimbs, including the stifle and tarsal joints, to accept progressively larger loads. Combining movements such as backing and laterally flexing (backing in a circle) allows the core stabilizing muscles to become coordinated with the large trunk and limb prime movers, to resist fatigue, and to maintain correct motor coordination throughout the movement routine.

These exercises are progressed by increasing the amplitude of movement (larger degree of lateral bending; transferring more weight to the caudal spine and hindlimbs; deeper backing and stopping with increased degree of spinal flexion and hip, stifle, and tarsal flexion) and the speed at which they are performed. The key theme of training for any motor skill is *specificity*. Specificity includes practicing a required movement in a progressive manner to ultimately allow the movement to be practiced within the same range of motion, speed, and load as is required during actual performance (Young et al., 2001). The working horse will not be sufficiently skilled, strong, and prepared in terms of soft tissue and bone integrity to perform at the level required for competition performance until well into age 3 years and after 24 months of consistent progressive training. The trainer should be diligent in assessing the performance of the horse at each training level to ensure that the horse has developed the necessary skills and condition before progressing to the next level. This approach to training will better protect the horse from injury and make the progression through to more advanced skills more rewarding.

INJURY PREVENTION STRATEGIES

SELECTION OF THE HORSE FOR THE PURPOSE

Although yearlings that have been overfed for the purpose of sale appear to be mature and good prospects, their musculoskeletal composition may be imbalanced because of high growth rate and such diseases as OCD. Horses should be selected on the basis of conformation and soundness. Horses carrying the breeding of sound performance horses are more likely to withstand rigorous training and performance programs.

FIGURE 26-7 **A,** Laterally flexing in a forward circle. This exercise provides a combination of lateral spinal flexion with spinal flexion to stretch the horse. The young horse starts in a large circle, and the radius is reduced to progress the exercise. The exercise is further progressed by increasing the magnitude of both lateral flexion and flexion. Progressively increasing the speed of the exercise promotes motor skill learning closer to the specific speed required in competition. **B,** Circling forward and stepping across. Adding shoulder abduction (stepping into the circle) requires the horse stabilize the spine and pelvis as more weight is taken on the caudal structures. **C,** Backing with spinal flexion. This top line stretch is progressed by increasing cervical flexion. Lumbosacral flexion may be increased by backing the horse faster, thus increasing stride length. **D,** Backing in a circle. This is a progression of Figure 26-7, *C*. It combines the action of backing with lateral spinal flexion and abduction or adduction of the hips. As the exercise progresses, the forelimbs are fixed and the hindlimbs move around the pivot front end.

AVOIDANCE OF OVERFEEDING

Overfeeding is an issue which should be easily addressed but, in practice, is difficult to resolve. Generally, horse owners and horse caregivers like to keep horses fat, in the belief that a "fat" horse is a "well" horse. Human athletes do not carry excess body fat. The same applies to the equine athlete. The disadvantage to the equine athlete being overfed is a higher load on immature musculoskeletal structures, as well as accelerated growth and risk of nutrition-related diseases such as OCD. Particularly in the first 12 months of training, the weight of the horse and the weight of the rider should be kept at moderate levels. The consequent loading on peripheral joints by each kilogram of excess bodyweight of the horse and rider weight can be substantial.

FOOT CARE

Foot care is important in any equine discipline. The long toe–low heel foot conformation reported in the working horse (Scott, 2008) is not unique to this discipline and is common in Thoroughbred racehorses suffering catastrophic injury (Kane et al., 1998). Palmar foot pain is the most significant foot problem of the working horse (Scott, 2008). The long toe–low heel foot conformation is the most common cause of palmar foot pain, which is often remedied by raising the heel and shortening the toe (O'Grady, 2006), by trimming or by trimming plus shoeing.

ARENA SURFACE

Arena surface is an important issue for the training of the working horse. Racing track and arena surface properties have attracted recent research (Petersen and McIlwraith 2008; Setterbo et al., 2008), and hard surface tracks have been found to promote bone injury from increased concussive forces and deep sand surfaces tend to be associated with more soft tissue injuries. Working horse training requires a soft surface to allow rapid changes in direction and speed (stopping) without slippage. However, not all training requires these types of activities, so training can be shared between a soft sand arena and a more firm arena or paddock. Working in very deep substrate should be avoided because of the extra strain placed on soft tissues. Deep substrate (sand) can, however, also be utilized to the trainer's advantage to add resistance and, thus, power training

for the young horse. The horse should be introduced progressively to the extra power requirements of starting, stopping, and changing direction in deep substrate. Training sessions in deep sand should be kept short, with the specific goal of power training. This means that horses should not be warmed up (loped) or trained for cardiovascular performance in soft sand.

TRAINING REGIME AND REST PERIODS

An unfortunate disadvantage of high-performance futurity competitions at age 3 years is the lack of available time in the preparation program of the working horse to allow for rest and adaptation periods. Reining, cutting, and other working horses are trained for a period of 24 months following starting to saddle at age 18 to 24 months. Six-week recuperation periods should be scheduled after key training milestones to allow the horse time to recover from low-grade injuries and to avoid low-grade injuries progressing to more significant overuse injuries. During these recuperation periods, the horse should be kept aerobically fit by three to four light training periods per week, consisting of steady trotting and cantering. Low stress flexibility exercises should be continued to maintain suppleness and range of movement.

SPECIFIC INJURY-PREVENTIVE EXERCISES

Equine athletes must remain flexible for top level athletic performance in an injury-free environment (Hampson et al., 2005). The working horse trainer is in the unique situation wherein he or she has significant control over each major part of the horse's anatomy. Therefore, suppling exercises and long-sustained stretches can be done more effectively in the saddle than is possible from the ground, using the horse's own weight, strength, and combined movements. As previously discussed, the maneuvers performed by the working horse require a great deal of flexibility. The stop, for instance (see Figure 26-4), requires the full amount of spinal and hindlimb flexion available to the joints involved. It would be injurious for the horse to be placed in the stopping position without months of training and musculoskeletal preparation. The stopping position can be gradually obtained by backing the horse with progressive amounts of neck, thoracic spine, and lumbosacral spine flexion (see Figure 26-7, *C*). Increasing the speed of the backing maneuver will increase hindlimb excursion and flexion of the hip, stifle, and tarsal joints to add increased specificity to the dynamic mobilizing technique. The training exercises described in Figure 26-7 are as important for suppling and stretching activities as they are for strengthening, control, and motor coordination techniques.

CONCLUSION

The working horse is specifically bred and selected for the purpose of working with cattle and performing powerful and agile maneuvers at high speed. Even though the duration of the competition event for these horses is relatively brief (2–3 minutes), the anaerobic workload is demanding, as is the skill level and requirement for committed concentration for a high level of performance. The working horse competes at a high level at age 3 years, so training in saddle is commenced at age 18 months. Because of the requirement for a high musculoskeletal load at a young age, the injury rate and wastage in the discipline is high. This chapter identifies the specific issues that should be addressed to properly select and train a working horse and to obtain a high level of performance with less risk of injury. The chapter also describes some shortfalls of typical training procedures in the industry and suggests practical strategies for training and injury prevention.

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CHAPTER

27 Training the Racing Quarterhorse

BRIAN D. NIELSEN

The American Quarterhorse derived its name from its origin in racing short distances—notably a quarter of a mile (402.3 meters [m]). At this distance, the Quarterhorse is considered the fastest land athlete. Racing of American Quarterhorses became established in the southern and southwestern parts of the United States but now is also conducted in other parts of the world (Caudill, 2008). Quarterhorses are regularly raced on three continents, with races being conducted in the United States of America, Canada, Mexico, Brazil, and Australia.

UNIQUENESS OF THE RACING QUARTERHORSE

DISTANCES

Although the presence of Quarterhorse racing is still somewhat limited, especially when compared with the racing of Thoroughbreds, this style of racing probably more closely mimics the naturally evolved "flight" behavior of the horse, namely, in terms of running distances that would be covered when escaping a predator. In this sport, horses sprint for relatively short distances ranging from 91.4 to 795.5 m (100 to 870 yards) with the more common distances raced being 229, 274, 302, 320, 366, and 403 m (250, 300, 330, 350, 400, and 440 yards, respectively). When racing at longer distances such as 795.5 m, both Quarterhorses and Thoroughbreds sometimes compete against each another in the same race.

SPEEDS

Along with racing shorter distances, Quarterhorses are also known for their speed at these shorter distances. Despite the world record for both Quarterhorses and Thoroughbreds being within 0.15 seconds at 402 m at the time of this writing, Quarterhorses reach a greater top speed in their races (Nielsen et al., 2006). The relative lack of difference in record times is attributed to Quarterhorse races beginning when the starting gates open (Nielsen et al., 2006) compared with Thoroughbred races that officially begin when the horses cross in front of a flagman stationed a short distance in front of the gates (Ainslie, 1986). Thus, Quarterhorses are timed from a standing start, whereas Thoroughbreds are timed from a running start. Using a high-speed camera, Pratt (1991) was able to determine that it takes approximately 0.35 seconds for the starting gates to fully open and a nearly 0.6-second time elapse from the beginning of a race until a Quarterhorse has taken a step away from the starting gate. This difference in the methods used to time races explains why Quarterhorses are recognized as being faster than Thoroughbreds despite having similar record times at the quarter-mile (402-m) distance. Quarterhorses accelerate rapidly as they sprint away from the starting gates, and Pratt (1991) calculated that they reach their peak speeds by about 230 m in a 402-m race. Peak speeds in Quarterhorse races have been calculated to be around 90 kilometers per hour (km/hr) by both Pratt (1991) and Nielsen et al. (2006).

SELECTION OF HORSES

To develop horses capable of such speeds, careful consideration must be given to bloodlines. Given that many decades have been spent in developing bloodlines of horses that excel at running these short distances, it is highly improbable that horses from nonrunning bloodlines would have the speed necessary to be worthy of being put into race training. That being said, a number of Thoroughbred stallions, particularly those that have excelled as sprinters, have contributed greatly to the development of the running lines of Quarterhorses. Examples include Top Deck (sire of American Quarterhorse Hall of Fame members Go Man Go and Moon Deck) and Beduino (sire of racing champions Brigand Silk, Chingaderos, Indigo Illusion, and Tolltac, as well as prominent sires Runaway Winner and Chicks Beduino). Some exceptional stallions such as First Down Dash command the greatest stud fee but have had outstanding offspring, a number of which have earned over a million dollars to justify the high cost of stud fees. In contrast, some horses that were derived from inexpensive breeding or that were purchased for a minimal amount have gone on to be extremely successful on the track. Examples of such horses are Refrigerator ($2,126,309 by Rare Jet) and Winalota Cash ($1,952,848 by Light On Cash). However, even in these cases, the horses had the racing bloodlines as well as the genetics to be fast. Even with proper genetics, having the horse reach its potential is challenging, though proper management and correct training methods will help.

EXAMPLE TRAINING PROGRAM

In 2008, the 2-year-old gelding Stolis Winner (Stoli–Veva Jean, by Runaway Winner) earned $1,820,437 by winning 8 out of 9 races, including the grade 1 Heritage Place, Rainbow, and All American Futurities, while achieving a speed index of 105 in the process (Figure 27-1). His earnings were more than any 2-year-old Quarterhorse in history and are surpassed by only one Thoroughbred, Boston Harbor, with earnings of $1,928,605. In addition to being chosen Champion

FIGURE 27-1 Stolis Winner winning his trial to the 2008 All American Futurity with jockey G.R. Carter, Jr. aboard. *(Photograph courtesy of Andrea Caudill.)*

Two-Year-Old Gelding and Champion Two-Year-Old, Stolis Winner was named the 2008 World Champion Racing Quarterhorse—one of the few 2-year-olds to ever hold that distinction. Stolis Winner is the product of the breeding program of Jerry Windham, who was named the 2008 Champion Owner and Champion Breeder. The preparation and racing of Stolis Winner involved two trainers. The first to work with Stolis Winner was Tommy Zarate, the on-farm trainer for the Windham Ranch of College Station, Texas. Zarate started the horse under saddle and raced him in his first two outs. The other trainer to work with Stolis Winner was Heath Taylor, who raced Stolis Winner when the horse left Texas. Both trainers have had great success in Quarterhorse racing and have many stakes winners to their credit. Both trainers recognize that every horse is an individual and that training must be customized to fit each horse's personality, athletic ability, and limitations. However, both were willing to share some of their general training practices for this chapter. Although all racing Quarterhorse trainers may not share these practices, the programs detailed could probably be viewed as relatively standard among most trainers.

The success a trainer experiences is greatly dependent on the animals he or she must train. Thus, the horse having the right genetics is important. Zarate gives most of the credit to his success on the racetrack to the breeding program of Windham Ranch. Zarate concedes that he, as a trainer, cannot make a horse any better than that horse's genetic potential will allow. His job is simply to help the horse achieve that potential. Beyond that, Zarate suggests one factor that makes a runner great is having "heart"—a desire to run and a willingness to exert maximal effort during a race. Taylor emphasizes the need for symmetrical balance in the horse. Regardless of whether a horse is large or small, if it has good balance throughout its body, this will allow it to be a better runner. Taylor also indicates that what he selects a horse for may depend somewhat on the goals of the owner. If the horse's owner desires to win a futurity in March that is contested at a shorter distance (such as 274 m), he may select a horse that will mature earlier, as opposed to a goal of winning the All-American Futurity contested in September, which covers a longer distance (402 m).

Regardless, to help achieve goals, both Taylor and Zarate believe that it is important to provide consistent and regular riding during the early stages of training. They believe that the most important aspect of early training involves mental training of the horse, the physical conditioning aspect of it initially being less important. Typically, during these early stages, horses are ridden 5 to 6 days per week. This schedule may continue for up to 6 weeks. Most trainers conduct their first rides in a round pen and, after the horse appears ready, will progress to riding the young horse on the racetrack (Nielsen et al., 1993). This can occur within the first week of training or may take several weeks, depending on the horse. Also, as horses progress and become accustomed to being ridden and trained, riding may be reduced to an alternate-day schedule, although some individuals will require more frequent training time to allow for more successful mental training.

As the horses become conditioned, the distance they are ridden at a "gallop" (i.e., a moderate to fast canter) increases to a distance of about 1200 to 1600 m following a warmup at the trot of about 400 to 800 m. Additionally, as soon as horses start to be ridden on the racetrack, they are often gradually introduced to the starting gates. Frequently, during the early stages of training, a horse is walked through the starting gate each time it is ridden on the track. While walking through the gates, horses may be asked to stand quietly before being allowed to proceed slowly out. Once the horses are comfortable standing in and walking out of the gates, they may be encouraged to trot away from the gates, and they will eventually be asked to gallop away from the gates. Typically, at least 45 days of such training (often even several months) will occur before the horses' trainers close the doors of the starting gates while their charges are inside. At that point, the starting gates may be manually opened (to allow for an environment in which the trainer can better control the gates to minimize startling the horse) and the horse then is asked to sprint away from the gates. After this phase of training has been completed successfully, with the horse at an acceptable comfort level, the gates may be mechanically opened and the horse asked to sprint away again. If all of this is performed correctly, the horse is ready to do the same in the company of other horses. Learning to sprint away from the starting gate is often done over a period of several weeks and is a gradual process. Taylor indicates the first two or so times he works (i.e., "sprints") a young horse, the distance is only about 45 m and typically the work begins from a controlled gallop to allow the horse to gradually learn how to respond when asked. After that, the distance sprinted will likely be increased to about 90 m. Similarly, during early gate-work such as when the gates are being manually opened, horses are only sprinted a short distance such as 45 m before being slowed down. This gradually accustoms them to running fast with riders on their backs and helps ensure a positive experience for the horse. Only after a horse is comfortable with being asked for speed and has had several shorter sprints will the distance be increased and official works, necessary to qualify for racing, will be performed. These official works tend to be from 201 to 229 m in length. However, as the distance worked increases, the time between works also increases so that when the 2-year-old is ready to race, it may be sprinted only every 14 to 21 days. Taylor indicated that he does not like to work a horse any closer to a race than 2 weeks, thus

placing great importance on the training regimen prior to race day. Also, most of the horses are being ridden only every other day at most, unless they need more riding time for their mental well-being. Although they are only galloped a few days per week, the horses are usually walked on nonriding days for anywhere between a half hour and an hour. After galloping, many are also walked for approximately a half hour or so or until the horses are cooled down. If a horse does need to be ridden more than a few times a week, the focus of training shifts from a conditioning aspect to a state of mental preparation to enable the horse to handle the mental stresses of racing. Zarate emphasizes that it is important for 2-year-olds to run error-free races, hence the importance of mental preparedness.

From the time the horse is initially started under saddle to when it can first race may be as short as 120 days if nothing interferes with training, but Taylor suggests it may be advisable to plan on 5 to 6 months to have a young horse ready for racing. This additional time allows for issues that may arise, for example, various types of lameness or respiratory issues, to be resolved while still allowing enough time for a horse to be properly prepared for racing. Because the largest monetary purses are offered in races for 2-year-olds, a large percentage of Quarterhorses are started under saddle in the autumn of their yearling year. In the United States, Quarterhorse races for 2-year-olds do not begin until March. On commencement of racing, it is unusual to race more often than every 2 weeks, and often, the horse is given a break of several weeks to a month between races. The frequency of racing is usually dependent on soundness of the horse, whether the horse is being prepared for stakes races, and whether races with appropriate conditions are available in which to enter the horse.

Compared with the unraced 2-year-old, taking a previously raced horse and returning that animal to training involves a shorter training period before racing. A sound older horse that returns to training in good condition may be able to race in as little time as 45 to 60 days, although 90 days may be the more common time frame. Both Zarate and Taylor use a combination of trotting, galloping (typically a distance of about 1600 m), ponying, some form of swimming, or all of these to return older horses to racing form. Sprinting of older horses is not an often-used training technique, with the primary occasion being an official work. If an older horse is racing somewhat regularly, sprint work may not be needed between races.

A hallmark of Quarterhorse racing is the use of time trials associated with stakes races, in which the horses with the 10 fastest times from the time trials run in the finals of the stakes race. By having larger numbers of horses nominated for a stakes race, a larger purse is generated through the nomination fees. A trial heat 2 weeks prior to actually running in the finals is required, and therefore, proper management to help ensure that the horse runs well in the finals is crucial. A major issue that trainers encounter is the soreness experienced by horses following the trials. If the horse is sore and if it is not a problem that can be resolved or remedied within the 2-week period, the horse may need to be removed from the finals, in which case it will typically receive last-place money for the finals. Assuming that the horse is not removed, it is important that the animal is well rested coming into the finals. Taylor likes to rest a horse for at least 3 days after racing and may gallop a horse only two or three times between the trials and the finals. Zarate states that he may do more with a horse between the trials and finals than do many other trainers, as he likes to get a horse out of its stall and keep its body loose. As he rides his own horses, he has the advantage of knowing how they feel and what adjustments they may need in their training program. Being able to determine what a horse needs is important, and Taylor points out the great variation in horses, with some requiring little training between races while other horses may work better if they frequent the track regularly. Hence, the trainer's job is to do what it takes to help the horse keep its appetite and interest in racing so that the horse is happy and healthy and peaking at the time of the finals.

Another distinguishing factor about Quarterhorse racing is the importance of the start of the race. Quarterhorse races are run in a straight line for a short distance relative to Thoroughbreds. As a result, getting away quickly from the starting gates is critical, as the race does not last long. Leaving the gate last may result in situations where the horse may have to adjust its course and, therefore, sacrifice running the ideal straight-line path. As a result, having a horse stand calmly, but alertly, at the starting gate is important to having a successful break when the starting gates open. Both Zarate and Taylor often stand a horse at the starting gate at times other than when the horse is racing. This helps eliminate the horse's anticipation of a fast start and decreases the likelihood of the horse acting up at the starting gate and making a mistake. This process involves loading the horse at the starting gate, typically on just a regular training day, and letting the horse stand at the gates for a couple of minutes before being led out. By not being asked to sprint away from the gates, horses will typically be calmer the next time they are loaded. Taylor typically stands all of his horses once before each race—typically 2 to 4 days before racing. Although certainly not a requirement, such a practice helps reduce the nervousness of a horse at the starting gate on race day.

Both trainers appreciate the importance of turnout time. Because he is located on a training farm away from a racetrack, Zarate is able to provide some turnout for his young horses through the majority of the initial training. Also, Zarate often turns a horse out after a hard work when it is tired, and thus, it is less likely to hurt itself by playing too hard in the pasture. He feels this is especially useful for the mental state of the horse, as this allows "a horse be a horse." Zarate recognizes that the risk of a horse getting hurt during turnout is always present, but he believes that the benefits typically outweigh the risks. Taylor also appreciates the benefits of turnouts and feels that "more would be perfect." But he recognizes the challenges one faces when having a horse turned out, especially for longer periods, because of the potential difficulty in getting them back into race condition. Regardless, he would like to see all horses turned out at least once or twice a year to give them a break from training.

Such a break from training is a necessity if an injury develops. Both Zarate and Taylor indicate that knee and ankle chips are the major problems they encounter. Bucked shins (dorsal metacarpal disease) can be a problem with racing Quarterhorses, although Taylor suggests that a tremendous difference may exist in incidence rate among trainers. As with Thoroughbred racehorses, respiratory problems are encountered in Quarterhorses also.

CHANGES ASSOCIATED WITH TRAINING

MUSCLE

Though many not familiar with Quarterhorse racing believe that the limited training outlined above and experienced by racing Quarterhorses leaves the animal relatively unfit, a belief held by Quarterhorse trainers is that too much training can slow down a Quarterhorse, this may have some scientific support. In terms of evolution, the horse evolved as a sprinter. When threatened by a predator, the horse will typically sprint a short distance (commonly said to be about a quarter of a mile) before slowing down to evaluate the seriousness of the threat. As such, horses tend to have a predominance of fast-twitch muscle fibers, which play a prominent role in sprinting. The proportion of fast-twitch fibers has been reported to be nearly 75% in Thoroughbreds (Andrews and Spurgeon, 1986; Kawai et al., 2009), but it is likely the proportion is even greater in racing Quarterhorses as would be indicated by their larger muscle mass and greater speed. It is commonly recognized that with training, the majority of muscular changes involve a shift to increased oxidative capacity (Rivero et al., 2007). As oxidative capacity increases, glycolytic capacity likely decreases to the detriment of the speed needed by the sprinting Quarterhorse. As with interval training, which tends to increase oxidative capacity (Wilson et al., 1987), too much conditioning of the racing Quarterhorse may increase the oxidative capacity of muscles at the expense of glycolytic capacity and speed.

Because horses evolved to sprint short distances such as those covered during many Quarterhorse races, conditioning needed for maximum performance is less than is required for other equine events that require increasing the animal's endurance capacity. For the Quarterhorse, the greater concerns often are the attempt to maintain soundness and mental preparation of the horse for competition, although the conditioning aspect should not be overlooked.

SKELETON

One of the main concerns of many Quarterhorse trainers is the avoidance of skeletal injuries. Contrary to popular belief, bone is a dynamic tissue and responds to whatever forces are applied to it. The lack of any high-speed exercise in the early stages of training, typical of most race training programs, likely predisposes many horses to injuries. In a study of 53 Quarterhorses, mineral was lost from the third metacarpal, as reflected by a decrease in optical density, when training was commenced at age 18 months (Nielsen et al., 1997). The bone loss continued until the horses entered the period of training at which speed was introduced. As the training continued and the horses began to race, mineral content of the third metacarpal began to increase, although even after racing nine times, the mineral content of the third metacarpal was still lower than it was 244 days prior to the start of the study, suggesting it may have been stronger before the horses even entered training. Not surprisingly, the greatest number of bone-related injuries occurred during the period when mineral content of the third metacarpal was at its lowest point in the study. This was also the time when speed was first introduced. This additionally reflects what happens in a practical setting. Rarely do bone-related injuries occur during the period when horses are being "legged up" and simply trotted and galloped. Injuries have a tendency to develop when speed is being introduced. In most cases, the time when horses are being managed traditionally and kept in stalls with minimal turnout is also a period when bone is probably at its weakest. The decline in mineral content in the third metacarpal in the cited study likely was simply the result of the change in housing as horses were moved from pasture into stalls to commence their training. To test this, a follow-up study reported that long yearlings kept in stalls with 1 hour of walking per day on a mechanical walker lost mineral from the third metacarpus within only 1 month of being transferred into stalls (Hoekstra et al., 1999). By comparison, no such loss occurred in pair-matched horses maintained on pasture. Even after 3 months, when the study horses were put into a conditioning program of trotting and galloping that would be typical of racing Quarterhorses, no increase occurred in bone mineral content. By the end of the 5-month study including 2 months of training, the horses kept in stalls had lower bone mass as determined by radiographic photodensitometry than at the start of the study. Many racehorses are stalled with only walking exercise "in hand" while being prepared for sales. These horses then enter a training program that affords no opportunity for the horse to sprint during the program's early stages. Such management likely compromises bone strength and may make such horses prone to skeletal injuries. By contrast, studies have clearly shown that young animals which are sprinted relatively short distances (between 50 and 80 m) only once per day, 5 days per week, have greatly increased bone mass in the lower leg compared with ones denied any access to exercise. This was seen even when all study animals were stall-housed and afforded no other exercise (Hiney et al., 2004a; 2004b; Figure 27-2). Short bouts of speed, either as free choice during turnout or under more controlled circumstances for horses housed in stalls without access to turnout, are needed to maintain skeletal strength. Contrary to popular belief, substantial amounts of trotting or slow cantering do not contribute substantially to bone strength. This was evidenced by a lack of difference in mineral content of the third metacarpal between horses trained to complete 60-km endurance tests and their pasture mates (Spooner et al., 2008). Simply put, it is crucial that horses routinely have access to sprint exercise to maintain bone strength. It has not been determined how often horses need some speed work to avoid losing bone strength, but periods longer than 2 weeks without any speed should probably be avoided. Additionally, it should be reiterated that speed exercise does not need to cover great distances. As stated earlier, sprinting distances ranging from 50 to 80 m were adequate to increase bone strength compared with animals similarly housed, but not sprinted. For prevention of dorsal metacarpal disease, the need for an early but gradual introduction of short bouts of speed is warranted (Verheyen et al., 2005).

TENDON

The incidence of bowed tendons is seemingly lower in racing Quarterhorses than in racing Thoroughbreds (Goodman, 1987). Several likely reasons exist for this. The average Quarterhorse is shod in a manner in which the toe length is shorter compared with that in many Thoroughbreds. This method of trimming and shoeing results in a quicker breakover of the hoof while running (Weishaupt et al., 2006). Given the need for rapid acceleration, this type of trimming and shoeing could provide advantages for performance as

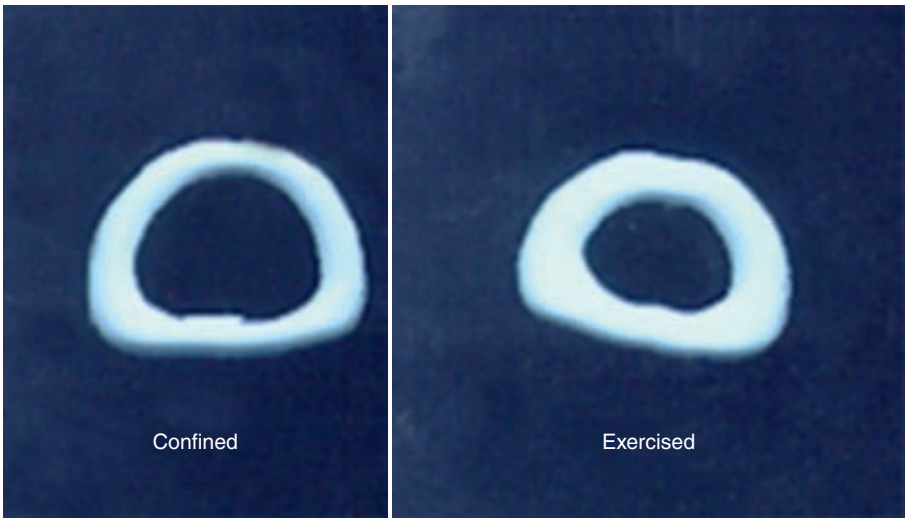

FIGURE 27-2 Example of cross-sectional image of fused third and fourth metacarpal bone from confined and exercised calves illustrating the changes resulting from the management program. The increase in cortical width seen in the exercised calf can be readily seen compared with the large medullary cavity and thinner cortical widths in the confined calf. *(From Hiney KM, Nielsen BD, et al.:* High-intensity exercise of short duration alters bovine bone density and shape, *J Anim Sci 82:1612-1620, 2004.)*

stride frequency could be increased. From a soundness standpoint, shoeing or trimming a horse in such a manner as to give it a shorter toe should also decrease the strain on the superficial digital flexor tendon—the tendon in which bowing occurs most often.

Racing Quarterhorses also train over shorter distances compared with Thoroughbreds (Nielsen et al., 1993; Nunamaker et al., 1990). As overuse and accompanying fatigue can lead to the development of a bowed tendon, the shorter distances used in training Quarterhorses logically result in a lower incidence of bowed tendons.

Interestingly, tendon, like bone, has some ability to adapt to training. However, it has been proposed that the ability to adapt is decreased once a horse is mature, as the greatest ability for tendon to increase in strength is before the horse reaches age 2 years (Smith et al., 1999). The specific type and quantity of exercise needed to elicit the most beneficial changes have yet to be determined. However, research suggests that failure to provide any exercise during the critical growth period may result in tendon that lacks structural strength compared with that in horses that have had some training while young.

RESPIRATORY SYSTEM

The coupling of stride to breathing creates a unique issue in racing Quarterhorses. In video replays in slow motion, particularly those of short races, stride frequencies of over three strides per second can be observed. Considering that horses typically inhale and exhale once per stride, this suggests that the horses are also breathing in and out over three times per second. As a result, it is possible that the pressure differences between inhalation and exhalation are even greater in racing Quarterhorses than in racing Thoroughbreds, which have a slower stride rate and, thus, slower respiratory rate. Consequently, even while racing a shorter distance, Quarterhorses commonly experience problems with exercise-induced pulmonary hemorrhage.

MENTAL CONDITIONING

The mental conditioning of racing Quarterhorses can also play a critical role in their success. Unlike with Thoroughbred or Standardbred racing, pace is not a factor in Quarterhorse racing. Quarterhorses are typically encouraged to run as fast as they can for the entire distance of the race. Also, with the exception of some races that are longer than 402 m, races are conducted in a straight line with each horse expected to stay in its own path. As a result, probably more so than in other forms of racing, the start of the race is vitally important. Given that races can last from just over 6 seconds in the 91-m races to between 21 and 22 seconds in the 402-m races, any additional distance that the horse needs to cover as a result of not being able to run in a straight line can greatly decrease the chances for success. Being accustomed to the commotion that is encountered during a race and breaking quickly from the starting gates can both be factors in a horse being able to maintain a straight path to the finish line.

The need to accelerate sharply from the starting gates is anticipated by trained racing Quarterhorses. One study showed Quarterhorses had a heart rate averaging around 165 beats per minute (beats/min) after warmup that increased by about 10 to 15 beats/min while the horses were in the starting gates (Reynolds et al., 1993). Interestingly, in that study, the heart rate did not increase much from the time the horses were in the starting gates until the completion of the race, but did tend to increase slightly after the race to an average high of 211 beats/min. Whether this failure to recognize a significant increase in heart rate from the point while horses were standing at the starting gates to when they were running maximally reflects what truly happened, or whether it was a failure of the heart rate monitors to capture an increase in such a short period, could be debated. Also, given that these heart rates are averages from horses of varying athletic ability, the primary point that can be drawn from these data is that tremendous anticipation occurs with racing Quarterhorses as they approach the starting gates and while they are standing

at the gates. This is supported by an average hematocrit of 54% in the same study during warmup. Though racing caused a slight increase in hematocrit, the apparent near-complete splenic contraction during warmup suggests that prerace excitement can have tremendous psychological and physiologic effects. While some anticipation can result in beneficial changes such as the increasing hematocrit, it can be important to try and have a horse as relaxed as possible before racing.

MANAGEMENT OF HORSES IN TRAINING

NUTRITION

The feeding program for racing Quarterhorses is not substantially different from that for racing Thoroughbreds. Horses are fed with the goals of maximizing muscle glycogen and minimizing excess weight. With that in mind, most racing Quarterhorses should be fed to maintain a body condition score (BCS) of near 5 on the 1-to-9 scale as described by Henneke et al. (1983). Research suggests that glycogen stores are not maximized when a horse is in a thin condition (Jones et al., 1992; Scott et al., 1992). Given that glycogen will be one of the primary substrates used for energy production during a Quarterhorse race, maximizing stores is crucial. No advantage to glycogen stores seems to exist above a BCS of 5, but a number of disadvantages to performance exist. These include the carrying of extra weight and a decreased ability to dissipate heat generated in muscle during exercise. The carrying of extra weight serves to slow the horse down and also increases the load upon the skeletal system, thereby increasing the risk of injury.

Because of the need to maximize glycogen stores, feeding diets containing significant amounts of soluble carbohydrates may be necessary. Although this would normally be accomplished through a high grain diet, it might be possible to provide such carbohydrates through certain forages that are relatively high in soluble carbohydrates.

Electrolyte supplementation is commonly provided to performance horses. However, assuming horses are fed a balanced diet in which salt (NaCl) is provided in the concentrate or is available as free choice, electrolyte deficiencies are unlikely, given that both racing and training involve relatively short bouts of exercise. Considering that in the United States, the majority of Quarterhorses are raced in the south or southwest where racing and training occur in relatively warm temperatures, possible supplementation could be warranted if substantial sweat loss is experienced beyond what horses experience racing or training.

COMMON TYPES OF UNSOUNDNESS

Estimates of the incidence rate of various types of injuries in racing Quarterhorses is limited compared to similar estimates in racing Thoroughbreds. Details of an equine practice that only deals with racing Quarterhorses was presented in 1987 by Goodman. It was indicated that lameness associated with the carpal joints and the distal interphalangeal joints were two of the most common types of lameness observed. With the carpal joint, problems ranged from synovitis and capsulitis to chip fractures to complete collapsing carpal slab fractures. Although not as common, arthrosis of the distal interphalangeal joint was present in a relatively large number of horses. It was suggested that Quarterhorses may be more prone to this problem because of a combination of factors such as their small feet, large body, and short, upright pasterns, as well as tremendous speed. The lameness associated with arthrosis of the distal interphalangeal joint presents itself as bilateral forelimb lameness characterized by a short gait, which worsens when the animal treads on a hard surface. The incidence rate of dorsal metacarpal disease (bucked shins) ranged from 5% to 50%, depending on the trainer. By contrast, bowed tendons were rare. Upper respiratory problems were frequent and similar in nature to those experienced by Thoroughbred racehorses.

At the time of the Goodman paper, the use of corticosteroids to treat some of the types of unsoundness, particularly joint unsoundness, was quite common. This appears to remain true for present times. Depending on the medication used, a return to racing sometimes averaged as little as 3 days (Goodman, 1987). Though some research has shown the therapeutic benefits of corticosteroid use, it is doubtful that complete cartilage repair could occur in such a short period. Hence, if the damage is severe, the use of corticosteroids could mask the pain and inflammation resulting in considerable problems. These problems endanger horses and their riders as the self-protective property of pain is removed. Thus, the use of corticosteroids is controversial (Nielsen, 2008).

SHOES

At the time of this writing, many racing jurisdictions in the United States have begun to implement new rules and guidelines regarding the types of shoes allowed on racing Quarterhorses. The main concern as well as focus of these rules is the use of toe grabs. These traction devices on the toes of the shoes come in various lengths and have been implicated in an increased incidence of injuries, particularly to the suspensory ligament (Kane et al., 1996). Research findings have been very convincing that the risk of catastrophic breakdown in Thoroughbreds is increased with the use of toe grabs. Given that fewer Quarterhorses racing could be studied, it has not been possible to determine whether toe grabs cause similar issues for Quarterhorses, although such a conclusion is reasonable. In Thoroughbreds, as toe grab length increases, the risk for injury also increases. Given that the Quarterhorse toe grab is longer than those used on Thoroughbreds, the risk of injury is also high. Seemingly, one of the reasons for an increased risk of injury is the delay in breakover, as the use of a toe grab can have the same mechanical effect as having a long toe (Weishaupt et al., 2006). Hence, the delay in breakover would cause the suspensory ligament to be subjected to greater strain and be at an increased risk for injury. The advantage a Quarterhorse would have over a Thoroughbred is that by running a shorter distance and taking fewer training strides, the accumulated damage should be less in the Quarterhorse.

Despite the convincing evidence in Thoroughbreds that the use of toe grabs increases the risk of injury, many Quarterhorse trainers believe the use of toe grabs actually serves to prevent injury. Their rationale is that it decreases slippage so the horse is less likely to take a bad step. This has not been proven. However, Quarterhorses have been raced barefoot (Caudill, 2009), seemingly without any increased injury risks. Furthermore, it has been suggested that rim shoes provide sufficient traction while decreasing injury rates (Kane et al., 1996), so the use of long toe grabs may not be justifiable.

FULFILLING GENETIC POTENTIAL

Racing of Quarterhorses is a fascinating subject in the area of equine exercise physiology. The extreme speeds they reach, coupled with their rapid stride and respiratory rates, make them unique among equine athletes. However, these same traits that make racing Quarterhorses interesting to study also make them prone to injuries. Achieving maximal fitness to perform well does not appear to be the major concern of most trainers. Instead, most would probably agree that injury prevention is one of the greatest challenges they face in trying to have their horses reach their genetic potential. Ironically, many of the practices used to prevent injury to the horses may actually make them more prone to sustaining an injury. When being prepared for sales, horses that are deemed extremely valuable on the basis of bloodlines and conformation are often handled carefully to avoid injury. This would include maintaining them in stalls and never putting those animals through any high-speed exercise. However, those animals are not given the chance to maximize skeletal strength because of this. A few strides at speed are needed, but many horses in race training, especially during early training, often are not afforded the opportunity to experience these crucial sprints. A skeletal system not properly conditioned for speed is prone to injury, and the typical management training programs used have probably contributed greatly to the injuries seen in racing Quarterhorses. However, by better understanding how to alter various physiologic systems, it should be possible to decrease injuries and improve performance in these remarkable racing animals.

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CHAPTER

Evaluation of Performance Potential

28

DAVID R. HODGSON

ATHLETIC PERFORMANCE

Successful athletic performance requires a complex interaction of physiologic mechanisms involving the musculoskeletal, nervous, respiratory, and cardiovascular systems. From a simplistic point of view, exercise demands the imposition of increased loads on the respiratory and cardiovascular systems to support the dramatic increases in metabolic rate occurring in contracting muscles during exercise. It is essential that the responses of each of these systems are appropriately integrated to ensure optimal physiologic performance. Dramatic increases occur in ventilation and cardiac output with increasing metabolic rate, and not surprisingly, the capacity and health of these systems will play a substantial role in determining the performance potential of a horse. Since superior athletic performance depends on tight integration of a number of body functions, it is to be expected that many elite equine athletes have metabolic characteristics indicating this potential for superior performance. For example, top-class Thoroughbred and Standardbred racehorses usually have values for maximal oxygen consumption ($\dot{V}O_{2max}$) in the range of 150 to 200 milliliter per kilogram per minute (mL/kg/ min). Conversely, although a high $\dot{V}O_{2max}$ indicates substantial cardiorespiratory capacity, it does not ensure superior athletic performance. This is demonstrated in the horse that has a large cardiorespiratory capacity and yet is endowed with a conformation that does now allow the musculoskeletal system to withstand the rigors of training and racing. However, we also have conducted exercise tests on horses in which the cardiorespiratory capacity or conformation may not be considered ideal, yet these horses performed at a level above that predicted. Parallels for this latter observation exist in human athletes, and the reasons are likely to be related to (1) our inability to measure the metabolic determinants critical to performance, (2) physiologic integration, whereby the sum of all the components contributing to exercise capacity is greater than those indicated by measurement of the parts, and (3) intangible factors such as desire, capacity to withstand discomfort, and so on.

Evaluation of performance potential requires an understanding of the physiologic mechanisms involved in the energetics of exercise. Muscular work requires that the physiologic systems of the horse are integrated to minimize the stress imposed on the component mechanisms supporting the energetics. Muscular respiration depends on complex interactive systems that allow gas exchange between muscle cells and the atmosphere. Optimal gas exchange between muscle cells and the atmosphere requires (1) efficient lung function, (2) effective pulmonary circulation, which is able to match the requirements of ventilation, (3) blood with an adequate hemoglobin concentration, (4) a cardiovascular system that can deliver an appropriate quantity of oxygenated blood to the periphery to match tissue respiratory requirements, and (5) control mechanisms capable of regulating arterial blood gas tensions and pH.

Energy for muscular contraction is obtained predominantly by the oxidation of fuels in the mitochondria, with an additional portion delivered via biochemical mechanisms in the cell cytoplasm. This energy is used to form high-energy compounds, predominantly phosphocreatine (CP) and adenosine triphosphate (ATP). The energy from the terminal phosphate bond can be made available for cellular reactions involved in synthesis, active transport, and muscular contraction.

OXYGEN-TRANSPORT CHAIN

$\dot{V}O_{2MAX}$

During exercise, dramatically increased loads are placed on muscle bioenergetics, creating the need for the respiratory and cardiovascular systems to respond to support the increased gas-exchange requirements. Transfer of oxygen (O_2) and carbon dioxide (CO_2) between the mitochondria and air requires a finely coordinated interaction between the cardiovascular and respiratory mechanisms that is integrated with the cellular metabolic activity. The large increase in muscle O_2 requirements during exercise demands that O_2 flow to muscle increases. A simultaneous increase in CO_2 production occurs, and CO_2 must be removed from tissues to ensure that acidosis is avoided, since this can have profound adverse effects on muscular contractile activity. Components of the oxygen-transport chain that are integral to superior athletic performance include the airways and lungs, the cardiovascular system, blood volume and hemoglobin concentration, and the musculoskeletal system.

AIRWAYS AND LUNGS

Following the onset of exercise, the increase in respiratory drive is thought to be mainly caused by increased neural stimuli. In elite athletic horses, this may involve an increase in minute ventilation from about 100 liters per minute (L/min) at rest to greater than 2000 L/min during strenuous exercise. The increase in ventilation occurs as a result of a small increase in tidal volume and a large increase in respiratory frequency of up to 150 breaths per minute. Entrainment of stride and respiratory frequencies restrict any greater increase in respiratory rate. As a result, peak airflows will be more than 6500 L/min. Achievement of flows of this magnitude will require production

of transpulmonary pressures of more than 60 centimeters of water (cmH_2O). For such enormous flows to occur, it is important that the upper respiratory tract be optimally dilated during exercise. This active dilatation allows a reduction in upper airway resistance during exercise. Not surprisingly, restrictions to the upper airway (e.g., idiopathic laryngeal hemiplegia) may substantially alter airflow dynamics and, therefore, gas exchange, resulting in reduced exercise capacity. Similarly, disorders that may alter elasticity of the lung or gas exchange in the alveolus (e.g., chronic obstructive pulmonary disease) also will reduce gas exchange and, therefore, restrict performance.

CARDIOVASCULAR SYSTEM

Integration of cardiovascular and respiratory function during exercise is essential if superior athletic performance is to be achieved. In the transition from rest to exercise, dramatic alterations occur in the vascular system to accommodate the large increases in cardiac output. Initially, metabolic vasodilation occurs in the vascular beds of working muscle, resulting in an increase in $\dot{V}_{O2}$ and stimulation of heart rate and cardiac output. Almost simultaneous dilatation of capillary beds occurs in the pulmonary vasculature to support the increased gas-exchange requirements imposed by the exercise. During intense exercise, heart rate increases to greater than 230 beats per minute (beats/min), which, in elite racehorses, is associated with increases in cardiac output to more than 350 L/min. Blood flow to working muscle has been shown to increase by more than 75-fold in ponies in response to intense exercise. These blood flows exceeded 160 mL/kg/min, almost twice the values reported to occur in humans.

BLOOD VOLUME

Blood is the conduit for transport of O_2 to and CO_2 away from working muscle. O_2 is carried by hemoglobin, and the volume of O_2 that can be carried in the circulation is related to the total blood volume and total hemoglobin concentration. Horses possess a substantial splenic reserve of erythrocytes, which are released into the circulation in response to exercise. This reserve is such that the blood O_2 concentration can increase from values of about 180 mL/L at rest up to 280 mL/L in response to maximal exercise. This is associated with an increase in hematocrit from about 0.40 L/L at rest to more than 0.60 L/L during maximal exercise. Total blood volume also increases from about 65 mL/kg at rest to more than 130 mL/kg with intense exercise.

MUSCULOSKELETAL SYSTEM

During exercise, the major end point of the oxygen-transport chain is the contracting skeletal muscle. In its simplest form, skeletal muscle can be regarded as the apparatus that is fueled by the chemical energy sources derived from ingestion of food. As described above, most energy for muscular contraction is derived from the oxidation of fuel in the mitochondria. In horses, skeletal muscle has an intrinsically high oxidative capacity compared with humans and most other domestic species, and this may be enhanced by training.

For the oxidative metabolic pathways to be able to meet the energy demands imposed by exercise, delivery of oxygen to the working muscle via the pulmonary and cardiovascular systems must be adequate. Working muscle consumes the O_2, and in response to the increased extraction of O_2 and addition of CO_2 to capillary blood by muscle, an almost immediate increase occurs in muscle blood flow. The initial vasodilation is thought to be centrally induced, with subsequent dilation occurring under the influence of local humoral control. This process is selective, allowing vasodilation in the muscle units with the highest metabolic rates.

ANAEROBIC ENERGY DELIVERY

Although the majority of energy during most intensities of exercise is provided by aerobic means, maximal exercise requires a substantial contribution from the anaerobic bioenergetic pathways. For this purpose, horses are endowed with intrinsically high activities of the enzymes involved in anaerobic energy production, with horses with the highest proportion of fast-twitch (type II) muscle fibers also having the greatest glycolytic potential.

From an energy point of view, it is important to consider that induction of energy production by the anaerobic pathways does not signal the downregulation of energy supply by the aerobic pathways. Lactate is a byproduct of anaerobic energy production, and at low exercise intensities, little or no change occurs in blood lactate concentration. As exercise intensity increases, a consequent formation of lactate takes place, with increases in the concentration of this metabolic byproduct in muscle and blood. The higher the intensity of exercise, the greater is the concentration of lactate accumulating in these tissues.

MUSCLE POWER

Superior athletic performance requires that locomotor muscles be able to generate sufficient force for an appropriate time during the competition. To do this, equine muscle is divided into muscle fibers that may have widely variable metabolic and contractile characteristics. On the basis of contractile properties, muscle fibers have been classified into two major types, type I and type II, with type II fibers often being further subdivided into subtypes. Type II fibers are the most powerful, having the fastest speeds of contraction and relaxation. The speed of contraction for type II fibers is up to tenfold greater than those recorded for type I fibers. The horse has adapted such that fast-twitch fibers are endowed with great muscle power, a characteristic that exists at the expense of energetic efficiency. As a result, activities requiring great muscle power are normally associated with production of large proton (acid) and lactate loads by the anaerobic pathways. Therefore, although induction of the anaerobic pathways allows large amounts of energy to be produced rapidly, the associated acidosis may have detrimental effects on the muscle contractile apparatus, which, in turn, directly contributes to fatigue.

The possession of substantial muscle power in horses is likely to result from the need for horses in the wild to possess the capacity for rapid bursts of high-intensity exercise when attempting to escape from predators. Domestic breeds have been selected mainly for short-term, intense exercise and, therefore, possess high proportions of fast-twitch fibers in key locomotor muscles. For example, Thoroughbred, Standardbred, and Quarterhorses have more than 80% fast-twitch fibers in the major muscles of locomotion. In

contrast, horses selectively bred for endurance capacity, such as Arabian horses, often have up to 50% slow-twitch fibers in the locomotor muscles.

BUFFERING CAPACITY

The proton load produced during intense exercise will exert detrimental effects on the contractile apparatus via a reduction in local pH. Horses possessing the capacity of superior athletic performance are able to offset these deleterious effects at least to some degree because they possess local intracellular and circulating buffer systems. Local buffering systems are the result of hydrolysis of CP or occur via physiochemical means. Additional buffering, in particular that occurring outside the muscle cell, is related to the bicarbonate buffering system. The high buffering capacity of muscle in horses is thought to be related to the high concentration of carnosine in muscle fibers. Carnosine contributes approximately 30% of the nonbicarbonate buffering, with the greatest concentrations of this dipeptide being found in type IIb fibers. Buffering capacity has been hypothesized to be a key determinant in the potential for sprint performance in humans, an association likely to translate to the horse, and this would, in part, explain the predominance of type II fibers and high buffering capacity in the muscles of Thoroughbreds and Quarterhorses.

CONFORMATION

Successful athletic performance is not possible unless the relationships between the functional capacities of the metabolic systems described here are combined with appropriate conformational characteristics of the locomotor system to allow effective propulsion of the horse. This interrelationship is summarized by reflecting that proper functioning of the locomotor system depends on precise synchronization of the movement of each part on every other part and in relation to the body as a whole. Pathologic changes may occur whenever improper synchronization occurs.

Many volumes exist on what constitutes appropriate conformation for the athletic horse and, indeed, which factors are most likely to result in musculoskeletal infirmities. Ideal conformation may vary considerably, depending on the breed and expected use of the horse. However, excessive loading forces on limbs of horses attempting to maintain racing speed is likely a major cause of breakdowns in racehorses. One school of thought suggests that appropriate conformation for an elite horse, when performing at racing speeds, involves the animal having a skeletal shape that allows it to have body support on the lead hindlimb as the nonlead forelimb makes contact with the ground. The load of the body weight is then pushed smoothly over the forelimb. In addition, he suggests that appropriate conformation of the shoulder is essential to ensure dampening of the loading forces. In contrast, horses that do not have this "appropriate" skeletal shape are forced to dissociate their hindlimbs and forelimbs when attempting to maintain near-maximal speed. It has been suggested that this dissociation is the factor that results in breakdown, since the horse is forced to "almost leap from hindlimb support to forelimb support," thereby dramatically increasing the stresses imposed on the forelimbs. This may explain why some horses that possess this coupling between forelimbs and hindlimbs are able to perform at high levels despite the presence of other musculoskeletal *conformational defects,* for example, backward deviation of the carpal joints (calf knees) or upright pasterns.

THE INTANGIBLES

Elite athletes demonstrate great competitiveness. In all probability, this characteristic is inherited, and although bad management will undoubtedly diminish the competitive spirit of a horse, whether this characteristic can be enhanced is still being debated.

Many highly regarded trainers see this intangible quality as being integral to successful athletic performance. Horses possessing a strong will to win have an intrinsic desire to dominate other horses during a race and are able to continue exercise when physiologic and psychological inputs should signal a reduction in performance.

Diminution of this will to win may occur as a result of infirmities, as occurs with chronic pain, boredom, or overwork ("overtraining"). Trainers have a profound influence on this aspect of competitiveness, and good trainers somehow find the correct balance between appropriate physical and psychological training for the horses under their care.

CONCEPTS OF FATIGUE

Elite athletic performance is the result not only of optimal function of key body systems, as discussed previously, but also of the capacity to develop resistance to fatigue. In most competitive athletic events, horses become fatigued and either have to stop the exercise or reduce the intensity. Thus, a discussion of some of the factors responsible for fatigue in both high-intensity and low-intensity events is important for understanding the limitations to performance and performance potential. One of the important training adaptations is a delay in the onset of fatigue.

Fatigue is manifested as the inability of the horse to continue to exercise at the intensity required. Superior athletes possess the capacity to offset these processes and maintain exercise intensity for longer than less capable athletes. Fatigue is a complex process that appears to involve central (psychological or neurologic) and peripheral (muscular) contributions. Peripheral aspects of the process have been afforded the greatest interest, possibly because they are easier to define compared with central contributions to reductions in performance.

Evidence suggests neurophysiologic contributions to fatigue in humans, involving an activating and an inhibitory system. However, further confirmation of this mechanism is required. Central fatigue also may have a psychological component. In humans, factors such as lack of motivation have been described as causes of fatigue. Since horses undergoing intense training will sometimes lose their competitive edge (overtraining) without evidence of organic disease, it is likely that central or psychological components are responsible for the apparent fatigue. Mental freshness and a positive psychological approach are, of course, vital contributors to elite performance in humans, and similar factors are probably critical for optimal performance in horses.

Studies investigating the effects of peripheral fatigue demonstrate that it is task specific and that its causes are multifactorial. Processes implicated in the cause of fatigue include impairment of excitation–contraction coupling, impaired energy production, and limitations to fuel supply. The processes involved in fatigue are related to the intensity and

duration of the exercise that the horse is required to perform. A number of factors have been associated with muscular fatigue, including the following:

1. Depletion of substrates for energy production
2. Interference in energy (ATP) production as a result of alterations in the internal milieu of the muscle fiber
3. Changes in neuromuscular irritability because of changes in electrolyte gradients
4. Interference with the contractile process which is the result of alterations in calcium (Ca^{2+}) uptake or release by the sarcoplasmic reticulum
5. Decreased blood flow, excessive increase in muscle temperature, or both

In many cases, a combination of these factors is likely to operate. In horses, much of the current understanding on the likely contributing factors to muscle fatigue comes from analysis of muscle biopsy samples following different intensities and durations of exercise.

FATIGUE IN RESPONSE TO HIGH-INTENSITY EXERCISE

Intense exercise results in fatigue within seconds to minutes. Depletion of the phosphagen pool (ATP and CP), reductions in intracellular pH, and possibly accumulation of lactate appear to be important factors contributing to fatigue. Cellular homeostatic mechanisms are designed to maintain intracellular ATP concentrations within a reasonably tight range. However, with short-term intense exercise, muscular CP is initially depleted, and this is followed by reduction in ATP concentrations. The greatest reductions in the concentration of ATP occur in fast-twitch fibers. Whether reduction in the phosphagen pool directly induces fatigue remains to be clarified, but intense exercise that produces ATP depletion is also associated with significant reductions in intramuscular pH from around 7.0 to 7.1 at rest to 6.4 or below at fatigue. Reduced pH is known to reduce the respiratory capacity of muscle and have direct effects on the contractile apparatus. Acidosis and the substantial increases in muscle temperature that occur are likely to be associated with impaired function of the sarcoplasmic reticulum. Loss of potassium from the contracting muscle and accumulation in the plasma also may be a contributory factor to fatigue. Intense exercise induces increases in plasma potassium concentration to greater than 10 millimoles per liter (mmol/L). This change in potassium homeostasis also may alter the function of the sarcoplasmic reticulum and thereby influence calcium handling within the cell. Whether acting singly or in combination, decreased pH, a decreased nucleotide pool, increased temperature, and altered electrolyte gradients are likely to exert deleterious effects on a number of metabolic processes in muscle, resulting in fatigue.

FATIGUE DURING PROLONGED SUBMAXIMAL EXERCISE

A number of factors, including altered fluid and ion balance, hyperthermia, and depletion of muscular fuel stores, have been implicated singularly and collectively as causes of fatigue during this type of exercise. During exercise, about 80% of the energy produced is released as heat. The horse possesses a finely tuned thermoregulatory system, which, under most situations, allows dissipation of this metabolic heat load. However, as ambient temperature and humidity increase, the demands placed on thermoregulation become progressively greater. Heat stress results in diversion of blood flow away from working muscle to skin to dissipate heat. This reduction in blood flow is proposed to contribute to fatigue. In addition to the cardiovascular demands produced by heat stress, significant volumes of fluids (more than 10 L per hour) may be lost as sweat during exercise. If this is not replaced, these losses produce reductions in total body water. Equine sweat is hypertonic, and thus substantial electrolyte losses accompany the fluid losses. These alterations in fluid and electrolyte balance are related directly to reductions in thermoregulatory efficiency and performance capacity.

Depletion of the intramuscular glycogen stores is a frequently reported cause of fatigue during prolonged exercise of moderate intensity. Glycogen depletion occurs in a selective manner in particular fiber types as a function of the duration or intensity of the exercise. Depletion of glycogen from within a muscle fiber is associated with decreased capacity for force production in that fiber. Although muscle fibers are selectively recruited during submaximal exercise, with additional fibers being recruited as others become exhausted, eventually a sufficient number of fibers are depleted of their carbohydrate stores that the overall force-producing capacity of the muscle falls below that required to maintain exercise intensity.

The various factors responsible for fatigue in both low-intensity and high-intensity events should be considered when assessing performance potential. Aspects of energy supply are also important. Because successful athletic performance is multifactorial, no single measurement will accurately predict exercise capacity. However, a number of measurements, which range in complexity and sophistication, are available and are useful for providing an indication of the capacity or function of key body systems in the oxygen-transport chain.

MEASUREMENTS FOR EVALUATION OF PERFORMANCE POTENTIAL

ESTIMATION OF HEART SIZE

Heart size is a major determinant of maximal cardiac output and maximal aerobic capacity. Given this relationship, a number of techniques are designed to assist in the determination of heart size and, thus, predict cardiac output and performance capacity. For horses, initial techniques were based on measurements derived from electrocardiographic recordings and are the basis of the *heart score* concept. More recently, echocardiographic variables have been measured in resting horses in an attempt to predict performance potential. Measurement of $\dot{V}O_{2max}$ also provides an index of cardiorespiratory function, whereas maximal oxygen pulse ($\dot{V}O_{2max}/HR_{max}$) is a valuable indicator of maximal stroke volume.

Heart Score

The concept of heart score, an electrocardiographic measurement of heart size, was developed in Australia in the late 1950s. The concept was based on the idea that heart size would be reflected by ventricular depolarization time. The proposal was that the heart size could be determined by measurement of the QRS duration, in milliseconds, in electrocardiographic leads I, II, and III and then averaging the values. This system has now been discredited, as the Purkinje system

in the equine ventricle allows for almost instant depolarization of the ventricular myocardium and, as such, the size of the QRS complex bears little or no relation to the size of that organ.

Echocardiography

Initial interest in the use of echocardiography for determination of cardiac dimensions to indicate performance potential was aroused following the first report of its use in a horse in the 1970s. This technique has expanded dramatically since then.

Positive relationships between body mass and left ventricular size measured at necropsy have been established in horses. Similarly, it has been shown that fit Thoroughbred horses have larger left ventricular internal dimensions compared with other warm-blooded breeds. On the basis of this, estimates of cardiac size can be made by using echocardiography, and from these estimates, some appraisal of performance capacity could be made. However, to date, an association of echocardiographic variables measured in the resting horse and that horse's performance potential has not been clearly established.

TOTAL RED BLOOD CELL VOLUME

Because the total volume of red blood cells in circulation is a major determinant of oxygen-carrying capacity in horses, measurement of the total red blood cell volume (CV) may provide some index of the exercise capacity. Measurement of CV is most frequently based on the use of Evans blue, a dye that enables measurement of plasma volume using the technique of dye dilution. From determination of the plasma volume and hematocrit following intense exercise, CV is determined as described. Good correlations have been shown between the exercise capacity of Standardbred Trotters and total hemoglobin or red blood cell volume. This, however, has not been proven to be true for all breeds of horse.

MUSCLE BIOPSY

Fiber-type proportions within muscle are genetically determined, with type II fibers being powerful, rapidly contracting fibers, whereas type I fibers are more slowly contracting, with high aerobic capacity and are, therefore, most suited to endurance activities. On the basis of these characteristics, it is logical to suggest that horses with the best sprinting capacities will have the greatest number of type II fibers in the muscles of locomotion, whereas successful Endurance horses will possess increased numbers of type I fibers. A number of studies have been performed to test this hypothesis. Although trends within the different athletic groups could be identified (e.g., successful racing Quarterhorses were found to have a higher proportion of type II fibers compared with unsuccessful cohorts), the differences were rather small. Similarly, successful Endurance horses have been shown to have greater numbers of type I fibers in the muscles of locomotion than horses that perform less well.

EXERCISE TESTING

Evaluation of athletic performance using treadmills or field testing has gained popularity in recent years and may provide potentially useful information in the selection of horses.

In general, information related to the functional capacities of the cardiorespiratory and musculoskeletal systems is most valuable when attempting to evaluate performance potential. To be useful to practicing veterinarians and horse owners, measurements must be relatively easy to perform and the information easily understood and applied. Variables that can be easily measured during exercise and are potentially valuable include heart rate, blood lactate concentration, oxygen uptake, and stride length.

CONCLUSION

Prediction of performance potential is one of the elusive ideals of the science of exercise. Therefore, it is not surprising that selection of horses at the elite level is often based on breeding or genes rather than on scientifically measured variables. Currently, none of the available scientific measurements discussed here provides an accurate assessment of performance potential, but some may provide a guide. It seems likely that ongoing refinement of field exercise testing, with the capacity to measure physiologic indices important to athletic performance, offers the best means for performance prediction. However, until further prospective studies are performed with large numbers of horses allowing relative weighting of the various indices, performance prediction will remain elusive.

CHAPTER

29 Clinical Exercise Testing

A. COUROUCÉ-MALBLANC AND DAVID R. HODGSON

Exercise testing has been used routinely for the past 50 years in human medicine to evaluate fitness and the significance of a range of diseases on exercise capacity. Testing usually has been performed in laboratories equipped to perform cardiovascular and respiratory measurements by using either bicycle ergometers or treadmills to vary the intensity of exercise. More and more information from field tests is available to athletes, with well-documented cases from cycling and triathlon, where athletes are able to determine work output by using a combination of heart rate (HR) meters, speed, and inputs of other variables.

Exercise testing to evaluate the physiologic responses of athletic horses to exercise has followed that in humans. The Swedes were the first to describe standardized protocols for investigation of exercise capacity, with research performed both on the track and using a treadmill, in Swedish Standardbred Trotters. This research began in the 1960s and became more routine in the 1970s and established normal responses of HR, oxygen uptake, blood lactate, and total red blood cell volume in this breed. However, in the exercise tests performed on the treadmill, horses were not exercised at maximal exercise intensities. In other studies using track testing, horses with lower airway disease were studied by other groups in Europe.

During the 1980s and 1990s, a dramatic increase occurred in the number of studies investigating cardiorespiratory and metabolic findings in athletic horses by using exercise tests undertaken either on the track or treadmill. Such studies provided important information on expected normal physiologic responses to exercise and data on the effects of some diseases. However, measurements regarding the physiologic responses of elite athletic horses to exercise are still few. The majority of studies have been performed using experimental horses of moderate to poor athletic ability. More recently field testing of racehorses has become more common and as such the depth of available information has increased. Despite these limitations, exercise testing has reached a point where important conclusions can now be drawn. This chapter will review material on exercise testing in athletic horses. It is a primer and in no way attempts to provide specifics on all specific methodologies available for exercise testing in health and disease.

INDICATIONS FOR EXERCISE TESTING

Exercise testing provides a mechanism for evaluating a range of body systems under standard exercise conditions. Measurements of cardiorespiratory and metabolic functions during an exercise test provide information about the capacity and efficiency of key body systems involved in energy production. Thus, some conclusions may be drawn about the athletic potential, or lack thereof, of the horse based on measurements of oxygen transport or estimates of anaerobic capacity, depending on the duration and intensity of the competitive event. Additionally, changes in levels of fitness may be evaluated by using exercise testing because resting measurements of hematology or biochemistry provide little or no indication of improvements in fitness. Exercise testing is probably of most use from a clinical point of view to assess the effect on performance of abnormalities found on physical examination or to determine the reason(s) for reduced athletic capacity in horses that have no abnormalities on resting examinations. Whatever the reason for the testing, one important premise is that standardized procedures are followed so that the data derived from each test can be compared against subsequent tests for the same horse or with measurements from other horses of similar age and fitness level.

TRACK VERSUS TREADMILL EXERCISE TESTS

Measurements from horses at the track are obviously much simpler and can be performed more readily without access to sophisticated equipment than investigations using treadmills. Track testing is not only more easily performed than treadmill testing but also has the advantage of being undertaken in an environment similar to that in which the horse has to perform. Originally, it was considered that these advantages were outweighed by the disadvantages of track testing, which included the relatively limited range of measurements that can be performed, variations in track and environmental conditions, and the influence of the rider or driver. This has changed in recent years with the advent of sophisticated telemetric HR meters, global positioning system (GPS) to track speed and changes in elevation, horse-side lactate analyzers, more durable and accurate portable respiratory masks, and dynamic, horse mounted video-endoscopy allowing real-time appreciation of upper respiratory function during intense exercise.

Measurement of lactate in blood may be performed on either whole blood or plasma. Plasma values will be about one third higher than whole-blood values, although the relationship between plasma and blood lactate is variable from horse to horse. If samples are not to be analyzed immediately, it may be best to collect blood into tubes containing fluoride or oxalate as an anticoagulant so that glycolysis is inhibited and lactate values do not continue to increase after collection.

However, it has been found that provided the blood samples are kept refrigerated and the samples are analyzed within 48 hours of collection, sodium or lithium heparin is a suitable anticoagulant.

One of the areas where great advances have occurred in the past decade is related to pulmonary function testing (Evans, 2007). Tests of lung function are particularly important in racehorses because minor compromise of oxygen transport may have profound effects on performance. A great advance now available to the practicing veterinarian is dynamic video-endoscopy (DVE), which is now more commonly used for assessment of possible upper respiratory limits to performance. With the development of these systems, exercise horses may be examined at training sites under more normal conditions. In general, DVE systems consist of a semi-rigid yet malleable insertion tube (approximately10 mm diameter) with light-emitting diodes (LEDs) in the tip (lower power needs). The tube is attached to a purpose-made bridle. This fits over standard tack. Key electronic components are housed in a permanent virtual circuit box. This interacts with a remote receiver or video display allowing real-time visualization of the upper airways. The electronics and lavage system are stored in a backpack, on the sulky or saddle blanket. Newer versions have remote control to allow adjustment of the head of the scope during exercise such that the best image is maintained. Digital images are readily downloaded and transmitted for review.

Breath-by-breath measurement of pulmonary ventilation with a suitable spirometer is the ideal method of assessing the likely clinical impact of a respiratory problem on athletic performance or for assessment of response to treatment. Likewise, tests of function in resting horses are unlikely to be helpful unless the horse has a severe respiratory problem such as recurrent airway obstruction. Clearly, the future of exercise testing will be based around accurate and sensitive pulmonary function tests in horses when exercising under field conditions emulating those occurring during racing. Studies of the maximal breathing capacity (the product of maximal tidal volume and respiratory frequency during maximal exercise) and the ratio of pulmonary ventilation to oxygen consumption during exercise (ventilatory equivalent for oxygen) are likely to have potential as tests of lower airway function in horses. Studies of pulmonary ventilation and flow volume loops during the recovery period after maximal exercise may also have potential diagnostic use. A byproduct of development of these lightweight masks is the accurate measurement of maximal oxygen consumption under field conditions.

Thus, the gap between what can be determined in the field versus the treadmill has narrowed substantially in the past two decades. One advantage of treadmill testing, however, is that if the machine is situated in a room, climate control is possible, as are standardized conditions for testing and the opportunity to perform a range of measurements during exercise as well as before and after exercise. Previously, it was considered that these advantages outweighed the disadvantages of the artificial nature of the treadmill environment and the fact that energy expenditure during exercise on the treadmill is quantitatively different from that during exercise on the track. Proponents suggested that treadmill exercise testing allowed more precise identification of disturbances to particular body systems if a wide range of measurements of the function of key body systems is undertaken. As mentioned above, with the advent of more accurate, lightweight technologies, a huge amount of useful information can be garnered from horses exercising in the field. This, of course, has huge advantages, as the investigator can go to the horse as opposed to the horse being shipped to a laboratory. Also, the horse can be exercised in its normal environment under ambient conditions similar to those in which it is expected to perform. Both field testing and treadmill testing have a place in the assessment of performance in horses.

TRACK EXERCISE TESTING

Track exercise tests involve various measurements undertaken during or after a standardized bout of exercise. The simplest track test is assessment of exercise capacity by timing the horse over the competition distance. A fast track time and good recovery are good evidence that the horse is fit for a particular race or event distance. This approach has the advantage of simplicity, the only piece of equipment required being a means to accurately determine exercise duration the simplest being a stop watch. However, in most cases where exercise testing is considered, more information is usually required.

One of the key issues in track exercise testing is the measurement of speed. For ridden horses, linear and temporal markers need to be located on the track for the rider to regulate the velocity and cadence of the horse. Velocity is then measured with a stopwatch. Also, velocity may be measured by the use of a GPS carried by the rider. In driven horses such as Standardbred Trotters or Pacers, velocity may be measured by using a tachometer placed on the wheel of the sulky connected to the meter or the GPS. HR response to exercise is an important indication of metabolic capacity. It may be easily measured and registered by means of two electrodes placed on the horse and connected to a heart rate monitor. HR response to graded exercise is linear, between 120 and 210 beats per minute (beats/min^{-1}), as shown in Figure 29-1. Many factors

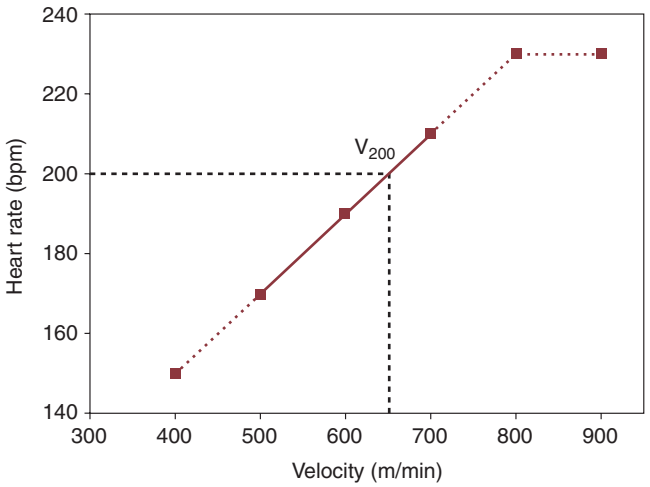

FIGURE 29-1 Average heart rate (HR) values in French Trotters with a three-step track exercise test. Because of the linear relationship of HR to velocity (V), linear regression may be used to calculate the speed at an HR of 200 beats per minute (V_{200}). The dotted part of the graph represents the projected HR at lower and higher velocities than those during the test. Note that at higher velocities, maximal HR is eventually reached.

such as exogenous factors (e.g., geometry and length of the track, environmental conditions), stage of training, and disease may influence the regression line of HR on work speed. However, the regression of HR on speed is very precise and reproducible when measured during a standardized exercise bout.

Blood lactate concentration may be measured by taking blood samples at the end of the exercise period, from the jugular vein into tubes containing fluoride-oxalate. Lactate is a product of muscular metabolism and accumulates in muscle and blood at high intensities of exercise. The concept of anaerobic threshold, extrapolated from plotted curve of blood lactate concentration against speed has been defined as the level of work just below that at which metabolic acidosis occurs. The aerobic–anaerobic transition or onset of blood lactate accumulation (OBLA) has been defined empirically as 4 millimoles per liter (mmol/L^{-1}) blood lactate concentration. At this level of activity, the initial increase in lactate production is followed by a steady state in which lactate utilization and lactate production are equal. At higher levels of activity, lactate production exceeds its utilization, and so it accumulates in blood (Figure 29-2).

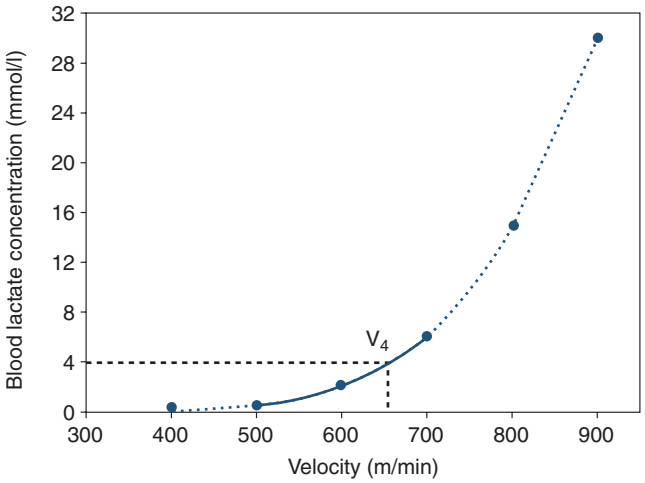

FIGURE 29-2 Average blood lactate concentration values in French Trotters with a three-step track exercise test. The exponential relationship of blood lactate concentration to velocity (V) allows calculation of the speed at a blood lactate of 4 mmol/L (V_4). The dotted part of the graph represents the projected blood lactate concentration at lower and higher velocities than those during the test.

OVERVIEW OF TESTING PROCEDURES

Numerous and varied testing procedures (Table 29-1) have been described for horses involved in different disciplines such as 3-day eventing, endurance, show jumping or racing (flat and Standardbred). Whatever the horse's discipline, field exercise test protocols should always be rigidly defined to calculate meaningful fitness measurements and to limit variability. Following standardized procedures is of great importance as the data derived from each test can be compared with those from subsequent tests for the same horse or with measurements from other horses of similar age and training status. Results may vary according to the methodology

TABLE 29–1
Characteristics of Some Field Exercise Tests in Horses

			TESTING PROCEDURE	
Authors	Horses	Track	Steps	Rest
Isler et al., 1982	12 Warmblood Stallions	Sand track	3 steps of 1650 m : 350–450, 450–500, 550–600 m/min^{-1}	Rest : 8 minutes
Wilson et al., 1983	12 trained Standardbred Trotters	Race track	4 steps of 3 min : 450–500, 600–700, 700–800 and > 800 m/min^{-1}	Rest : 5 minutes
Sloet van Oldruitenborgh-Oosterbaan et al., 1987	6 Endurance horses and 6 saddle horses	Sand track, 864 m long	5 steps of 1100 m : HR from 140 beats/min^{-1} to maximal heart rate (HR)	Rest : 8 minutes
Auvinet et al., 1991	34 three-event horses	Galloping track	6 steps of 3 min : 350 to 600 m/min^{-1}	Rest : 1 minutes
Bourgela et al., 1991	7 untrained Standardbred Trotters	Sand track 436 m long	7 to 9 steps of 3 minutes each : 200 to 500 or 600 m/min^{-1}	Rest : 1 minutes
Castejón et al., 1993	11 Andalusian 9 Arabian 4 Anglo-Arabian	Sand track 1000 m long	4 steps of 5 minutes each : 15, 20, 25, 30 km/hr^{-1}	Rest : 5 minutes
Lindner and Wittke, 1993	6 Thoroughbreds 7 Thoroughbreds	Sand track	2 steps of 1100 m : 10.5 and 13 m/s^{-1} 4 steps of 2100 m : 7.4, 8.4, 9.4 and 10.4 m/s^{-1}	Rest : 20 minutes Rest : 1 min

used and with factors such as number and duration of steps, resting time between steps, and increment from one step to the next.

Therefore, the following points are important to consider when designing a possible testing procedure:

- Velocity should be controlled and maintained as constant as possible during the exercise test. This is a requisite of the rider or driver and may be difficult to achieve. Regardless, the aim is to maintain a constant speed during each step of the test.
- The duration of each step should be sufficient to provide a steady-state heart rate and blood lactate response. Generally 3 to 5 minutes are necessary for humans to reach a steady-state blood lactate concentration. In contrast, shorter steps may be possible in the horse.
- Because an efflux of lactate from muscle to blood occurs following exercise, consistent periods between each step is essential.
- The responses of HR and blood lactate concentration to speed are dependent on the age and training level of the horse.

STANDARDIZED TRACK-BASED EXERCISE TEST FOR STANDARDBRED TROTTERS

The experience of one of the chapter authors, A. Couroucé-Malblanc, relates mainly to the testing of Standardbred Trotters in Europe. Given this, we provide an example herein widely used by this author.

TESTING PROCEDURE

Various protocols have been described for Trotters. However, the example described here is a test of three steps each of 3 minutes, with a 1-minute rest between steps one and two. Depending on the age of the horse, the velocity of step 1 varies from 440 to 500 meters per minute (m/min^{-1}) with the lowest speeds used for the two-year olds. The increment within each step varies from 40 to 80 m/min^{-1} (Table 29-2). The aim of the highest speed increment is to induce a blood lactate concentration greater than 4 mmol/L^{-1}.

CALCULATION OF INDICES OF EXERCISE CAPACITY

From the measured variables HR, V (speed, or velocity), and blood lactate concentration, derived variables may be calculated to permit simple comparison of test results. Examples are described below.

TABLE 29–2

Velocity (M/Min^{-1}) of the Three Steps of the Standardized Field Exercise Test*

Age (years)	Duration of Training (months)	Velocity Step 1	Velocity Step 2	Velocity Step 3
2	0 to 4	440	480	520
2	4 to 8	470	530	590
3	8 to 24	490	560	630
4 and more	More than 24	500	580	660

*Taking into account age and duration of training (Demonceau and Auvinet, 1992).

Velocity and Blood Lactate Concentration

For comparison of blood lactate values between horses or in the same horse during training, the velocity at a blood lactate concentration of 4 mmol/L^{-1} (V_4) generally has been used. V_4 is considered a reference value for horses, as it is considered by many to be a good predictor of aerobic capacity. A high value for V_4 (see Figure 29-2) is an indication of superior exercise capacity and is related to racing performance. In the 1990s, Evans et al. (2007) studied the relationship between the blood lactate response to exercise and performance in Thoroughbreds during a submaximal exercise test on a 5% inclined treadmill. These authors showed that the blood lactate concentration 2 to 5 minutes after exercise was correlated to racing performances as assessed by Timeform rating ($r = -0.68$; $p<0.01$). In another study undertaken in Sweden, it was shown that in a small group of Trotters, those first two to begin their racing careers were the ones that had the lowest lactate concentration after submaximal tests. In another study, Casini and Greppi (1996), studying 20 Trotters completing a field exercise test were able to compare 10 good performers and 10 poor performers, on the basis of best time. These authors found significantly higher V_4 values in the first group and a negative correlation between V_4 values and best time (0.61). Couroucé et al. (1997) also showed that 96% of horses with low V_4 values according to their age were poor racing performers. Finally, Davie et al. (2002) studied the relationships between V_4 values and earnings in a population of 16 pacers. A significant correlation was found between V_4 values and log earnings and log earnings or start. Overall, the higher the V_4, the fitter is the horse and the greater is its exercise capacity.

Velocity and Heart Rate

A useful reference point for comparison of cardiovascular capacity in Standardbred horses, is the V_{200} (see Figure 29-1), which represents the velocity at an HR of 200 beats/min^{-1}. According to many, even though individual variations may be found, at an HR of 200 beats/min^{-1}, most Standardbreds are close to the point of onset of blood lactate accumulation (OBLA, blood lactate concentration of 4 mmol/L^{-1}). A retrospective study carried out with 194 French Trotters that performed 1105 field standardized exercise tests on a sand training track, permitted calculation of V_4 and V_{200} mean values (± standard deviation [SD]) according to age groups (Table 29-3). It was also shown that in most cases, the

TABLE 29–3

Mean Values (± SD) of V_4 and V_{200} Expressed in M.Min^{-1} According to Age-Groups (Couroucé et al., 2002)

Age (years)	V_4	V_{200}
1	512^a (± 21)	498^a (± 39)
2	551^a (± 29)	539^b (± 40)
3	590^a (± 41)	586^a (± 37)
4	611^a (± 26)	614^a (± 34)
5	616^a (± 26)	617^a (± 31)
6 and more	626^a (± 30)	641^b (± 35)

Comparing V_4 and V_{200} for each age group, values with different superscript are significantly different ($p<0.05$).

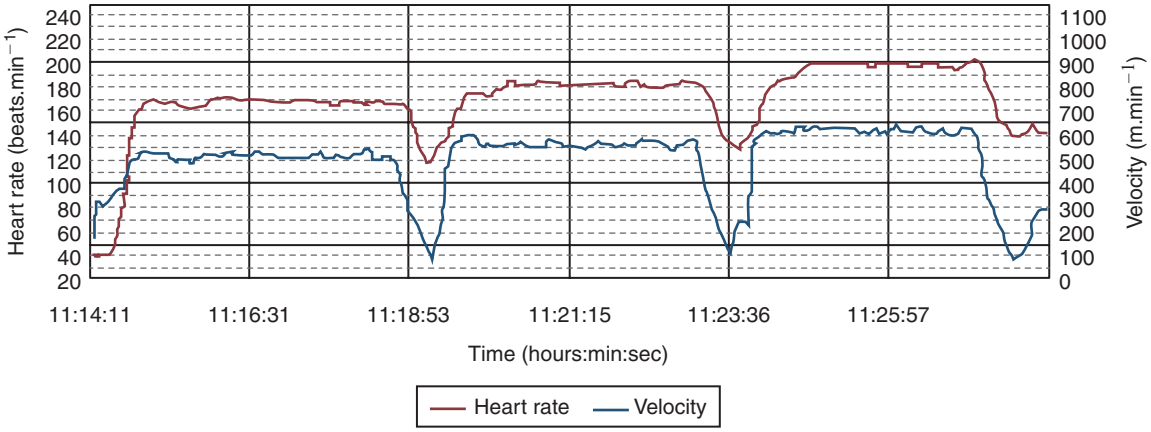

FIGURE 29-3 Heart rate and velocity graphs recorded using a Bauman Speed Puls Equus meter during a three-step track exercise test. Each step lasts for 3 minutes; a 1-minute rest between one step and the next is provided. *(Fitsoft software, Bauman and Haldi).*

workload carried out at V_{200} is close to V_4. For 1-, 3-, 4-, and 5 year-olds, no significant difference was seen between mean V_4 and V_{200} values. In contrast, for 2- and 6-year-olds, a significant difference was seen between mean V_4 and V_{200} values, V_{200} being lower for 2-year-olds and higher for 8-year-olds and those older.

For saddle horses, the velocity for an HR of 170 beats/min^{-1} has been suggested to be more appropriate, as it is often difficult for these horses to reach an HR of 200 beats/min^{-1}.

REPRODUCIBILITY

On the track, a number of the conditions may vary, for example, track quality and temperature, humidity, or other weather related factors. However, standardized field exercise tests should represent reference data for trainers in the evaluation of the fitness level of their horses and in evaluating their response to training. For this standardized field exercise test for French Trotters, we have found that results are reproducible, at least on the same track. Under standardized conditions, we have found that no significant differences exist between V_4, HR_4, and V_{200} measurements from one test to the other.

Interpretation of V_4 and V_{200}

It is particularly important to be able to compare data obtained from one test with measurements from other horses of similar age and level of training. In this author's experience with French Trotters, V_4 is the most important measurement to assess the fitness level because of the relationship between this variable and racing performance. In fact, it seems that a horse with a low V_4 measurement according to its age and state of training is likely to be a poor performer because of a low aerobic capacity.

V_4, V_{200}, and Track Testing

The track is an important variable to consider, as it may influence the calculation of V_4 and V_{200}. In a previous study, five French Trotters performed standardized exercise tests at two different tracks (a 1250-m sand race track and a 720-m sand training track) and on an uninclined treadmill during the same week to determine the influence of exercise surface on different measured variables such as V_4 and V_{200} (Couroucé et al., 1999). No significant differences were found for the physiologic variables between the two tracks. In contrast, significant differences for these variables were observed between those occurring on the two tracks versus the those on testing on the treadmill, with horses showing lower HR and blood lactate responses on an uninclined treadmill (Table 29-4).

V_4, V_{200}, and State of Training

In the context of an athletic horse, a major point is to perform regular field exercise tests to measure the response of a horse to a specific training program by the measurements of V_4 and V_{200}. As outlined above, state of training influences HR and lactate response to exercise, a normal horse showing lower HR and blood lactate concentration for the same submaximal work load after a training period.

To illustrate this, here is an example. Let us assume that in response to the first exercise test, Horse 1 (H_1) had a V_4 = 580 m/min^{-1} and a similar V_{200} (Figure 29-4). It was trained for 6 weeks involving a training program that included mainly aerobic exercise three times a week. The intensity of these training sessions was defined by HR_4 or V_4 on the assumption that this provides a maximum working speed utilizing the horse's aerobic capacity while avoiding the development of fatigue associated with the onset of lactate accumulation. H_1 performed a second field exercise test after this training period and showed a V_4 = 620 m/min^{-1} indicating a lower blood lactate accumulation for the same work load (Figure 29-5).

TABLE 29–4

Mean Values (±SD) for Derived Physiological Variables on a 720-M Long Sand Training Track (Test 1), on a 1250-M Long Sand Race Track (Test 2) and on the Uninclined Treadmill (Test 3) (Couroucé et al., 1999)

Variable	Test 1	Test 2	Test 3
V_{200} (m/min^{-1})	571^a (±20)	587^a (±17)	662^b (±44)
V_4 (m/min^{-1})	588^a (±12)	599^a (±23)	640^b (±18)
HR_4 (beats/min^{-1})	205^a (±7)	204^a (±8)	196^{b,a} (±11)

Variables with different superscripts are significantly different (p <0.05), with HR_4 = heart rate corresponding to V_4.

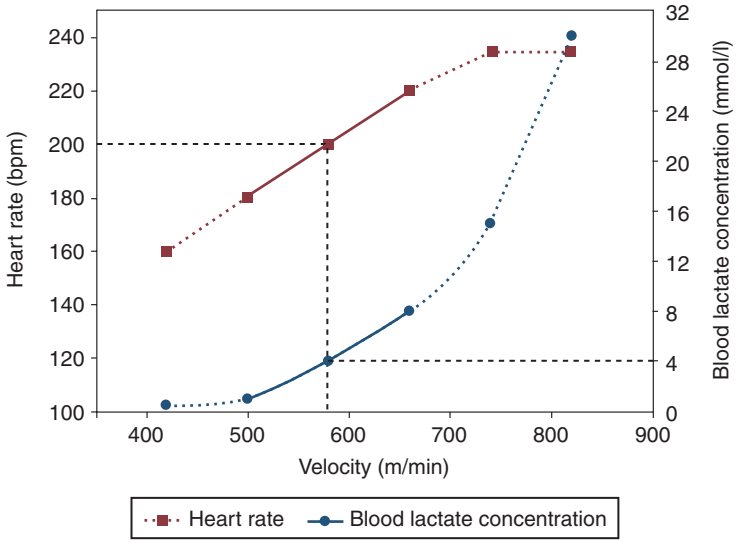

FIGURE 29-4 Mean heart rate (HR) and mean blood lactate concentrations related to velocity (V) in three French Trotters (H_1, H_2, and H_3) performing a three-step standardized exercise test after 3 weeks' training. The relationship of these variables to velocity allows calculation of V_{200} and V_4 values, which, in this example, are the same. The dotted part of the graph represents the projected HR and blood lactate concentration at lower and higher velocities than those during the test. Note that at a higher velocities, a maximal HR is eventually reached.

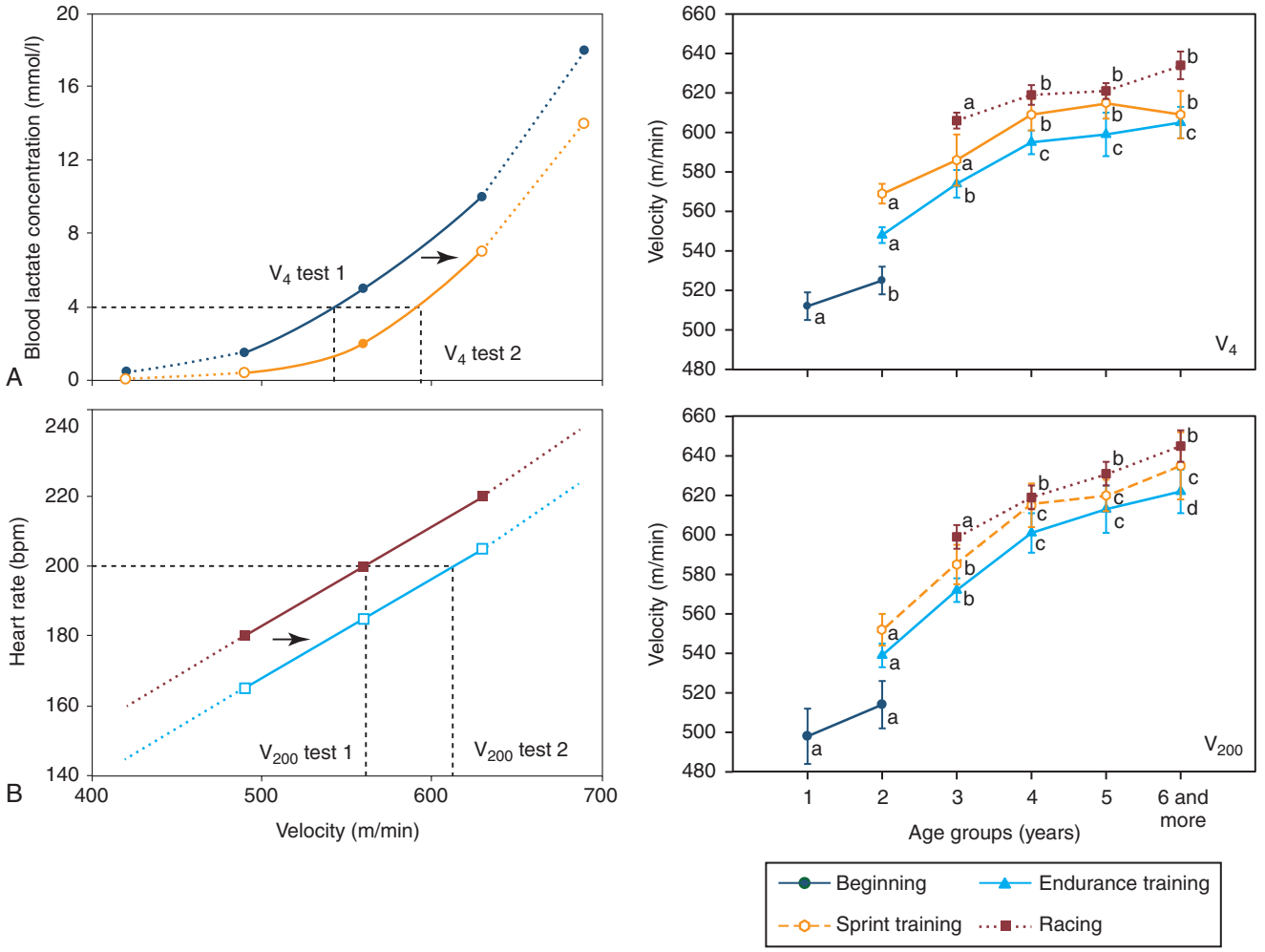

FIGURE 29-5 Influence of training state on heart rate (HR) and blood lactate relationship to speed on V_4 and V_{200} values in a "normal" horse (horse H_1). Closed symbols indicate the results of the first standardized exercise test and open symbols indicate the results after a 6-week training program. The results indicate a shift to the right, with improved fitness, of both of the blood lactate concentration (*A*) and HR values (*B*) related to velocity on the track.

Also, H_1 had similar V_4 and V_{200} values in both tests, demonstrating a stable HR_4 of 200 beats/min^{-1} before and after the training period (see Figure 29-5). In fact, H_1 continued to respond well to the training program and showed a high V_4 value for a 3-year-old after it completed 9 weeks of training. The low V_4 value measured on the first exercise test was to be expected, given the limited amount of trained undertaken previously. As training state is of great importance in the interpretation of the results of an exercise test, careful questioning of the trainer should always be done to evaluate the frequency, duration, and intensity of training sessions. This is helpful to determine whether or not a training problem exists and, more particularly, if the training period or exercise intensity is inadequate.

A high V_4 value according to the age group and state of training usually reflects a good level of fitness. Furthermore, if V_{200} is close to V_4, horses are considered to have a good cardiac response to exercise. Horses with such measurements are considered to have good performance potential, as their large aerobic capacity reduces the their reliance on anaerobic metabolism and delays the onset of fatigue. A standardized exercise test may be useful to define precisely the training intensity level represented either by HR or speed. On the basis of results obtained from different trainers, this author has found that optimal training improvement in horses occurs when horses are exercised according to their individual HR_4 or V_4 results.

In another study conducted by our group (Couroucé et al., 2002) showed V_4 and V_{200} results for 194 horses which performed 1105 standardized exercise tests on a 720-m sand training track. Horses were grouped according to age and state of training. Four groups were based on state of training: (1) beginning (for horses age 1 or 2 years commencing training), (2) endurance training (for horses in training for 3 to 8 weeks involving aerobic exercise), (3) sprint training (for horses in training for more than 8 weeks and now participating in bouts of exercise at higher speeds), and (4) racing (for horses involved in races: mean distance = 2700 m). The results showed that a age and training has a significant influence on V_4 and V_{200}, with these variables increasing with training and age (Figure 29-6).

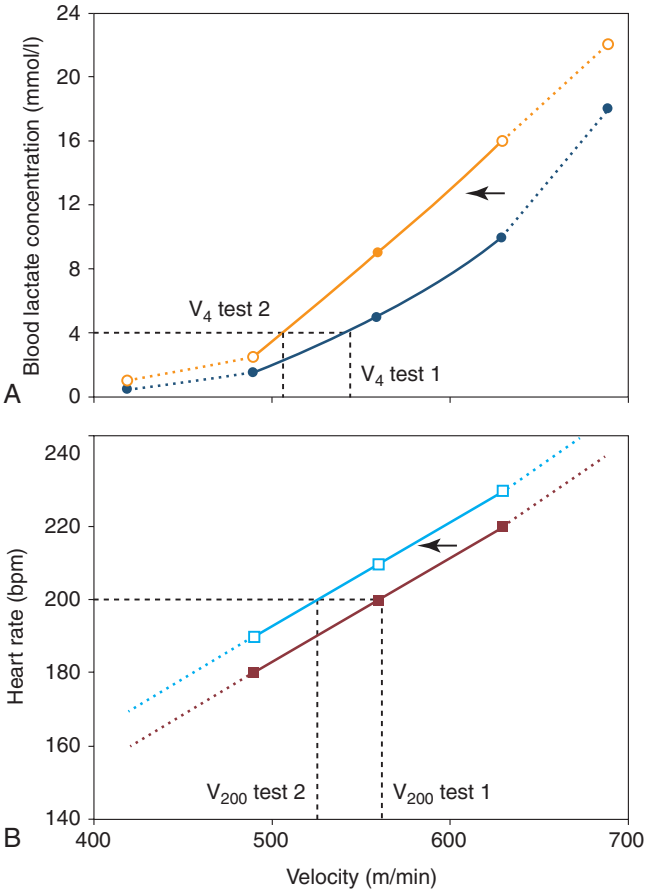

FIGURE 29-6 Mean and 95% confidence interval values for V_4 and V_{200} according to age and training-groups in 194 French Standardbred Trotters. At each age and for a given training state, values with different superscripts are significantly different ($p < 0.05$).

V_4, V_{200}, and Clinical Usefulness

In the context of an athletic horse, another major point is to perform regular field exercise tests to detect underlying problems. To explain further the clinical usefulness of track testing, results from 3-year-old horses performing two exercise tests at a 6-week interval are presented. These horses were in training for 3 weeks at the time of the first test, and performed the exercise tests on a trotting race track (see Figure 29-4).

V_4, V_{200}, and Respiratory Disease

In response to the first exercise test, Horse 2 (H_2) showed the same V_4 and V_{200} values as H_1 ($V_4 = V_{200} = 580$ m/min^{-1}). Both horses undertook the same training program, with H_1 showing a decrease in V_4 and V_{200} after 6 weeks of training (Figure 29-7). A clinical examination performed at the time of the second exercise test revealed a low-grade subclinical infectious respiratory disease. It has been shown variously that abnormalities of respiratory pathways, for example, laryngeal hemiplegia, epiglottic cysts or pulmonary problems, may limit oxygen exchange. Horses with such abnormalities often show an increased blood lactate concentration for the same work intensity and, compared with normal horses, have lower V_4 values. This is of great importance, as respiratory diseases are frequent in horses and a great number of such disorders are subclinical. These diseases may lead to a decrease in gas exchange during exercise and limit performance.

With regard to HR response to exercise, it has been shown that high HR during exercise, particularly submaximal exercise, may result in a decrease of V_{200} in association with respiratory diseases such as chronic obstructive pulmonary disease (COPD) or other pulmonary problems. In this author's experience, the most important variable to consider when one has a suspicion of underlying respiratory disease is blood lactate accumulation, as this is a byproduct of anaerobic metabolism and its accumulation is governed by the availability of oxygen to the tissues during exercise. Furthermore, some horses with respiratory diseases may have low submaximal HR during exercise and, thus, high V_{200} values.

V_4, V_{200}, and Orthopedic Disease

Here is another example in Trotters. In response to the first exercise test, Horse 3 (H_3) had the same V_4 and V_{200} values as H_1 and H_2 ($V_4 = V_{200} = 580$ m/min^{-1}). H_3 undertook the

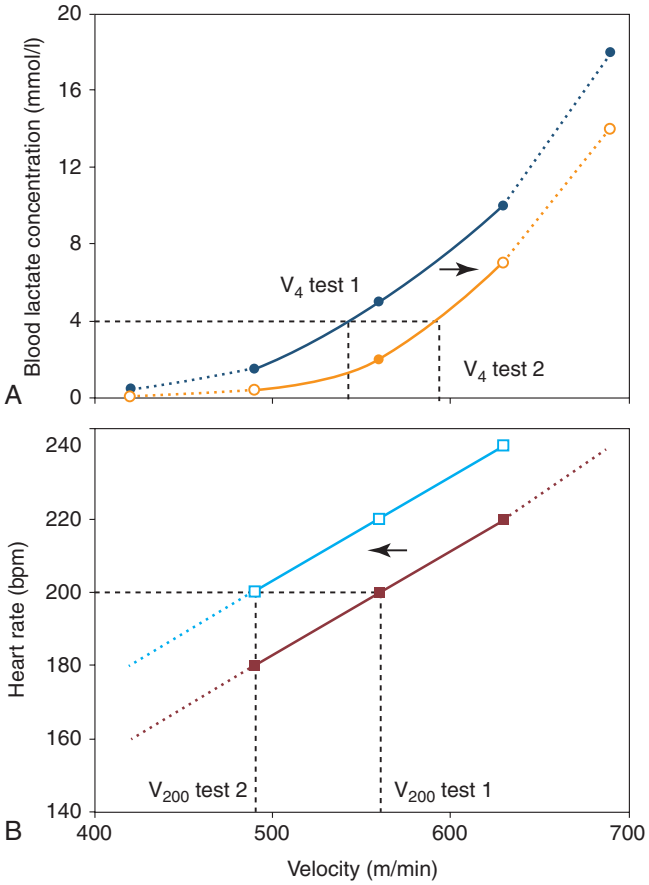

FIGURE 29-7 Influence of a low-grade subclinical infectious respiratory disease on heart rate (HR) and blood lactate relationship to speed on V_4 and V_{200} values (horse H_2). Closed symbols indicate the results of the first standardized exercise test and open symbols indicate the results after a 6-week training program. The results indicate a shift to the right, with improved fitness, of both of the blood lactate concentration (*A*) and HR values (*B*) related to velocity on the track.

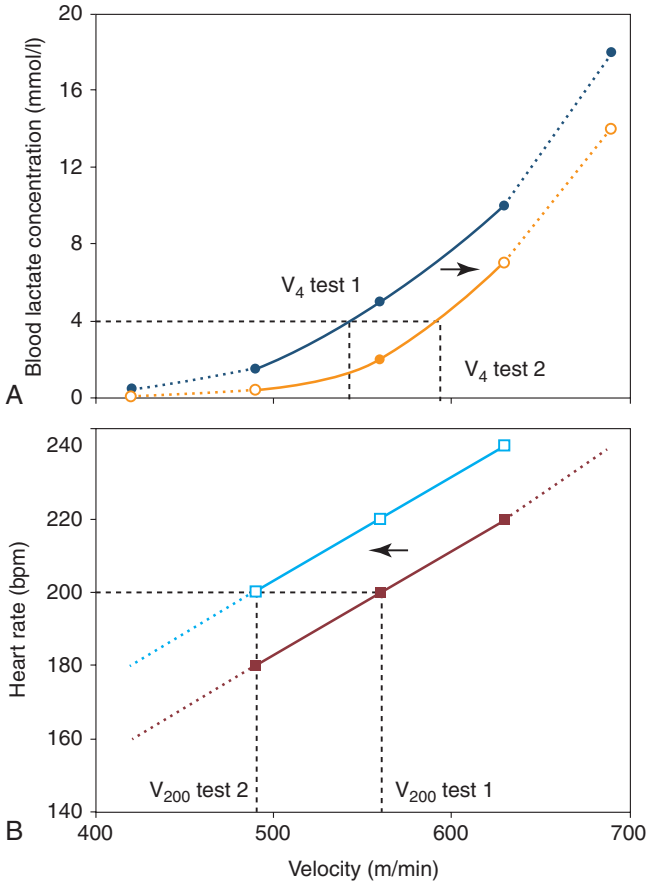

FIGURE 29-8 Influence of a low-grade hindleg lameness (osteochondrosis in the right hind fetlock and in the left hock) on heart rate (HR) and blood lactate relationship to speed on V_4 and V_{200} values (horse H_3). Closed symbols indicate the results of the first standardized exercise test and open symbols indicate the results after a 6-week training program. The results indicate a shift to the right, with improved fitness, of both of the blood lactate concentration (*A*) and HR values (*B*) related to velocity on the track.

same training program as H_1 and H_2. Horse H_3 showed similar improvements in V_4 as H_1 but a decrease in V_{200} (V_{200} = 520 m/min^{-1}; Figure 29-8). Because of this high HR during submaximal exercise, a veterinary examination was performed, and it revealed low-grade bilateral hindleg lameness, subsequently diagnosed as osteochondrosis in the right hind fetlock and left hock. High submaximal HR values and, thus, low V_{200} have been previously described during exercise in the case of lameness and are considered a good indicator of pain and orthopedic disease. Couroucé et al. (1996) also showed that in a population of 100 French Trotters, 66 showed high HR during submaximal exercise. Among this group of 66 horses, 43 were subjected to a thorough physical or clinical examination, with 40 of them having confirmed orthopedic disease. High HR during exercise, particularly higher than expected values during submaximal exercise, is considered useful in the detection of underlying problems or to evaluate how a horse responds to a known disease process. To highlight this, a horse such as H_3, with high V_4 values according its age and training state, shows good aerobic capacity and, thus, should have a good performance ability once the orthopedic problems are rectified. However, as high submaximal HR during exercise often is related to orthopedic diseases, a detailed veterinary examination should always be carried out.

These three examples show how the results of a standardized field exercise test can be useful to identify progress or problems in relation to training and disease states. They also show the importance of careful questioning of the trainer or owner to determine the previous performance level of the horse (total earnings in relation to age and number of races), to evaluate the state of training of the horse (frequency, duration, intensity, and type of exercise) and existence of previous diseases. These examples show that V_4 and V_{200} largely depend on the horse's training level, on the existence of underlying diseases such as respiratory, muscular, or orthopedic diseases, not to mention the physical ability of the horse. We must never lose sight of the fact that a horse that has never shown good training or racing performance might just have a lack of ability. The old adage that "the greatest cause of poor performance is unrealistic expectations" should always be front of mind.

Without doubt, the degree of metabolic fitness plays a critical role in determining the level of racing performance achieved. As is the case with human athletes, a wide range of baseline metabolic fitness is found in athletic horses of all

disciplines, independent of the level of training. These differences are responsible for some of the variability in racing performance observed in otherwise normal horses. However, decreased metabolic fitness associated with pathologic changes in any of the systems involved in exercise or inadequate training can be a primary cause of poor performance.

TRACK EXERCISE TESTS FOR HORSES UNDERTAKING OTHER DISCIPLINES

THOROUGHBREDS

Recently, Evans (2007) reported interesting methods for assessing performance or, more importantly, poor performance in Thoroughbred horses exercising on training gallops. He utilized GPS to determine speed (sender included in the saddle blanket) and a telemetric HR monitor fixed under the saddle, with the HR recorded on a portable digital recorder fixed to the rider's wrist. This allows easy determination of the speed at which maximal heart rate is reached (VHR_{max}). Additionally, this author described in detail determination of derived variables involving the relationship between speed, HR, and blood lactate concentration. From this, Evans drew a number of conclusions, particularly in terms of factors limiting performance in Thoroughbreds. Many of the factors resulting in poor performance in Thoroughbreds mimic those outlined for Standardbred Trotters. These include the following:

- Orthopedic disease
- Respiratory disease
- Loss of fitness due to lack of training or injury
- Cardiovascular disease, including anemia
- Overweight

Additionally, Evans provided an explanation for one of the most problematic issues related to poor performance in horses, which he referred to it as "poor sprint or stamina capacity." Poor racing performance could be attributed to either poor sprint speed or poor stamina (i.e., the capacity to maintain high speed) or a combination of these. Table 29-5 presents a model of these two main factors influencing racing performance; stamina and sprint speed. Evans described the three most important physiologic measures of stamina as HR, blood lactate concentration, and oxygen uptake during exercise. In this scenario, the term *sprint* refers to the fastest speed over a short distance such as 400 to 600 m. Sprint speed is easily measured with a stopwatch or onboard GPS. Poor racing performance may also be caused by a mismatch between the horse's inherent physiology and the racing strategies used. An example cited by Evans refers to a Thoroughbred with only a moderate sprint capacity but superior stamina. In this case, poor performance could have been the result of this horse racing over short distances, whereas ideally it should have been racing over greater distances and should not be relying on a sprint at the end of the race. Assessment of poor performance cases should always include consideration of the trainer's assessment of the horse's sprinting ability in combination with physiologic assessment of stamina.

EVENT HORSES

Various procedures have also been described for event horses (with maximal velocities from 450 m/min to 600 m/min on galloping or all-weather tracks or on grass race tracks. However, these procedures appear to provide difficulty in maintaining consistent steps, particularly their duration, thereby leading to difficulty in interpreting the often-calculated variables or to the test having too many steps.

Thus, a standardized field exercise test for event horses might look something like this: The horse is subjected to a 5- to 10-minute warmup period at slow trot. Then, it undertakes three or four 3-minute steps at velocities between 350 to 600 m/min. Each step will require an increase in velocity of 50 to 80 m/min, depending on the type of track, age of the horse, state of training, and experience. A 1-minute rest is allowed between each step, with a 5-minute active recovery period after the test is completed.

- *Example 1:* Three steps of 3 minutes each: (a) step 1: 400 m/min; (b) step 2: 450 m/min; (c) step 3: 500 m/min, for young horses beginning training or adult horses with lower ability
- *Example 2:* Three steps of 3 minutes each as in example 1, with the steps being (a) 440 m/min; (b) 520 m/min; and (c) 600 m/min, for high-level horses.

SHOW JUMPING HORSES

For show jumpers, a similar testing protocol to that described for eventing horses may be used. However, it must be kept in mind that these horses generally do not undertake the same length or intensity of exercise as do eventers. Thus, step duration may be decreased from 3 minutes to 2 minutes. An example of procedure for these horses might be as follows: a 5-minute warmup at slow trot, followed by three or four 2-minute steps at a velocity between 300 and

TABLE 29–5

A Model for Considering the Physiological Limits to Performance in Racehorses Competing in Events of 1- to 3-Minute Duration

			600 m SPRINT (s)				
Field VHR_{max} m/s	VO_{2max} ml/min/kg bwt	Blood Lactate* mmol/l	<34	34-35	35-36	37-38	>38
>15.3	>150	<4	Good stamina, fast sprint			Good stamina, slow sprint	
14.7-15.3							
13.6-14.4							
<13.6	<120	>10	Low stamina, fast sprint			Low stamina, slow sprint	

VO_{2max} = maximal rate of oxygen consumption; VHR_{max} = the velocity at which the horse reaches its maximal heart rate. *Using method of Evans *et al.* (1993). (From Evans, 2007.)

550 m/min, with 50 m/min increment according to the track and the age and state of training of the horse. A 1-minute rest is allowed between steps and a 5-minute active cool down following the test.

- *Example 1:* 3 or 4 steps of 2 minutes each : 300 – 350 – 400 m/min and sometimes 450 m/min.
- *Example 2:* 3 or 4 steps of 2 minutes each : 350 – 400 – 450 m/min and sometimes 500 m/min.

For saddle horses, the velocity for an HR of 170 beats/min^{-1} appears to be a more reasonable upper limit, as it is often more difficult for horses of this age and breed to achieve an HR of 200 beats/min^{-1}.

TREADMILL EXERCISE TESTS

INCREMENTAL VERSUS HIGH-SPEED TESTS

The majority of studies on exercise testing in athletic horses have used rapid incremental tests. In these tests, the speed of the machine usually is increased every 60 to 120 seconds until the horse can no longer keep pace with the speed of the machine or until a predetermined HR has been obtained. This type of test has proven most popular because data can be gathered during both submaximal and maximal exercise. However, such a test has been criticized on the basis that exercising over sprint distances (less than 1600 m or 1 mile), tests where the speed is gradually increased, may not be the most appropriate for racehorses. In these cases, a "run to fatigue" test, in which horses are exercised at a near-maximal speed or intensity until they can no longer maintain this intensity of exercise, may be used. However, such testing requires that the horse be fully acclimated to treadmill exercise so that rapid acceleration (5 to 6 seconds) to a galloping speed is possible. This type of test has been used in an attempt to measure anaerobic capacity in human athletes by determination of maximal accumulated oxygen deficit (MAOD). This type of test may be adapted for horses, with anaerobic capacity assessed by exercising the horse at 115% to 120% of velocity for maximal oxygen capacity ($\dot{V}O_{2max}$) and the accumulated $\dot{V}O_2$ measured during exercise and subtracted from the total oxygen demand. For measurement of MAOD, submaximal $\dot{V}O_2$ measurements have to be made so that the oxygen demand can be extrapolated above the point of $\dot{V}O_{2max}$.

SPECIFIC PROTOCOL FOR EXERCISE TESTING

No "gold standard" treadmill exercise protocol exists. In summary, two general types of exercise test are available. An incremental velocity exercise test commences with a suitable warmup, perhaps trotting and slow canter (at speeds of 3–5 meters per second [m/s]), followed by increases in velocity every 60 seconds. Measurements may be made during each stepwise increase in velocity. This approach enables description of the relationships between speed and HR, blood lactate concentration, and other variables. This protocol can be terminated during submaximal exercise, before fatigue sets in. However, many exercise tests do necessitate continuing with incremental addition of higher speeds to demonstrate the maximal values of oxygen uptake and HR. The treadmill is rapidly decelerated when the horse is unable to maintain the same speed as the treadmill, despite verbal encouragement.

An alternative protocol employs the warmup, and then the horse runs for 120 seconds at a speed that is chosen carefully to make the desired measurements. For example, 120 seconds of exercise at 10 m/s on a treadmill at an incline of 10% produces a large range of HR and blood lactate concentrations at the end of the run, enabling description of the trained horse's physiologic response to strenuous submaximal exercise. Horses with superior performance have demonstrated lower blood lactate concentrations after such a treadmill test. Single-step exercise tests have also been used in the field in Standardbreds and Thoroughbreds.

MEASUREMENTS DURING A TREADMILL EXERCISE TEST

While treadmill exercise testing may provide a range of information, the most important relates to function of the cardiopulmonary and musculoskeletal systems. These body systems are the ones that are most intimately involved in determination of successful athletic performance. However, the central nervous system is important in exercise fatigue. A number of measurements are possible during treadmill exercise tests, but it is important that the measurements be useful and practical. To be useful in a clinical setting, the measurements must be easy to perform and provide information that is relevant to performance. Such measurements include HR, blood lactate concentrations, arterial blood gases, blood volume, stride length, and oxygen uptake. Additionally, endoscopic examination of the upper respiratory tract during exercise may be valuable in certain cases.

HEART RATE DURING EXERCISE

HR is one of the easiest measurements that can be taken during exercise and provides an indirect index of cardiovascular capacity and function. Many HR meters are available to measure exercising HR. Care should always be taken to ensure good electrode contact with skin. Since stroke volume does not change greatly with increasing exercise speed, HR provides a guide to changes in cardiac output. This has been described in the previous section. The increase in HR as speed increases is greatest in young horses, with HR_{max} being reached at relatively lower exercise intensities. V_{200} can be determined by linear regression analysis using a variety of spreadsheet based programs. As shown earlier, in general, horses with the highest cardiovascular and metabolic capacities will have the highest V_{200} values. Measurement of V_{200} also may be useful for evaluation of improvements in fitness. A high correlation ($r = 0.75$) between V_{200} and $\dot{V}O_{2max}$ has been found in Thoroughbred racehorses.

However, care should be taken in using V_{200} to assess exercise capacity. At an HR of 200 beats/min, horses may be exercising at quite different proportions of their HR_{max} and, therefore, their $\dot{V}O_{2max}$. For example, in a horse with an HR_{max} of 215 beats/min, an HR of 200 beats/min represents 93% of HR_{max}, whereas in a horse with an HR_{max} of 240 beats/min, an HR of 200 beats/min would be only 83% of HR_{max}. In these two cases, the former horse would be exercising close to its maximal cardiovascular capacity at a heart rate of 200 beats/min, whereas in the latter, substantial cardiovascular reserve and different metabolic requirements are present. Additionally, V_{200} values may be affected by excitement and apprehension because of variability in HR at speeds involving trotting and slow cantering, where HR values may be elevated. In determinations of submaximal HR in horses presented for exercise testing, we have found variations in V_{200} of more than 1 m/s in individual animals during repeated tests on sequential days.

Another measurement of cardiovascular capacity is the speed at which the horse reaches HR_{max} ($\dot{V}HR_{max}$). The $\dot{V}HR_{max}$ is correlated with $\dot{V}O_{2max}$ and exercise capacity and is a better indicator of cardiovascular capacity than the V_{200} because $\dot{V}HR_{max}$ is not a relative measurement. The disadvantage with $\dot{V}HR_{max}$ is that unlike determination of V_{200}, the exercise test used must involve horses exercising up to their maximal speeds so that a plateau in HR can be identified. Measurement of V_{200} requires only four submaximal exercise speeds, with the maximum intensity of exercise being equivalent only to about three quarters of racing speed.

CARDIOVASCULAR DISEASE

We have found HR measurements during exercise to be useful in the assessment of the functional significance of some heart murmurs as well as assessment of electrocardiographic conduction abnormalities. Horses with functional cardiac disease will have elevations in submaximal HRs because of reductions in stroke volume. Values for V_{200} less than 7 m/s are abnormal and, if found in a fit horse, indicate decreased cardiac capacity.

TELEMETRIC ELECTROCARDIOGRAPHY

An alternative to using an HR meter for measuring the exercising HR is telemetric electrocardiography (ECG). Today, the quality of the tracings obtained using telemetric ECG during exercise is often excellent. This may provide additional information to simple HR measurements because dysrhythmias may occur during exercise (Figure 29-9).

BLOOD (PLASMA) LACTATE MEASUREMENT

Lactate is produced in exercising muscle during all intensities of exercise. However, above half pace (speeds above 6 m/s on a treadmill set at a 10% slope), lactate production will increase as the aerobic energy contribution becomes insufficient to meet total energy requirements. Additionally, the increased energy demands of exercise result in rapid glycogenolysis, with large amounts of pyruvate production. The result is an increase in lactate production, simply by a mass action effect. Because lactate diffuses from muscle to blood, measurements of blood or plasma lactate concentrations will reflect muscle lactate concentrations. The rate of increase of lactate in the blood, therefore, may be used as an indirect indicator of cardiovascular and metabolic capacity. Horses with the highest aerobic capacities caused by a high maximal cardiac output will tend to have lower lactate values at submaximal exercise intensities compared with those with low aerobic capacities.

Because little information is obtained at the lower treadmill speeds, when we perform incremental treadmill tests and are measuring blood lactate, we collect samples only at 6, 8, and 10 m/s. Alternatively, a single sample collected immediately after the 10-m/s exercise step can be used or at fatigue in the 120-second test.

As discussed above for comparison of blood or plasma lactate values between horses or in the same horse during training, the treadmill speed at a plasma lactate of 4 $mmol/L^{-1}$ (VLA_4) generally has been used. Overall, the higher the VLA_4, the fitter is the horse and the greater is the exercise capacity. Plasma values for $\dot{V}LA_4$ in normal, fit thoroughbred horses that are 3 years of age and over range from 8 to 9.5 m/s (Figure 29-10).

FIGURE 29-9 A horse undergoing telemetric electrocardiography (ECG) during exercise on a lunge for investigation of a suspected cardiac-related cause of poor performance.

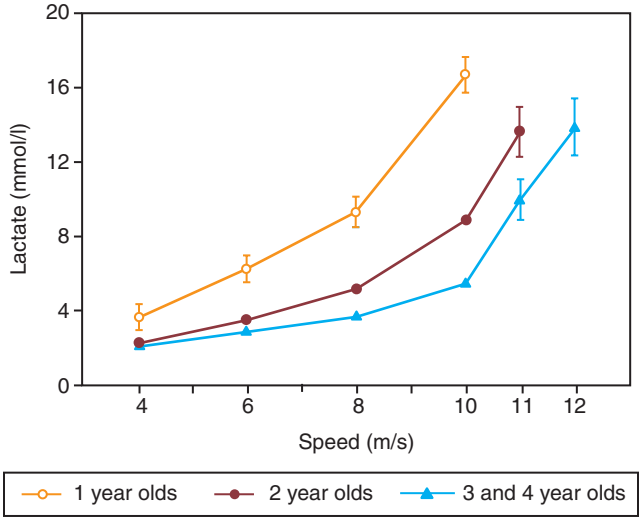

FIGURE 29-10 Normal plasma lactate values in yearling (O), 2-year-old (•), and 3-year-old (Δ) Thoroughbred horses. The 2- and 3-year-old horses were in racing condition, but the yearlings were untrained. Values were obtained with the treadmill set at a 10 percent slope. *(From Rose RJ, Hendrickson DK, Knight PK:* Clinical exercise testing in the normal thoroughbred racehorse, Aust Vet J *67(10):345-8, 1990.)*

ARTERIAL BLOOD GAS ANALYSIS DURING EXERCISE

At exercise intensities above 65% $\dot{V}O_{2max}$ athletic horses become hypoxemic, with arterial blood gas tensions, at sea level, falling from mean values of around 100 mm Hg at rest to 70 mm Hg at or above intensities equivalent to $\dot{V}O_{2max}$. Although this is generally true, the extent of the hypoxemia appears to be much less in some individuals than in others. In horses with low $\dot{V}O_{2max}$ values, the decrease in arterial oxygen tensions may be very small.

Venous blood, although appropriate for assessing acid-base status, is inappropriate for evaluation of gas exchange during exercise. Arterial blood may be collected during exercise by catheterization of the transverse facial artery near the lateral canthus of the eye. Because blood temperature influences blood gas and pH results, a central venous thermistor also must be placed to determine blood temperature during exercise. This allows correction of the values determined at 37°C in the blood gas machine to the blood temperature, which may reach 42°C during maximal exercise.

Arterial blood gas analysis during exercise may be indicated in horses with poor performance suspected to be caused by respiratory disorders (Figure 29-11). On occasion, it may be useful to undertake arterial blood gas determination during exercise in horses that have abnormalities on bronchoalveolar lavage cytology or in the presence of suspicion of an upper respiratory tract abnormality that could interfere with gas exchange.

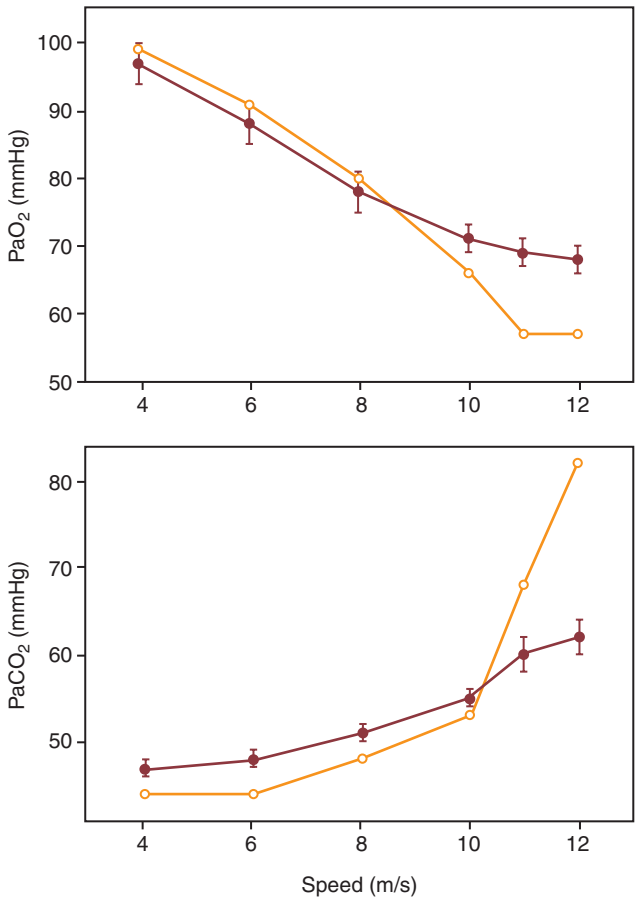

FIGURE 29-11 Arterial blood gas results during exercise in normal racehorses (•) and a horse with idiopathic laryngeal hemiplegia (○).

ENDOSCOPIC EXAMINATION OF THE UPPER RESPIRATORY TRACT DURING EXERCISE

Treadmill endoscopy was proven and continues to be a useful diagnostic aid in horses in which partial upper respiratory tract obstructions are suspected. Treadmill endoscopy was the most useful method for assessing the significance of many cases of upper airway (larynx or pharynx) dysfunction during exercise for most of the 1990s and early 2000s. However, with the advent of DVE for use in the field the need for treadmills to undertake this type of evaluation is diminishing (Figure 29-12 and 29-13).

FIGURE 29-12 Schematic of a horse undergoing dynamic videoendoscopy during exercise under saddle in an enclosed arena.

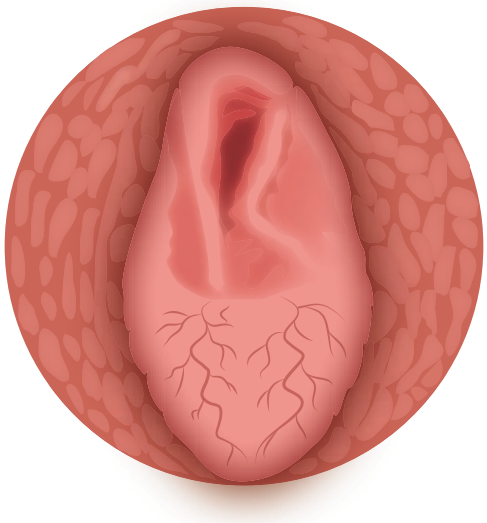

FIGURE 29-13 Left laryngeal collapse in a horse during dynamic videoendoscopy of the larynx druing intense exercise on the training track.

OXYGEN UPTAKE

The measurement of $\dot{V}O_2$ is critical in the assessment of athletic performance. Not only are measurements of submaximal $\dot{V}O_2$ important in calculating the energy cost of exercise, but maximal oxygen uptake ($\dot{V}O_{2max}$), a key indicator of exercise capacity, can be measured. Measurement of $\dot{V}O_2$ is still likely best performed using a gas-collection mask without valves via an open flow method because masks with valves generally have the capacity to impair respiration. The open-flow method for $\dot{V}O_2$ measurement uses a loose-fitting mask or a mask with holes around its perimeter so that respiration is not restricted. A large vacuum motor is used to suck air through the mask at flow rates between 6000 and 10,000 L/min, and a differential pressure transducer is used to ensure that the flow rate across a Venturi device remains constant during exercise. It has been established that gas flow rates in excess of 6000 L/min are adequate to prevent rebreathing of expired gas. Samples of gas are collected, and after dehumidification, the oxygen and carbon dioxide concentrations are determined using appropriate analyzers. From measurement of the flow, gas temperature, oxygen, and carbon dioxide, the $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) can be measured reliably.

$\dot{V}CO_2$ increases linearly as the intensity of exercise increases in a similar fashion to $\dot{V}O_2$. At higher exercise intensities, $\dot{V}CO_2$ increases more rapidly than $\dot{V}O_2$, resulting in an increase in the respiratory exchange ratio, *R*. At intensities approaching $\dot{V}O_{2max}$ values for *R* exceed 1.0, and at $\dot{V}O_{2max}$, *R* values are usually around 1.2 to 1.3 because of the buffering of lactate by bicarbonate as lactate moves from exercising muscle into blood.

Determination of $\dot{V}O_{2max}$ has been a key measurement in assessment of exercise capacity of human athletes since the 1950s. The $\dot{V}O_2$ increases linearly with increasing treadmill speed, and $\dot{V}O_{2max}$ can be identified where a plateau in $\dot{V}O_2$ is present despite an increase in speed (Figure 29-14). In most horses, a plateau in $\dot{V}O_2$ occurs despite an increase in speed (see Figure 29-14), but in some, no such plateau may be found, but rather an asymptote. Where no plateau can be identified, we take the $\dot{V}O_{2max}$ as the maximum $\dot{V}O_2$ recorded in an incremental exercise test in which the horse exercises to fatigue. In humans, $\dot{V}O_{2max}$ is regarded by the majority of human athletes as the "best predictor of athletic potential." A number of studies have shown that the Thoroughbred horse has $\dot{V}O_{2max}$ values that are higher than those of many other mammalian species when expressed on a mass-specific basis. The major factor responsible for the high $\dot{V}O_{2max}$ in athletic horses is their high oxygen-carrying capacity, which arises from a large arteriovenous oxygen content difference and a high stroke volume. Given that the HR_{max} and arteriovenous oxygen content difference do not increase with training, a high value for $\dot{V}O_{2max}$ probably represents a high maximal stroke volume. Because of the rapid kinetics of $\dot{V}O_2$ a high $\dot{V}O_{2max}$ would seem likely to be advantageous for most athletic horses. Although the best athletic horses are likely to have high $\dot{V}O_{2max}$ values, it is unlikely that $\dot{V}O_{2max}$ by itself will be a useful predictor of performance. So, as has been shown in humans, although $\dot{V}O_{2max}$ is a reasonable predictor of performance in human athletes when one is evaluating subjects of different abilities, $\dot{V}O_{2max}$ is less useful in predicting performance of a homogeneous group of athletes.

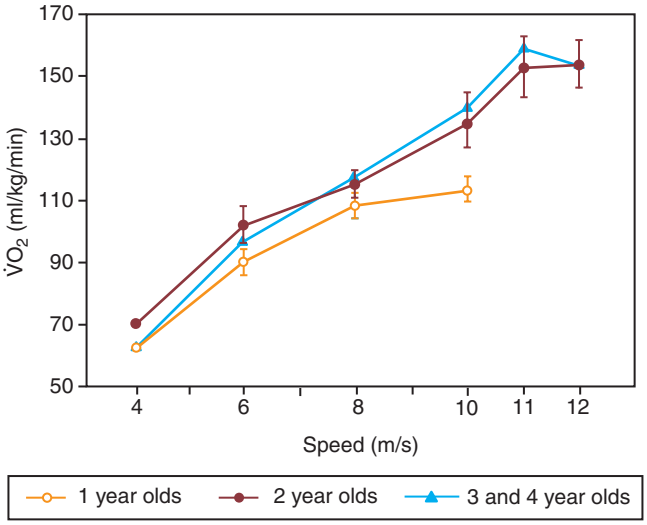

FIGURE 29-14 Oxygen uptake increases linearly with increasing treadmill speed, and velocity for maximal oxygen capacity ($\dot{V}O_{2max}$) can be identified as the plateau in velocity for oxygen capacity ($\dot{V}O_2$) despite an increase in speed. Values for $\dot{V}O_2$ are showin in yearling, 2-year-old, and 3-year-old. Thoroughbred racehorses. *(From Rose RJ, Hendrickson DK, Knight PK:* Clinical exercise testing in the normal thoroughbred racehorse, Aust Vet J *67(10):345-8, 1990.)*

CONCLUSION

Clinical exercise testing provides a way of assessing exercise capacity in an objective way, with the ability to undertake important physiologic measurements under conditions of peak metabolic demand. Such testing continues to be an important advance to our traditional assessments of the athletic horse with reduced performance, as well as providing a method for evaluation of performance potential.

The reason for poor performance often is multifactorial, with the respiratory and musculoskeletal systems being most commonly involved. A careful workup is indicated for horses presented for assessment of poor performance, and in some cases, a clear role for clinical exercise testing may exist. The role of exercise testing in young horses to attempt to determine performance potential is still to be determined. However, it seems likely that exercise testing of horses prior to training has the potential to discriminate among animals of poor, moderate, and superior metabolic capacities. Simple exercise tests also enable trainers to objectively assess the effect of training on improving fitness. This type of testing, with blood or plasma lactate samples collected in response to a standardized test, has considerable potential for both track and treadmill testing.

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