Muscling in on the Cause of Tying-Up

Stephanie J. Valberg, DVM, PhD, Diplomate ACVIM, ACVSMR

Author's address: Professor, Director, University of Minnesota Equine Center, St. Paul, MN 55108; e-mail: valbe001@umn.edu. © 2012 AAEP.

1. Introduction and History of Tying-Up

Horses are supreme athletes whose beauty and performance depends on their powerful musculature. Through careful genetic selection, equine breeders have shaped muscle form and function to produce breeds with muscles fine-tuned for extreme endurance, an elegant piaffe, or a bold stretch across the finish line. Along with selection for positive traits, deleterious traits can also be inadvertently incorporated into the genome. Even minor or intermittent perturbations in muscle function can have a major impact in horses because they are constantly being exercised and pushed toward maximal performance. Fortunately, muscle remains a highly plastic tissue that is capable of adjusting in response to growth, hormonal influences, exercise, disease, and diet. Thus, horses may be able to compensate for deleterious traits if appropriate changes in environment, diet, and training are instituted. The past 30 years have offered exciting discoveries with regard to equine muscle form and function, genomics, inherited causes of muscle disorders, and regimens to manage these traits. These scientific advances built up on a wealth of new knowledge of equine exercise physiology, muscle biopsy, and genomics that led to the discovery of equine muscle disorders, as well as provide an overview of the current state of knowledge of exertional rhabdomyolysis in horses.

History of Exertional Rhabdomyolysis

The dogmas of the quiet past are inadequate to the stormy present. The occasion is piled high with difficulty, and we must rise with the occasion. As our case is new, so we must think anew and act anew.

—Abraham Lincoln

Exertional rhabdomyolysis (ER) in horses is a centuries-old condition that is well described in the literature of the 19th century. In 1883, the clinical signs of ER, or as it was then termed “azoturia,” were described as “sweating and trembling, scarcely able to turn in the stall, the muscles of the back and loins in a state of spasm, tail quite stiff.”1 Azoturia was known to develop in “animals rather handsome, well-shaped, and good thrivers, than others differently constituted.”2 It occurred most often in the fall and winter in horses fed legumes or corn. Further, veterinarians had already recognized that “azoturia
does not, or very rarely, attacks horses roaming at large in the fields, whether young or old; also that in all cases it is more apt to occur under conditions succeeding a period of rather smart or active work that followed enforced idleness.1

The variety of terms used for this condition over centuries is as diverse as its proposed etiologies. Initially “hysteria,” “lumbago,” and “black water” were applied.2 In 1883, the coffee-colored urine found with severe ER was believed to be due to excessive nitrogenous waste and thus the term azoturia was used.1 Veterinarians readily admitted, however, that “in our day azoturia is freely spoken of as something new or strange, but it has apparently been well-enough recognized by many who have preceded us.”1 “Holiday disease” or “Monday morning disease” described the common occurrence of ER after a day or more of rest.3,4 Lay terms “set fast, cording up, and tying-up” well describe the tightening of muscles during episodes. “Paralytic hemoglobinuria” was coined in the late 19th century, based on finding heme pigment in the urine, and this term was replaced with “paralytic myoglobinuria” when myoglobin was identified in urine.2,4 ER is now the most commonly used term that describes the degeneration of striated skeletal muscle with exercise.

The development of ER was a common and catastrophic occurrence during the 19th century and early part of the 20th century before cars replaced draft and carriage horses as a means of transportation. Mortality rates as high as 50% were reported in some clinics.2,4 Although it was an important disease, little was really known about its etiology, and speculation abounded. In 1917, Steffin stated that “No one disease [ER] in the horse has been subject to so many theories, theoretical treatments and hypothetical suggestions as this one.”5 Most early theories were based on observation. A kidney disease was initially believed to cause ER because of the “black water” passed by horses and pain palpable in the loins over the kidney.6 The higher incidence in horses rested, fed a full ration, and then exercised led to a popular theory of nutritional intoxication. “A disturbance or perversion of function, not very well understood, produced by a superabundant supply of a particular nutritive material, leads to the exaltation and ultimate loss of function of particular parts of the voluntary muscular system and the extensive disturbance of the nervous system.”1 Others proposed a streptococcal infection as the basis for ER and thought that cold predisposed horses to the disease.

In the early 20th century, elegant scientific studies by Birger Carlström definitively established that azoturia was a muscular disease with myoglobinuria arising from damaged muscle.2,4 Further, Carlström was able to reproduce ER in draft-type horses by feeding 3 kg of molasses during a period of rest before exercise. Muscle biopsy samples obtained during an acute episode of ER had muscle glycogen concentrations that were twice normal and muscle lactate concentrations up to 3 times normal. As a result of these well-designed studies, Carlström’s theory that a period of rest on a high grain diet brought about a lactic acidosis with exercise that coagulated muscle tissue was persuasively held to be true for all breeds of horses for 60 years. His observations were astute precursors to the discovery of polysaccharide storage myopathy in horses.

As lighter breeds of horses increased in popularity in the mid 20th century, confusion abounded as to whether the milder clinical signs of “tying-up” in lighter breeds of horses had a similar basis as azoturia in draft-type horses. Robertson reported in 1886 that “when developed in lighter animals, and those employed for fast work, the same general causes seem to be in operation which we have already indicated as observed in agricultural horses.” However, a higher incidence was noted in mares than geldings and as a rule, “the female is more susceptible of nervous excitement than the male.” Debate ensued among practitioners as to whether these were one or two conditions, and opinions often varied based on the type of clinical practice. In 1917, Steffin recognized that the development of ER varied with circumstances, localities, environment, feeding customs, breeds, and individuals.5 Meginnis in 1957 further substantiated the view that tying-up and azoturia were different muscle disorders, noting from his practice that there were four significant differences between ER in light versus draft breeds: (1) seasonality (none in racehorses), (2) history (fit racehorses worked daily unaffected by a change in ration), (3) handling (racehorses were less severely affected if walked during an attack, and (4) mortality (much lower in light breeds).7 Many others, however, disagreed and thought that lighter breeds had a milder form of the same disease, azoturia, that afflicted heavier breeds.8,9 One point most authors agreed on was that “until the physiologic chemists can determine the changed chemistry in the metabolism of the normal and ‘tying-up’ horses, prevention and treatment will be merely guesswork.”7

From 1980 onward, there was a dramatic increase in the number of scientific and clinical investigations of ER in horses. Koterba and Carlson10 established that light breeds of horses with ER did not have a lactic acidosis but rather a hypochloremic metabolic alkalosis. Roneus11 and Lindholm determined that deficiency in Vitamin E, selenium, or both were not probable causes of ER in Standardbreds. The proposed theory of hypothyroidism causing ER12 was not substantiated by thyroid-releasing hormone stimulation tests.13 T3 activity measured when horses were not receiving nonsteroidal anti-inflammatories, or by thyroidectomy.14 Electrolyte disturbances including intracellular potassium depletion were also proposed to cause ER.15,16 Substantial electrolyte imbalances were apparent in serum samples from endurance horses.
but not horses having ER with light exercise.\textsuperscript{17–19} Fractional excretion of electrolytes measured in catheterized urine samples suggested that body stores of sodium or calcium were depleted in ER horses, and dietary supplementation was suggested to ameliorate episodes of ER.\textsuperscript{20,21} Subsequent studies, however, showed that there was marked variability in fractional excretion of electrolytes from day to day and horse to horse and found no difference in total daily electrolyte excretion between Thoroughbreds with ER and healthy horses fed the same diet.\textsuperscript{22,23}

A gradual recognition of the complexity of ER that affected a wide variety of equine breeds arose from these studies. To delve deeper into the mystery of ER, we needed a thorough understanding of normal equine muscle responses to exercise; better diagnostic techniques to capture physiologic disturbances in ER muscle; a better understanding of muscle pathology; and development of equine genome maps and sequencing of the equine genome to characterize potential genetic defects. During the later part of the 20\textsuperscript{th} century, an explosion of information and diagnostic techniques in human neuromuscular disorders would prove key to unraveling tying-up. Armed with these tools, we moved from theories of autointoxication or lactic acidosis to a more sophisticated understanding of multiple environmental and genetic causes of equine ER.

II. New Discoveries

1. Muscle Form and Function

A window into the world of equine muscle opened when Arne Lindholm (1974)\textsuperscript{24} and David Snow (1976)\textsuperscript{25} adapted the percutaneous needle biopsy technique for use in horses. A modified 6-mm Bergstrom needle (Fig. 1) made it possible to repeatedly sample unsedated horses and characterize the properties of equine muscle. The gluteus medius muscle was frequently studied in horses because of its major role in equine locomotion. It became possible to integrate knowledge from other species with equine studies to obtain a clear picture of the structure and function of equine muscle. Our understanding was also greatly enhanced when high-speed treadmills were introduced for horses and metabolic responses to standardized exercise were captured by obtaining muscle biopsies before, during, and immediately after various intensities and durations of exercise.\textsuperscript{19} The remarkably athletic ability of the horse was apparent, as was the diversity of muscle characteristics in different breeds.

Gross Arrangement of Myofibers

Locomotor muscles are supplied by motor nerves that branch to enervate a set of myofibers. The number of myofibers enervated by a single motor nerve varies, depending on the design of the muscle for dexterity (fewer fibers per motor unit) or power (many fibers per motor unit). Motor neurons differ in their rates of discharge, with fast-contracting myofibers supplied by fast-discharging phasic motor neurons and slow-contracting myofibers supplied by tonic motor neurons with a slow discharge rate. For efficiency, myofibers are grouped within the muscle such that the slower-contracting fibers used for postural support are frequently located deeper in the muscle and the faster-contracting fibers used for higher speeds are located more superficially.\textsuperscript{26}

In most locomotor muscles, however, the distribution of fiber types is always in a mosaic pattern, with varying percentages of fast and slow fibers along the depth and length of the muscle.

Structure of a Myofiber

Myofibers possess a number of structural adaptations that confer the ability to generate force through contraction. These include a cell membrane capable of propagating an electrical potential, a precise alignment of contractile proteins, and internal membrane structures and energy-generating pathways that can regulate the amount of calcium.
and adenosine triphosphate (ATP) available for excitation-contraction coupling.

**Sarcolemma**

A basement membrane surrounds muscle fibers and is directly linked to the sarcolemma. It provides a scaffold for myogenesis and myofiber regeneration as well as structural support. The sarcolemma maintains the intracellular milieu, actively transports substrates into the myofiber, 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 sarcolemmal occurs via glucose transporters (GLUT). GLUT-1 is constitutively present in the sarcolemmal and provides basal glucose uptake, whereas GLUT-4 is present in endosomes in the sarcoplasm, which migrate to the sarcolemmal when stimulated by insulin and contraction-dependent processes.27 Transport of long-chain fatty acids occurs via translocases28 located in the sarcolemma, whereas short- and medium-chain fatty acids enter the myofiber by diffusion.29

The sarcolemmal properties of excitation and conduction are largely due to the presence of membrane-spanning, ion-conducting pathways that regulate the selective and nonselective conductance of sodium, potassium, calcium, and chloride. Voltage-gated channels contain additional voltage-sensing transmembrane domains and are essential for the generation and modification of action potentials. Sodium/calcium exchangers and ligand-gated ion channels set myoplasmic calcium concentrations. Tubular invaginations of the sarcolemma called T-tubules project deep within each myofiber at regular intervals perpendicular to the cell length. The T-tubule membranes contain numerous voltage-gated calcium channels called dihydropyridine receptors (DHPR) and serve to transmit electrical impulses into the interior of the myofiber, where they can almost simultaneously initiate myofibrillar contraction.

**Sarcoplasmic Reticulum**

The sarcoplasmic reticulum (SR) is physically separate from the sarcolemma and surrounds each myofibril in a highly repeating pattern (Fig. 2). 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.

**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 release of calcium ions from the terminal cisternae into the sarcoplasm by opening the RYR in the SR membrane (Fig. 3). This elevates the calcium ion concentration surrounding the myofilaments in the sarcoplasm from $10^{-7}$ to $10^{-5}$ M.

Calcium released into the sarcoplasm binds to troponin, 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. Relaxation of myofibrils occurs...
through active transport of calcium ions into the lumen of the SR by the SR calcium-ATPase (SERCA).

Perturbations in the regulation of excitation-contraction coupling can lead to degeneration of muscle fibers through elevation in sarcoplasmic calcium concentrations. Although some excessive calcium can be sequestered by the mitochondria, eventually mitochondria become overloaded, and oxidative metabolism ceases; oxygen free radicals are generated; phospholipases are activated, inducing the arachidonic cascade; calcium-dependent proteases are stimulated; and complement is activated all leading to myofiber degeneration.

**Contractile Proteins**

The ability of skeletal muscle to shorten is conferred by a strategic alignment of contractile proteins called myofilaments that form a myofibril. The repeating unit of myofilaments within the myofibril is referred to as a sarcomere, the fundamental unit of contraction. Muscle contractions occur when, within each sarcomere, thin myofilaments slide over the thick myofilaments, bringing consecutive ends of the sarcomere closer together. Thick myofilaments consist of two myosin heavy chains (MHC) arranged in a double helix. At one end, each heavy chain forms a globular head that has binding sites for both actin and ATP, as well the enzyme ATPase. Thin myofilaments consisting primarily of actin have a complementary binding site for the myosin globular head. The interaction between myosin globular heads and actin is regulated by tropomyosin and troponin.

The composition of myosin heavy chains within sarcomeres varies between individual myofibers and confers a range of speed of contraction. Originally equine muscle fiber types were distinguished by ATPase stains, which relied on the distinct sensitivity of myosin ATPase enzyme in different fiber types to variations in pH. Preincubation of equine muscle at pH 4.6 before ATPase staining reveals three fiber types, darkly stained slow-twitch type I fibers, lightly stained fast-twitch type IIA fibers, and intermediate staining fast-twitch type IIB fibers (Fig. 4). Immunochemical techniques that use antibodies directed against various MHC isoforms also have been developed for fiber typing. (Roman numerals and capitals are used here to distinguish fiber typing using histological techniques.) Immunochemical techniques that use antibodies directed against various MHC isoforms also have been developed for fiber typing. (Numbers and small-case letters are used here to distinguish fiber typing using immunohistochemistry.)

**Equine Studies**

Equine skeletal myofibers may express the following distinct MHC isoforms: perinatal (or neonatal), slow, fast-type 2a, fast-type 2x (or 2d), or a hybrid of 2a/2x. The speed of contraction of these myosin heavy-chain isoforms increases in the order listed above. The gluteal muscle contains type 1, type 2a, and 2x fibers as well as hybrid 2a/x fibers. In general, type IIB fibers correspond to type 2x fibers,

![Diagram](image-url)
although hybrid 2a/x fibers are variably captured in ATPase stains as type IIA or type IIB fibers.\(^{34}\) (In this review, for simplicity, numbers and lower case letters will be used throughout and the term type 2x will be used recognizing that in many referenced studies fiber typing was actually done using histochemical techniques.)

Compared with human locomotor muscles, equine gluteal muscle has a smaller proportion of type 1 fibers and a much higher proportion of type 2 fibers, particularly type 2x fibers. There can be a range of fiber type composition in different equine muscles from 100% type 2x in the cutaneous trunci muscle to 60% type 2x in gluteal muscle to 0% type 2x in digital flexor muscles. Breed, training, and sex have all been found to affect the ratio of type 2a:2x fibers in gluteal muscle with, in general, breeds adapted to slower speeds, highly trained horses, and males having higher type 2a:2x fiber ratios.\(^{35}\)

**Muscle Mitochondria**

Mitochondria are primarily located under the sarcolemma and between myofibrils.\(^{36}\) The enzymes required for generation of ATP via the citric acid cycle, oxidative phosphorylation, and beta oxidation of fatty acids are strategically located within mitochondria (Fig. 5). The citric acid cycle enzymes (with the exception of succinate dehydrogenase) and beta oxidation enzymes are located in the mitochondrial matrix, whereas the electron transport chain is located in the inner mitochondrial membrane. Pyruvate produced by glycolysis is actively transported

![Fig. 4. A, Myosin ATPase stain pH 4.6 of normal gluteal muscle showing three muscle fiber types: black slow-twitch type 1 fibers, white type 2a fibers, and brown type 2b fibers. B, Myosin ATPase stain pH 4.6 of gluteal muscle obtained immediately after exercise from a Standardbred trotter that developed ER. Arrows indicate degeneration of fast-twitch type 2 fibers.]()

![Fig. 5. Anaerobic energy metabolism within the sarcoplasm derives ATP through the metabolism of glycogen to pyruvate and then lactate. Aerobic energy metabolism within mitochondria utilizes pyruvate or beta oxidation of fatty acids to generate acetylCoA, which is then metabolized in the citric acid cycle to generate electrons that are harnessed to produce ATP via the electron transport chain.]()
across the inner mitochondrial membrane and into the matrix, where it is oxidized and combined with coenzyme A. The citric acid cycle oxidizes the acetyl-CoA to carbon dioxide, and, in the process, produces reduced cofactors that are a source of electrons for the electron transport chain. The redox energy is transferred to oxygen in several steps, and the electrochemical gradient this produces across the inner mitochondrial membrane is coupled to the generation of ATP.

**Metabolic Properties of Equine Muscle Fiber Types**

Muscle fibers have characteristic oxidative and glycolytic properties (Table 1). These are determined biochemically by measuring the activities of citric acid cycle enzymes such as citrate synthase or succinic dehydrogenase and glycolytic enzymes such as lactate dehydrogenase or subjectively determined by histochemical staining with nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR). Across species, type 1 fibers have lower glycogen, lower glycolytic capacity, and higher lipid and oxidative capacity relative to type 2 fibers. Type 2a fibers have intermediate properties, whereas type 2x fibers have higher glycogen, higher glycolytic, and lower lipid and oxidative capacity than the other fiber types. It is important to note, however, that the metabolic properties of fibers can vary widely, especially with training. For example, type 2x fibers in some trained Thoroughbreds have a greater oxidative capacity than type 1 fibers in an untrained horse.

**Responses to Exercise**

**Muscle Fiber Recruitment**

For smooth, coordinated locomotion, muscles are recruited in an orderly manner. Within a particular muscle, myofibers 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 alpha motor neurons of specific types, 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. During low-intensity submaximal exercise, type 1 and 2a fibers are recruited; however, if extremely prolonged low-intensity exercise is performed, type 2x myofibers may eventually be recruited. As the speed of exercises increases, muscle fibers are recruited in the order of type 1, type 2a, and type 2x.

**Energy Metabolism With Submaximal Exercise**

Muscle glycogen concentrations fall steadily in type 1 and 2a fibers during submaximal exercise as a result of metabolism of glycogen to pyruvate, oxidation of pyruvate in the citric acid cycle, and close coupling with the electron transport chain to produce ATP. High rates of pyruvate oxidation inhibit phosphofructokinase (PFK) and slow glucose utilization in favor of free fatty acid (FFA) oxidation. Thus, as exercise progresses, the fall in glycogen is attenuated by oxidation of FFAs. A drop in plasma insulin and rise in cortisol with exercise facilitates release of FFA from adipose tissue, increasing their availability within 15 minutes of initiating submaximal exercise. Long-chain FFAs are actively transported into the myofiber by translocases and utilize a carnitine transport shuttle to traverse the inner mitochondrial membrane. Carnitine also serves to buffer excess fatty acid CoA moieties that may be formed. Beta oxidation of FFA is an essential process for prolonged exercise and spares the finite stores of glycogen with skeletal muscle. Amino acids may also serve as energy substrates within skeletal muscle under conditions of restricted energy intake or prolonged exercise. Fatigue usually occurs with extended submaximal exercise as a result of dehydration, electrolyte depletion, hyperthermia, glycogen depletion, or central fatigue.

**Equine Studies**

Using tissue snap-frozen in liquid nitrogen, Lindholm, Snow, and many other subsequent researchers captured metabolic activity of equine muscle exactly as it was at rest or when sampled...
after various intensities of exercise. The changes in glycogen, ATP, inosine monophosphate (IMP), and lactate concentrations that occurred with exercise were completely dependent on the speed, duration, training level, and muscle fiber type composition of the horse. The information derived from these studies of normal muscle metabolism was integral to compare subsequent studies of muscle metabolism in horses with ER.

In contrast to humans, horses were found to have higher resting glycogen concentrations, ranging from 450 to 650 mmol/kg dry weight (dw) (human, 300 to 400 mmol/kg), with the highest concentrations found after long term training. Further, in contrast to humans, muscle glycogen concentrations in healthy horses were found to be resistant to carbohydrate loading and slow to replete after exercise. Resting equine muscle lactate (equine, 24 to 40 mmol/kg) and ATP (22 to 28 mmol/kg dw) concentrations were slightly lower than in humans, and resting IMP concentrations in both species were found to be <0.25 mmol/kg dw. Submaximal exercise studies in horses lasting 90 minutes showed a decline of glycogen in the range of 25% to 40%, depending on the speed of exercise, whereas repeated bouts of submaximal and sprint exercise at 100% maximum oxygen uptake over 3 days produced glycogen depletion of 50%, and depletion was pronounced at 56% after 80-km endurance rides.

Energy Metabolism With Near-Maximal Exercise Intensity

For a few seconds of high-speed exercise, creatine phosphate (CP) and ATP serve as small reserves of immediate energy until ATP production from glycogenolysis begins. The rapid rate of energy generation required and potentially limited oxygen supply at high speeds drives the conversion of glycogen to pyruvate to lactate via anaerobic glycolysis. It is important to note that anaerobic glycolysis is not the sole source of energy of high-speed exercise. It produces energy over and above that simultaneously being supplied by oxidation of pyruvate in mitochondria. As the muscle reaches its maximum ability to generate ATP from oxidative metabolism (at point of maximum oxygen uptake), all three muscle fiber types are recruited and anaerobic glycolysis produces an exponential rise in lactate. With maximal exertion, ATP also may be generated by the myokinase reaction utilizing adenosine diphosphate (ADP), produced by the hydrolysis of ATP. This metabolic process is facilitated by removing adenosine monophosphate (AMP) to prevent enzyme inhibition accomplished through deamination of AMP to IMP. Fatigue with maximal exertion may occur as a result of hydrogen ion accumulation and a drop in muscle pH, which affects myosin-actin interactions, reuptake of calcium into the sarcoplasmic reticulum and PFK activity. In addition, ATP depletion can occur in individual muscle fibers, causing fatigue.

Equine Studies

Relative to humans, equine muscle has remarkably high muscle glycogen concentrations, high oxidative and glycolytic capacities, and a remarkable ability to produce and withstand high lactate and low ATP concentrations with intense exercise. Muscle biopsies obtained after near-maximal exercise over 800 m show a decline in glycogen of 28% and ATP by 30%, an 8-fold increase of IMP, and 9-fold increase in muscle lactate, dropping muscle pH to 6.4. Even with maximal exertion, only a proportion of myofibers in a gluteal biopsy are recruited and thus studies that measure the general change in substrates within a muscle biopsy may underestimate changes that occur in active fibers. Essen Gustavsson developed an intricate technique to dissect and type individual freeze-dried fibers from postexercise muscle samples. Single-fiber studies revealed that whole-muscle biopsies do not accurately reflect the metabolic events in individual muscle fibers. Of particular note was the fact that whole-muscle ATP concentrations of 18.3 ± 2.7 mmol/kg dw were measured in Standardbreds after a race, whereas ATP concentrations as low as 1 mmol/kg dw were found in individual fibers. This single-fiber technique would prove extremely useful for evaluating potential disruption in energy metabolism in ER-affected muscle.

Normally, safety mechanisms are in place in healthy horses to prevent metabolic processes from proceeding to the point of damaging the internal milieu of the myofiber. These mechanisms include the inhibition of PFK by AMP and a low pH, thereby slowing glycolysis and acidosis as well as nociceptors within the muscle that create discomfort and drive a centrally mediated decrease in locomotion.

Impact of Training on Skeletal Muscle

The major training adaptation of equine muscle is an increase in aerobic capacity. For this adaptation to occur, a threshold of training intensity is required over a minimum training duration. The benefits of training for horses performing at submaximal aerobic exercise intensities include enhanced delivery of oxidative substrates to muscle fibers as well as improved oxidative metabolism of glycogen and free fatty acids. Muscle strength and power output may also be improved. Especially in endurance horses, greater free fatty acid utilization leads to a glycogen-sparing effect and prolonged endurance capacity. The benefits of enhanced oxidative capacity for horses performing maximal intensity exercise include increased capacity to generate ATP aerobically, which allows horses to achieve higher speeds before the onset of lactate accumulation and ATP depletion.
These advances in exercise physiology in the later part of the 20th century fueled an explosion of information in the field of human and subsequently equine neuromuscular disorders. Specialized neuromuscular laboratories were established where neurologists began to personally evaluate frozen sections of muscle biopsy specimens from their patients, using histochemical and biochemical techniques, and closely correlate results with their clinical evaluation.

2. Neuromuscular Diagnostic Laboratories

Neuromuscular diagnostic laboratories formed the cornerstone for the identification of specific etiologies for ER in horses. A centralized site to receive muscle biopsies from across North America facilitated (1) assimilation of the astute observations of large numbers of practitioners submitting biopsies, (2) accumulation of hundreds of cases from which to identify patterns of disease, (3) banking of biochemical and DNA samples that could be used in investigating the pathophysiology of disease subsets, and (4) formation of a research hub where breeders, equine practitioners, physiologists, biochemists, and molecular biologists could collaborate on muscle diseases in horses.

The first neuromuscular diagnostic laboratory that provided routine diagnostics in veterinary medicine was developed by Dr. Cardinet III at the University of California, Davis, in the late 1970s. Dr. Cardinet’s training at the National Institutes of Health and his close collaboration with neurologist Dr. Terryl Holliday provided a unique, clinically focused platform to investigate muscle diseases in veterinary medicine. Dr. Cardinet’s laboratory served as the launching point for both the Comparative Neuromuscular Laboratory at the University of California, San Diego, and the Neuromuscular Diagnostic Laboratory at the University of Minnesota.

The interpretation of biopsies at Neuromuscular Diagnostic Laboratories combined findings from evaluating a battery of histochemical stains of frozen tissues with history, clinical signs, and, once developed, genetic testing. Findings differed from those gleaned by pathologists examining hematoxylin and eosin (H&E) stains of formalin-fixed tissues. Formalin cross-links proteins producing indefinite preservation of tissues; however, in muscle it also creates artifactual vacuoles (Fig. 6A), leaches substrates such as glycogen, and inactivates critical contractile and metabolic enzymes used for fiber typing. Further, formalin-fixed tissues are not useful for biochemical assays or isolation of mRNA for genetic studies. Thus new insights into specific muscle diseases were gleaned from histochemical studies of frozen equine muscle tissue (Fig. 6B).

3. Development of Genetic Tools for Studying ER

Equine genomics remains a new frontier in disease investigation in horses. The remarkable development of genomic maps and the sequencing of the equine genome in the past 15 years provided new tools to investigate potential heritable bases for ER in horses. The first suggestion that ER may have a heritable basis appears to have come from McLean in 1973, although an individual susceptibility to ER had been noted prior to this. The suggested heritability of ER was not based on observations of a familial history but on the discoveries in humans from the 1950s and 1960s that glycogen storage disorders and myoglobinuria could arise from inherited defects in glycogenolysis/glycolysis. A familial basis may have been difficult to recognize because clinical signs of ER develop years after horses are dispersed by breeders and because environment had such a strong impact on disease penetrance. A genetic basis for ER was initially investigated in Standardbred horses, using blood typing and in Quarter Horses and Thoroughbreds by pedigree analysis. These studies, however, were only suggestive and did not prove inheritance.
Breeding Trials

Breeding trials in Quarter Horses\textsuperscript{80,81} and Thoroughbreds\textsuperscript{79} were required to establish that some forms of ER are passed down through family lines. Breeding trials are ideal when the heritable nature of a trait is in question and the trait is believed to be caused by a single gene. The classic approach is to matе second-generation heterozygotes and analyze the proportion of affected and unaffected offspring to determine the probable pattern of inheritance. The numbers of animals required usually makes such trials cost-prohibitive to perform in veterinary medicine. Fortunately, however, the American Quarter Horse Association and the Morris Animal Foundation had the foresight to support breeding herds of Quarter Horses and Thoroughbreds with ER that were crucial to establishing the nature of inheritance of their specific forms of ER. Further evidence of the heritable nature of forms of ER in horses awaited development of equine-specific genetic tools.

Candidate Gene Approach

The first genetic approach available to equine researchers prior to development of equine genome maps was the candidate gene approach. This approach was first used by Dr. Sharon Spier to identify the genetic basis for hyperkalemic periodic paralysis in Quarter Horses\textsuperscript{82} and was successfully used to approach was first used by Dr. Sharon Spier to identify the genetic basis for hyperkalemic periodic paralysis in Quarter Horses\textsuperscript{82} and was successfully used to investigate the ryanodine receptor 1 (\textit{RYR1}) gene as a cause for ER by Dr. Monica Aleman.\textsuperscript{83} A candidate gene is usually identified comparatively, where another species is found to have a disease with similar clinical signs and a genetic defect has already been identified in that species. Dr. Aleman recognized that the clinical signs she observed in two Quarter Horses under general anesthesia were identical to those of pigs with malignant hyperthermia (MH).\textsuperscript{84} Selected exons of the putative candidate gene, the (\textit{RYR1}), were sequenced from cDNA prepared from snap-frozen muscle samples from healthy and affected horses. Polymerase chain reaction (PCR) primers designed on the basis of known sequence homology from other species were utilized to generate templates to sequence. A point mutation in exon 46 was identified in horses with MH, and horses with this mutation were subsequently found to show signs of ER.

Whole blood for isolation of genomic DNA and frozen tissue for mRNA are ideal for candidate gene approaches to muscle disorders. The availability of complete genome sequence helps to predict intron exon boundaries and sequencing of just the coding exons by PCR from a simple DNA sample can be achieved. Sequence polymorphisms are identified when comparing normal and affected individuals. It is important to note, however, that many base-pair substitutions are of little consequence because they do not change the amino acid code (silent mutations). On the other hand, missense, nonsense, frameshift, and splice site junction mutations can all cause altered structure, function, or synthesis of the encoded protein and potentially be causative of a disease. Roadblocks with this direct sequencing approach include sequencing the wrong gene and missing large structural rearrangements of a gene that result in no mRNA production or simply altered levels of mRNA. The larger structural rearrangements can, however, be detected using Southern blotting techniques on genomic DNA or large-scale DNA sequencing.

Genome-Wide Association Mapping and Sequencing

The ability to perform genome-wide mapping was highly desirable for studying ER because for most forms of ER, no likely candidate gene were readily apparent. It requires only the hypothesis that the disease is inherited and the mutant genes are somewhere in the genome. This approach seeks to identify genetic markers closely associated with the chromosome containing the as-yet unknown affected gene, and, through fine mapping, zero in on likely candidate genes. The initial limiting factor for its application in horses was the lack of readily available equine genome maps with polymorphic markers evenly spaced across the genome. Development of equine genome maps began in the 1990s, based mainly on short repetitive DNA sequences called microsatellites, in which alleles differ in the number of nucleotide repeat units. Collaborative international efforts generated two equine genetic linkage maps, each containing more than 740 microsatellite markers.\textsuperscript{85,86} A 4,000 marker whole-genome equine-hamster radiation hybrid RH map including 2,000 microsatellites, and 2,000 gene loci was also developed to enable both microsatellite and gene-based markers to be efficiently assigned to a horse genome map.\textsuperscript{87} This achieved the goal of a genome-wide coverage with an average marker spacing of less than 1 Mb and ready comparison of the same gene markers on the equine chromosome maps with their homologues on the chromosomes of other species. These microsatellite genome maps proved useful for the whole-genome association analysis that identified the genetic mutation responsible for one form of ER in Quarter Horses, polysaccharide storage myopathy.\textsuperscript{81} A panel of markers spaced across all 31 autosomes was initially screened in 48 control and 48 PSSM-affected horses. Associated markers were identified on equine chromosome 10 (ECA10), and reanalysis using a denser panel of markers on ECA10 narrowed the region to an area that contained an excellent candidate gene the glycogen synthase 1 (\textit{GYS1}) gene. Sequencing of this gene revealed the causative genetic mutation.

Linkage Analysis

Another approach that was also used to study the genetic basis for ER in horses is linkage analysis. Genetic linkage analysis ideally uses multi-genera-
tion pedigrees with many full or half-sib offspring, whereas genetic association utilizes populations of relatively unrelated individuals. Like the association studies described above, a subset of the DNA markers from the genome map are statistically analyzed for co-inheritance or association with the trait in appropriate resource populations. Genetic linkage would indicate that the gene for the disease is located close to the genetic marker and therefore it does not dissociate from the marker during random crossovers in meiosis. Due to widespread geographic distribution of related horses and the difficulties created in phenotyping and sample collection, it is very difficult to assemble sufficiently sized pedigrees to perform linkage analysis in horses. This approach, however, was used in Thoroughbred horses where samples were collected through breeder cooperation and from a breeding trial.\(^{88}\) Linkage analysis was used to exclude potential candidate genes involved in excitation-contraction coupling as causative of ER in that cohort of Thoroughbred horses.

**Single Nucleotide Polymorphism Chips**

In 2008, whole-genome sequencing of the female Thoroughbred Twilight was achieved by scientists at The Broad Institute under the auspices of the National Human Genome Research Institute.\(^{89}\) This sequence covered 2.42 gigabytes (Gb) of the 2.67 Gb horse genome and was of sufficient coverage to give almost complete, or at least partial, sequence of all the equine genes, as well as define repetitive sequence content and nature. This sequence was aligned with those of human and other mammalian genomes to define syntenic segments and predict the locations of equine genes with insufficient sequence coverage. To enhance the ability to discover genes associated with diseases and specific traits in horses, a denser set of polymorphic markers was required than the microsatellites identified previously. These new markers were selected from the approximately 1.5 million single nucleotide polymorphisms (SNPs) that were identified from the sequence of Twilight and seven breed representatives, derived from both recently developed and ancient breeds. SNPs exist on average at approximately every 1,500 base pairs (bp) of horse sequence. To further facilitate equine genetic discovery, a commercial chip that contained 50,000 SNPs was developed for automated whole genome mapping. Analysis of such SNP chip genotype data processed from affected and control horses searches for statistical associations between the frequency of alleles of SNP markers and the disease phenotype. SNP chips have been used to search for potential genes causing ER in Thoroughbreds. The University of Minnesota research group identified a region on equine chromosome 16 that was significantly associated with ER in Thoroughbreds.\(^{90}\) The next steps involve searching for potential candidate genes in the relatively short genomic regions, based on proposed function (basically as with candidate genes above). If the function of the genes in the region are known or their expression in various tissues is known, their potential likelihood for producing the physiologic basis for the disease helps to select genes for subsequent sequencing. Unfortunately, despite an enormous effort in human medicine, the function of a large fraction of all genes remains unknown, complicating selection of the appropriate gene for sequencing. It is highly likely, however, that further progress in developing tools for genome analysis in horses will lead to the unmasking of more genetic mutations that cause muscle disorders in horses.

### III. Clinical Approach to Horses With ER

The advances described in the preceding section were all essential for developing a modern approach to evaluating horses with ER. A thorough understanding of muscle contraction and relaxation as well as the documentation of metabolic responses that normally occur with exercise provided an ideal basis from which to compare muscle function in horses with ER. The development of a safe muscle biopsy technique and laboratories specialized in interpretation of muscle disease in frozen sections widened the ability to identify specific diseases. Advances in diagnostic techniques for equine internal medicine opened up new avenues to standardize the clinical approach to muscle diseases. Finally, the development of genome maps and sequencing of the equine genome provided tools to investigate the genetic basis for ER.

The modern approach to evaluating horses with ER that arose from many previous studies is described in this section.

**History**

A detailed history is the foundation for evaluating ER because the disorder can be intermittent in nature and not evident on physical examination and also because identifying the environmental stimuli that trigger ER is necessary to appropriately manage the condition. A careful description of the horse’s muscle tone, muscle mass, gait, degree of pain, exercise intolerance, and weakness while experiencing clinical signs as well as the duration and frequency of signs is important. Further, an effort to characterize possible eliciting factors such as the horse’s temperament, diet, previous performance, exercise schedule, evidence of exercise intolerance, type and duration of exercise performed when experiencing episodes, mental state when episodes begin, other possible precipitating factors, and current medications should be made. Accumulating historical information on a number of horses of a variety of breeds revealed that some horses seem to have an extrinsic cause for ER that was sporadic in nature, whereas in other cases, even under the best management, horses will have repeated episodes of ER.
Thus, a subset of horses appeared to have an intrinsic abnormality that caused chronic ER.

Physical Examination
Regardless of the cause of ER, horses with acute signs of ER share features of muscle stiffness, sweating, firm painful muscles, elevated respiratory rate, and reluctance to move. Even when acute signs are not present, however, the physical examination may reveal alterations in muscle mass and symmetry or symmetrical atrophy characteristic of a recent severe acute episode of ER. Inspection of the horse at a distance for symmetry of muscle mass while the horse is standing with forelimbs and hind limbs exactly square is imperative to assess muscle mass, symmetry, and signs of atrophy. Subsequently, palpation of the entire muscle mass of the horse provides valuable information regarding muscle tone, heat, pain, swelling, subtle muscle atrophy, and fasciculations. Firm, deep palpation of the lumbar, gluteal, and semimembranosus and semitendinosus muscle may reveal pain, cramps, or fibrosis. A lameness evaluation, including flexion tests, is often indicated because chronic lameness may be present that exacerbates ER and must be treated before normal fluid muscle function returns. Muscle pain may be secondary to changes in movement caused by lower limb lameness. A neurologic examination should be performed to determine if this is an underlying cause of abnormal movement in which owners have attributed a gait abnormality to ER.

Ultrasonography is potentially useful for identifying focal area of muscle trauma with physical disruption of the muscle. Horses with classic signs of ER and no external evidence of muscle stiffness may have rhabdomyolysis within the psoas muscles, which can be identified by rectal palpation and ultrasound. Careful comparisons must be made between similar sites in contralateral limbs in both transverse and longitudinal images because the typical striated echogenic pattern varies according to the muscle group. The appearance of muscle is also sensitive to the way the horse is standing and whether the muscle is under tension, so it is important that the horse is standing squarely and bearing weight evenly. Muscle fascia appears as well-defined, relatively echo-dense bands. Care must be taken in identifying large vessels and artifacts created by them. In an acute injury, muscle fiber disruption is seen as relatively hypoechoic areas within muscle, with loss of the normal muscle striation. The jagged edge of the margin of the torn muscle may be increased in echogenicity. Tears in the muscle fascia may be identified. The defect in muscle may be filled by a loculated hematoma that is slowly replaced by hyperchoic granulation tissue. Muscle repair shows a progressive increase in echogenicity. Relatively hyperechoic regions may develop due to fibrous scarring. Hyperchoic regions causing shadowing artifacts reflect mineralization.

Serum Creatine Kinase and Aspartate Transaminase
The most rapid diagnosis of severe acute ER for hundreds of years has been catheterization of the bladder and examining the urine for pigmenturia that is HB-positive on urine stick tests in the absence of intravascular hemolysis and hematuria. Diagnosis was greatly simplified by research that showed that elevations in serum creatine kinase (CK) and aspartate transaminase (AST) activities were indicative of rhabdomyolysis. The degree of elevation of these enzymes in serum is dependent on the severity of muscle damage as well as the length of time that has elapsed between sample collection and the occurrence of muscle damage. Limited elevations in CK (<1,000 U/L, high range of normal value = 380 U/L) may accompany training or transport. Extreme fatiguing exercise, (e.g., endurance rides or the cross-country phase of a 3-day event) may result in CK activities being increased to more than 1,000 U/L but usually less than 5,000 U/L. Under these circumstances, serum CK activities rapidly return to baseline (i.e., less than 350 IU/L in 24 to 48 hours). Recumbent animals also may have slightly elevated CK activities that are usually <3,000 U/L. In contrast, ER usually results in a sharp rise in serum CK activity, with substantial elevations from several thousand to hundreds of thousands of U/L in blood samples that peak approximately 6 hours after degeneration ceases and then decline over a period of days, depending on the height of the peak value (Fig. 7). AST activity rises more slowly in response to myonecrosis than does CK, often peaking between 12 to 24 hours after the insult. In addition, AST is cleared slowly by the reticuloendothelial system and may persist for 2 to 3 weeks after rhabdomyolysis (Fig. 7).
By comparing serial activities of CK and AST, information is derived concerning the progression of muscle degeneration. Elevations in both CK and AST reflect relatively recent or active myodegeneration; persistently elevated serum CK indicates that myodegeneration is likely to be continuing. Elevated AST activity accompanied by decreasing or normal CK activity indicates that myodegeneration has ceased. The degree of elevation of CK and AST does not necessarily reflect the severity of clinical signs, as some forms of rhabdomyolysis are more painful than others.

Serum Chemistry
With severe rhabdomyolysis, electrolyte abnormalities such as hyponatremia, hypochloremia, hypocalcemia, hyperkalemia, and hyperphosphatemia are identified. These derangements result from losses in sweat as well as shifting of fluid and electrolytes (sodium, chloride, calcium) down a concentration gradient into damaged muscle. Release of electrolytes such as potassium and phosphorus from damaged muscle cells can result in increased serum concentrations. A metabolic alkalosis was found to be the most common acid base abnormality with ER, as a compensation for hypochloremia. Lactic acidosis is rarely, if ever, observed. Azotemia has been identified in dehydrated horses from myoglobinuric nephrotoxicity. Azotemia is much less common in horses compared with humans because their alkaline urine provides some protection from myoglobin precipitation.

Exercise Response Test
An exercise response test proved to be useful for evaluating horses with a possible history of ER that have normal serum CK and AST activity at the time of evaluation. Blood samples are taken before exercise and about 4 to 6 hours after a light exercise test to evaluate peak changes in CK activity. A submaximal exercise test is used for detecting ER because more consistent evidence of subclinical rhabdomyolysis was found with this test versus maximal exercise tests. Fifteen minutes of trotting or, in unfit horses, 2-minute intervals of walking and trotting for a maximum of 15 minutes, is often sufficient to produce subclinical muscle damage in horses prone to chronic ER. If signs of stiffness develop before this, exercise must be concluded. A normal response is less than a 3- to 4-fold increase from basal CK to 4 to 6 hours after the exercise test.

This exercise test is not only useful to identify susceptibility to ER, it is also useful in defining the amount of exercise to use when horses are put back into training. The duration of exercise when beginning training should be less than that which produced abnormal elevations in serum CK. Serum CK activity is not measured immediately after exercise because at this time point it will not reflect the amount of damage occurring during the exercise test. A 4- to 6-hour postexercise sample is ideal because this is when the highest CK activity will occur in serum after muscle damage. Small fluctuations in serum CK activity may occur with exercise due to enhanced muscle membrane permeability, particularly if exercise is prolonged or strenuous and the horse is untrained.

Muscle Biopsy
The muscle biopsy technique has now become a routine part of equine practice. Most frequently, practitioners utilize an open surgical approach; however, neuromuscular laboratories that have the ability to freeze muscle biopsies in isopentane use the percutaneous technique. The percutaneous needle biopsy has the advantage of a no layup after biopsy.

Open Surgical Technique
The semimembranosus or semitendinosus muscle is usually selected for the open surgical biopsy because of the ability to easily align an incision parallel to the length of muscle fibers. After wrapping the tail and sterile preparation of the site situated at about the height of the lower commissure of the vulva, a local anesthetic is placed under the skin (5 mL) but not into the muscle. The objective is to obtain approximately a half-inch cube of tissue; hence, a suitably long skin incision and incision into the fascia is required. After undermining the fascia, two parallel incisions one-half inch apart are made longitudinal to the muscle fibers with a scalpel. The muscle is only handled in one corner using forceps to avoid crushing the sample and thereby creating artifacts (Fig. 8). The muscle sample is then excised by cross-sectioning incisions one-half inch apart. Closure of the fascia will prevent muscle hernia, and closure of the subcutaneous layer eliminates dead space preventing dehiscence. Interrupted sutures are used in the skin and can be removed after 14 days. Box stall rest for a few days is necessary to prevent dehiscence.

Percutaneous Technique
This technique is not commonly used in the field because needles are not readily available, experience is required to obtain an adequate sample size, and this type of sample does not ship as well as larger surgically excised biopsies. The percutaneous needle biopsy technique can be used to sample a variety of muscles, but the gluteus medius is most frequently evaluated because of its major role in exertion and ease of access. The skin over the site of the biopsy is steriley prepared, and 3 mL of local anesthetic is injected. The internal cutting cylinder of the needle is inserted into the muscle through a 1-cm skin incision and the cutting cylinder is partially withdrawn, exposing a window in the needle. The biopsy needle is pressed firmly against the muscle to trap a piece of muscle within the window, and the cylinder is depressed to excise a piece of muscle that moves into the hollow cylinder. The chopping
motion is repeated several times before withdrawing the needle, usually resulting in a 50- and 250-mg core of muscle. Standardization of a biopsy site accommodates the variation in muscle fiber types that occur along the depth and length of a muscle, making results comparable between time points and studies. The site often sampled in the gluteal muscle is 15 to 17 cm along a line from the highest point of the tuber coxae to the head of the tail, at a window depth of 6 to 8 cm. The depth and length must be reduced for studies of younger animals to reflect the same relative part of the gluteal muscle as examined in adults. Mild hemorrhage from the biopsy site is the only occasional complication.

Sample Preparation
Specimens for histochemical analysis must be handled gently to avoid crush artifacts that mimic abnormal glycogen and myodegeneration. If samples are being shipped, they are placed in a firm container, kept on ice packs, and sent by overnight courier to the laboratory. Samples should be either sent dry or wrapped in slightly damp gauze because samples wrapped in saline-soaked gauze have more artifacts. On arrival in the lab, biopsy specimens are first oriented in cross section and then rapidly frozen in isopentane chilled to the appropriate temperature in liquid nitrogen to avoid the formation of freeze artifact vacuoles. By avoiding formalin fixation, substrate levels and activities of enzymes within individual muscle fibers can be readily assessed.

The most valuable information is obtained from fresh muscle samples obtained by open surgical technique in the field that are shipped overnight on icepacks to specialized neuromuscular diagnostic laboratories. Muscle biopsies are best performed Monday through Thursday morning in order to arrive at the laboratory by Friday, where they can be frozen on arrival. If samples cannot be obtained with this time line, they are best kept chilled, wrapped in slightly damp gauze in a refrigerator, and shipped within 48 hours to the laboratory. The quality of samples declines with a delay before freezing, and some valuable information may be lost, so this should be avoided wherever possible.

The minimum battery of stains used to evaluate biopsies from horses with ER includes H&E and modified Gomori trichrome for morphology, cellular infiltrates, fibrosis, rimmed vacuoles, and myelination of nerve branches; PAS and amylase-PAS for glycogen and abnormal polysaccharide; and oil-red-O for intramyofiber lipid accumulation. Other stains that are of value include myosin ATPase or immunohistochemical stains for fiber type, nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) for mitochondria, alkaline phosphatase for degenerating fibers, and acid phosphatase for lysosomes and macrophages.

Muscle Biochemistry
Muscle tissues for analysis of substrates, enzymes, and mRNA must be snap-frozen in liquid nitrogen to capture metabolic activity exactly as it was when muscle is sampled. For accuracy, when analysis involves samples less than 10 mg in size, muscle should be freeze-dried and dissected under a stereomicroscope to remove extraneous blood, fat, and connective tissue before performing assays. Results are then expressed as dw, which can be roughly converted to wet weight by multiplying dw by 4.

IV. Sporadic ER
It became very clear when characteristic muscle biopsy features were identified in subsets of horses with ER that there were many causes for ER, and this conclusion was backed up when specific genetic mutations were found in some of these chronic subsets. Thus, ER, like colic and coughing, represents a clinical syndrome, and, to determine the cause in an individual horse, a proper diagnostic approach is required.

Horses with sporadic forms of ER develop rhabdomyolysis because of an extrinsic event or recurring
extrinsic events that induce muscle damage with exercise. Usually a horse is presumed to have sporadic ER the first time it develops ER. If ER recurs once the horse has been rested and gradually returned to exercise, then an investigation of an inherent chronic form of ER is usually pursued. Causes of sporadic ER include focal or generalized trauma to muscle, exercise performed beyond any training adaptation or performed to the point of exhaustion, and dietary imbalances that affect muscle function.

Signalment, History, and Diagnosis
Horses with sporadic ER may be of any age, breed, or sex and involved in a wide variety of athletic disciplines. Sporadic ER usually occurs in horses with a history of adequate performance prior to onset of ER, and a familial history is absent. A diagnosis is made by history, clinical signs, and elevations of serum muscle enzymes. Once external perturbations that affect muscle function are corrected, complete resolution and a successful return to performance are expected. Resolution occurs after a reasonable period of rest, provision of a balanced diet, and a gradual introduction of a training program matched with performance demands. If over time ER recurs despite reasonable management, a diagnosis of chronic ER becomes more likely, and further diagnostic testing is appropriate.

Focal or Generalized Trauma
Horses that strain a particular muscle may be very reluctant to move and show signs resembling more generalized ER. This is particularly true when lumbar, glutal, psoas, adductor, or semi-membranous muscles are acutely involved. Further, horses that struggle when cast, are caught in a fence, or are thrashed by another horse may have acute signs of rhabdomyolysis due to severe exertion or trauma. A diagnosis of focal trauma is established by history, physical examination, and serum muscle enzymes. Rectal examination and ultrasonography may help to localize the extent of muscle damage for focal lesions. On occasion, scintigraphy may help to identify specific muscle involvement.

Overexertion
A history of an increase in work intensity without a foundation of consistent training for this level of exercise is usually the basis for suspecting a training imbalance as a cause of ER. Signs of muscle stiffness and gait changes range from mild to severe, and severity is reflected by variable elevations of serum CK activity. Overexertion is a well-described cause of ER in polo horses, with 81% of cases of ER attributed to overexertion and 30% of cases occurring after a day of rest. The incidence of ER is as high as 9% in US polo horses, with most of these horses only having one episode of ER early in the polo season.

Pathological changes are often not evident in light microscopic evaluation of muscle biopsies from individuals performing unaccustomed exercise, but electron microscopy shows significant disruption of the alignment of muscle contractile proteins within muscle fibers. In more severe cases, overt segmental damage to myofibers may be apparent in muscle biopsy. Repetitive overuse of muscles, such as occurs with overtraining, may result in exercise intolerance and is associated with pathologic changes such as increased muscle fiber size variation and centrally located myonuclei in muscle biopsies.

Exhaustion
Exhaustion occurs most commonly in endurance horses or racehorses exercising in hot, humid weather. Signs of heat exhaustion include weakness, ataxia, rapid breathing, muscle fasciculations, sweating, and, in severe cases, collapse. The body temperature may be elevated to 105° to 108°F. Muscles are frequently not firm on palpation, although serum CK activity can be markedly elevated and myoglobinuria may be noted.

Dietary Imbalances
Episodes of ER may be triggered by diets with a high nonstructural carbohydrate (NSC) content and low forage content or by diets deficient in electrolytes and may be exacerbated by inadequate selenium and vitamin E.

Electrolyte Imbalances
Electrolyte balance within the body is difficult to determine accurately. One suggested means to practically assess electrolyte balance in horses is to measure urinary fractional excretion of electrolytes. Measurement of urinary electrolyte excretion as an indicator of electrolyte balance is complicated because marked variation can occur from diet, exercise, and sampling technique between individuals as well as within individuals from day to day. Furthermore, the high calcium crystal concentration of alkaline equine urine requires acidification to accurately assess calcium and magnesium content. The high potassium content interferes with sodium analysis, using conventional ion specific electrodes. Thus, although popular in the 1990s urinary fractional excretion is now rarely performed in the United States unless there is a strong suspicion that body depletion has occurred and if a laboratory can be located with gas chromatography mass spectrometry analysis of acidified urine. It is important to ensure that horses receive sodium chloride in the diet, with higher concentrations needed in horses that compete in hot, humid conditions and sweating extensively.

Episodes of ER may be triggered by diets with a high nonstructural carbohydrate (NSC) content and low forage content or by diets deficient in electrolytes and may be exacerbated by inadequate selenium and vitamin E.
The entire diet should be adequately balanced with calcium and phosphorus (ration of 2:1 Ca:P is ideal).

Vitamin E and Selenium Concentrations
Whole-blood selenium concentrations measured in EDTA or heparin tubes are of value in assessing selenium status in animals housed in areas where soil is deficient in selenium. In cases where selenium has been administered prior to blood collection, glutathione peroxidase activity can be used to assess potential selenium deficiency. Vitamin E concentration should be measured in serum samples kept chilled and protected from light; however, variability in serum levels can be quite large, and repeat sampling is recommended if marginal levels are identified in the first sample tested. Horses with ER are infrequently deficient in selenium and vitamin E, and, alone, it may not be responsible for ER; however, anecdotal reports suggest that in some cases, supplementation may help to further prevent episodes of ER.

Management
As described in the section on acute ER, rest with regular access to a paddock once stiffness resides and weekly monitoring of serum CK activity are recommended. Horses are much more susceptible to a second episode of ER in the 2 weeks after an acute episode, and allowing horses the ability to calmly determine their own exercise through turnout often avoids exacerbating rhabdomyolysis. If that is not feasible, hand-walking must be performed with caution and limited to no more than a few minutes initially. While the horse is rested, the diet can be assessed to ensure that the ration is fed in an amount recommended by the manufacturer for the corresponding level of exercise. This will ensure that the proper balance of vitamins and minerals is provided. If excessive calories are provided when fed at manufacturer-recommended levels, then the diet should be switched to a less calorie-dense vitamin/mineral balanced diet. A salt block or 30 to 50 g (1 to 3 tablespoons) of salt per day will provide the necessary additional sodium chloride, with the amount fed depending on the heat, humidity, and intensity of exercise. Once serum CK returns to normal, training can be resumed gradually. A regular exercise schedule, beginning with 20 minutes or less of exercise per day, is gradually increased to eventually match the expected amount of daily exercise to the underlying state of conditioning.

V. Chronic ER: Intrinsic Causes
Horses that have repeated episodes of ER from a young age, or from the time of purchase, or when they are put back into training after a long period of rest, may have an underlying intrinsic abnormality of muscle function. Many horses with intrinsic muscle defects will have repeated episodes of ER with minimal exercise even when the dietary and training recommendations for sporadic ER are followed. Five specific intrinsic causes of ER have been identified to date:

1. Recurrent Exertional Rhabdomyolysis (RER)
2. Malignant hyperthermia
3. Type 1 polysaccharide storage myopathy (PSSM1)
4. Type 2 polysaccharide storage myopathy (PSSM2)
5. Idiopathic chronic exertional rhabdomyolysis

The idiopathic group represents other causes of ER that have yet to be identified. In all of these intrinsic forms of chronic ER, it appears that there are specific environmental stimuli that are necessary to trigger muscle necrosis in genetically susceptible animals. Horses cannot be cured of their susceptibility to this condition, but, if the specific form of ER is identified, changes in management can be implemented to minimize episodes of rhabdomyolysis.

Pathogenesis
Rhabdomyolysis is triggered suddenly during exercise in RER horses, which results in a sharp rise in serum myoglobin and CK activity (Fig. 7). Clinically, the triggering event is often associated with excitement in a horse that already has an underlying nervous temperament. Segmental necrosis in single fibers or small clusters of necrotic fibers scattered throughout fascicles may be apparent in fewer than 5% of type 2A and 2B muscle fibers in a gluteus medius muscle biopsy. Serum cortisol concentrations are higher in RER horses than in normal horses prior to exercise and increase during an episode of ER. Serum concentrations of epinephrine and norepinephrine are normal prior to an episode but increase dramatically in horses with marked elevations in serum CK activity.
Exercise

In Standardbreds, ER commonly begins after 15 to 30 minutes of jogging at 5 m/s, although clinical signs may not be apparent until after exercise (Fig. 7). In Thoroughbreds exercising on the treadmill, ER most commonly develops with intervals of walk, trot, and canter and is less common if horses are allowed to gallop. At the racetrack, ER occurs commonly when RER horses are held back to a paced gallop. During eventing, RER commonly occurs after the excitement of the steeplechase or early in the cross-country phase, when horses are held to a predetermined speed. ER rarely occurs when horses are allowed to achieve maximal exercise speeds such as racing. A day or more of rest before this type of exercise results in higher serum CK activity after exercise.

Lactic Acid

There is no basis for an association between RER and a lactic acidosis. Thoroughbreds and Standardbreds rarely develop ER during near maximal exercise, when muscle lactates reach 240 mmol/kg dw and plasma lactates are as high as 25 mmol/L. Standardbred horses experience ER during jogging, when muscle lactate concentrations are less than 24 mmol/kg dw. Similarly, plasma lactate concentrations in RER Thoroughbred horses are less than 1.5 mmol/L when performing the walk, trot, and canter treadmill exercise that induces high serum CK activity. Thus, rhabdomyolysis in RER horses is not due to a lactic acidosis.

Intracellular Calcium Regulation

More recent research suggests that horses with RER may have an inherent abnormality in intramuscular calcium regulation that is intermittently manifested during exercise. Myoplasmic calcium concentrations are tightly controlled by channels and pumps in the sarcoplasmic reticulum and usually are unaffected by normal serum calcium concentrations in RER horses are similar to healthy horses, based on assays in muscle cell cultures. Higher intracellular calcium concentrations have been measured in horses of unspecified breeds during an episode of ER; however, this also could be secondary to any insult that impairs energy generation or cell membrane integrity to specifically study shuttling of calcium between the sarcoplasmic reticulum and myoplasm within the muscle cell, technically complex procedures such as muscle contracture testing, calcium imaging in muscle cell cultures, and calcium release by isolated muscle membrane preparations have been evaluated.

Muscle Contracture Testing

Contracture testing has been performed in horses, using surgically excised bundles of brachiocephalic, semimembranosus or external intercostal muscle. It is a tedious day-long process that involves dissecting 2-mm-diameter bundles of muscle fibers from the biopsy, which is placed in an aerated normal physiologic saline solution. Bundles are tethered on end, attached to a force transducer, and the length adjusted until maximal tetanic force is reached when stimulated electrically by horizontal platinum electrodes. Sequentially increasing concentrations of triggering agents are then placed in the bath, and, during each washout, muscle tetanic tension must return to its approximate original basal value. Potassium, caffeine, halothane, and 4 chloro-m-cresol have been evaluated as triggering agents for equine contracture testing. Results are reported as the threshold at which a significant increase in muscle tension occurs above baseline levels. Caffeine and halothane are most frequently used to trigger the release of calcium from the sarcoplasmic reticulum to the myoplasm via the ryanodine receptor. High myoplasmic calcium causes a conformational change in tropomyosin, revealing the myosin binding site on actin and

Fig. 9. Results of caffeine-induced contracture testing of normal Thoroughbreds and Quarter Horses, Thoroughbreds with recurrent exertional rhabdomyolysis (ER), and Quarter Horses with polysaccharide storage myopathy (PSSM). Horses with recurrent ER showed an increase from baseline in muscle fiber force at significantly lower caffeine concentrations than other horses.
inducing a contracture. This test remains an experimental procedure due to the difficulty in obtaining accurate and timely results.

Early studies by Hildebrand suggested that some but not all horses with ER showed a lower threshold for caffeine- and halothane-induced muscle contractures than healthy horses. Waldron-Mease and Beech found that caffeine contracture thresholds were lower in semimembranosus muscle from Thoroughbreds and Standardbreds susceptible to RER compared with breed-matched controls. Lentz et al. chose to study intact external intercostal muscle fibers, based on concerns that damaged ends of transected fibers may cause slight depolarization and affect the repeatability of the contracture results. Intact intercostals muscle bundles from six Thoroughbreds with RER had a lower contracture threshold for triggering agents such as potassium, caffeine, and halothane but not 4 chloro-m-cresol compared with control horses (Fig. 9). An abnormality in intracellular calcium regulation was also supported by a muscle cell culture study of RER Thoroughbred horses. A lower threshold for induction of myoplasmic calcium release by caffeine was found in myotube cultures from six RER Thoroughbreds compared with controls. Thus, all of these studies supported an abnormality in intracellular calcium regulation in most of the Thoroughbred or Standardbred horses studied. Contracture testing of PSSM horses did not reveal any difference in contracture threshold compared with controls (Fig. 9).

### Myofibrillar Sensitivity to Calcium

A potential alteration in the activation and regulation of the myofibrillar contractile apparatus by ionized calcium has been studied as a basis for RER. No difference was detected between the type II fibers of RER-affected and control horses in terms of calcium sensitivity of force production in chemically skinned muscle fibers or in myofibrillar ATPase activity. Thus, the basis for pathologic contracture development in muscles from RER-affected horses did not appear to be due to an alteration in myofibrillar calcium sensitivity.

### Isolated Membrane Preparations

To determine if calcium release and ryanodine binding is abnormal in RER muscle, sarcoplasmic reticulum membrane has been isolated from biopsies of RER and normal horses. An initial study found a difference in calcium-induced calcium release between RER and control horses. In another study, the time courses of sarcoplasmic reticulum calcium-induced calcium release and [3H]ryanodine binding to the ryanodine receptor after incubation with varying concentrations of ryanodine, caffeine, and ionized calcium were similar between muscle membranes obtained from control and RER horses. Furthermore, the maximal rate of SR calcium-ATPase activity and its affinity for calcium did not differ between muscle membranes from control horses and horses with RER. Thus, these studies did not conclusively identify any abnormalities in intracellular calcium regulation, indicating that there are distinct differences between RER and disorders such as malignant hyperthermia.

### Linkage Analysis for Genes Involved in Intracellular Calcium Regulation

To further explore the possibility that RER was due to an inherited defect in intracellular calcium regulation, genetic linkage analysis was performed using 96 horses in 4 families of Thoroughbred horses segregating for RER. No association was detected linking RER with the genes RYR1 (ryanodine receptor), SERCA (SR ATPase), or DHPR (dihydropyridine receptor).

### Twitch Characteristics

The twitch characteristics of muscle from RER Standardbreds and Thoroughbreds have been investigated and are different between RER and normal horses. Muscle bundles from RER horses had a slightly but significantly faster time to peak tension and a significantly shorter time to 50% relaxation than muscle from normal horses.

Thus, despite many detailed investigations of a small number of horses, the exact defect in regulating muscle contracture in RER horses has yet to be identified. RER appears to be a novel defect in muscle excitation-contraction coupling, calcium regulation, or contractility whose basis is yet to be defined. Alternatively, the increased sensitivity of RER horses in contracture testing may be a nonspecific indicator of an abnormality in other pathways governing muscle contraction.

### Prevalence and Risk Factors

Assuming that the majority of cases of ER at racetracks are due to RER, the prevalence in Thoroughbred racehorses is remarkably similar around the world, with estimates ranging from 4.9% in the United States, 5.4% in Australia, and 6.7% in the United Kingdom. The annual incidence of ER in Standardbred racehorses in Sweden is 6.4%. Exercise obviously increases the prevalence of RER in horses, and episodes are observed more frequently once horses achieve a level of fitness.

In Standardbreds and Thoroughbreds, mares more commonly have RER than males; however, no general correlation has been observed between episodes of rhabdomyolysis and stages of the estrus cycle. There is an interaction between age and sex in RER Thoroughbred horses such that the proportion of affected females to males is much higher in young horses compared with older age groups. Swedish Standardbreds with RER, which do not begin racing until 3 years of age, did not show the same age association with RER as Thoroughbreds. Temperament has a strong ef-
fect on the expression of RER in both Standardbreds and Thoroughbreds, with nervous horses, particularly fillies, having a higher incidence of rhabdomyolysis than calm horses.94,95,123 Young fillies are more likely to have a nervous temperament than mares or male horses. Diet also has an impact, with Thoroughbred horses fed more than 2.5 kg of grain per day being more likely to show signs of RER.125 One study also found a higher prevalence of rhabdomyolysis among Thoroughbred horses with lameness.84 A few days of rest prior to exercise increases the degree of elevation on serum CK activity with the next exercise session and in practice predisposes horses to an episode of ER.126

Genetics

A genetic susceptibility to RER appears to exist in Thoroughbred horses, in which RER-afflicted Thoroughbreds may pass the trait along to 50% or more of their offspring.78,79 A breeding trial conducted at the University of Minnesota evaluated the number of offspring of an RER horse that had a positive contracture test.79 Results supported an autosomal dominant mode of inheritance with an equal ratio of male and female affected Thoroughbred offspring. Using a clinical diagnosis of RER rather than contracture test, offspring of RER-affected dams also appeared to inherit the disorder as a dominant trait, with a predominance of females having clinical signs.79 The variable penetrance of the disease, however, and other potential modifying genes, makes this hard to firmly evaluate. A recent genome-wide association study in US Thoroughbred horses found a significant association between RER and SNP markers on equine chromosome 16.90 The location of this association is different from that reported by a Japanese group.140 Studies of allelic frequencies of genetic markers in Standardbred horses with RER were significantly different from healthy Standardbred horses, suggesting that there is potentially a heritable basis for this condition in this breed as well.76 Of note is the finding that Standardbred trotters with a history of RER actually have faster racing times from a standstill start than those without a history of RER, suggesting that the trait may have beneficial as well as deleterious effects.123 There are also anecdotal reports of higher prevalence of RER in certain Arabian horse families. Thus, although there is a familial tendency for RER, its genetic basis has not yet been defined.

Much remains to be done to determine the pathogenesis of ER in Thoroughbred and Standardbred horses and to what extent these horses suffer from an abnormality in the regulation of muscle contraction.

Diagnosis

A presumptive diagnosis of RER is based on clinical signs of muscle pain and the presence of risk factors commonly associated with RER. Muscle histopathology is nonspecific in RER horses; either no abnormalities or evidence of centrally located nuclei in mature fibers and potentially waves of myofiber degeneration or regeneration is found.109 There is a notable absence of abnormal amylase-resistant polysaccharide, although increased subsarcolemmal amylase sensitive glycogen may be present.102 The value of a muscle biopsy as a diagnostic tool in horses with suspected RER is largely confined to particularly recurrent unmanageable cases in which a need arises to rule in or out other forms of ER. Serum CK and AST activities serve as the basis for detecting muscle degeneration, and they often show intermittent abnormal elevations that return to normal relatively quickly during the course of training (Fig. 10). If CK elevations are mild, there are a number of factors to consider. Timing of the sample with regard to the onset of ER and exercise are important. Reliability is improved by obtaining blood samples at a standardized time after exercise, preferably 4 to 6 hours, when CK peaks, and consistently with regard to exercise on the preceding day, since serum CK activity is higher on exercise days.

Fig. 10. Serum CK activity in three Thoroughbreds with recurrent exertional rhabdomyolysis (RER) and three Quarter Horses with PSSM during 3 to 4 weeks of training.109 Note that RER horses show intermittent elevations in CK that return to baseline, whereas PSSM horses have more persistent elevations in serum CK activity.
that are preceded by a day or more of rest. In addition, normal values must be adjusted for the age and sex of horses. Two-year-old fillies generally have greater fluctuations in serum CK activity during race training than 3-year-old fillies or geldings.

Management

Prevention of rhabdomyolysis in horses susceptible to RER is complex, and multiple factors must be changed to decrease episodes. These factors include the environment, the exercise regime, and diet. In addition, medication may be needed at times to prevent further episodes of muscle damage.

Environment

Because excitement and nervousness often trigger rhabdomyolysis, stressful situations in the environment that can be modified must be identified. This may involve a change to a smaller barn with fewer horses and fewer handlers, compatible companions, and a more consistent daily routine. Many stressed RER-susceptible horses respond to a regular routine including feeding first before other horses and training first before other horses, especially if the horse becomes impatient while waiting. Other horses respond to being housed in a quiet area of the barn next to calm, companionable horses. The use of hot walkers, exercise machines, and swimming pools must be evaluated on an individual basis, as some horses develop rhabdomyolysis when using this type of equipment. Horses that develop rhabdomyolysis at specific events, such as horse shows, may need to be reconditioned to decrease the stress level associated with such events or tranquilized as part of the accommodation process. Providing daily turnout with other horses seems to be very beneficial for RER horses and may decrease anxiety and thereby the likelihood of rhabdomyolysis.

Exercise

Although a period of rest is recommended for sporadic forms of ER until serum CK normalizes, it is not recommended for horses with RER. Daily exercise is important in preventing episodes of rhabdomyolysis, and therefore when serum CK is less than about 3,000 U/L, horses are returned to regular daily exercise. Horses with more severe episodes of rhabdomyolysis may require additional time off in a paddock before gradually resuming exercise. Once back in training, some form of daily exercise is recommended. Avoiding fighting to hold racehorses to slower speeds during galloping may decrease rhabdomyolysis in RER-susceptible Thoroughbreds. Interval training and reduction of jog miles to no more than 15 minutes per session will benefit Standardbreds. For horses under saddle, a relaxed warm-up with intermittent periods of long, low stretching may be of benefit, and continued breaks to stretch between episodes of collection are recommended. Event horses may require training that incorporates calm exposure to speeds achieved during the steeplechase or cross-country phase, especially when used in an interval training program.

Diet

A nutritionally balanced diet with appropriate caloric intake and adequate vitamins and minerals is the core element of managing RER. For RER Thoroughbreds and Standardbreds in training, the challenge is usually supplying enough calories in a highly palatable form to meet their daily energy demands. This is in part because they often require >30 MCal of digestible energy (DE) a day (5 kg sweet feed, 1.5% of body weight in hay), and, because of their nervous temperament, they may be more discriminating in their eating habits. Out of the total daily calories required by RER horses, research suggests that less than 20% DE be supplied by nonstructural carbohydrates (NSC = starch and sugar) and at least 20% of DE be supplied by fat.

Selection of Forage

Thoroughbred horses do not appear to show the same significant increase in serum insulin concentrations in response to consuming hay with an NSC of 17%, as seen in Quarter Horses (Borgia L. 2010 PhD thesis, University of Minnesota). This fact combined with the high caloric requirements of racehorses may mean that it is not as important to select hay with very low NSC content in RER Thoroughbreds as it is in PSSM horses. Anecdotally, some trainers find horses with RER have more frequent episodes on alfalfa hay. The nervous disposition of some RER horses may predispose them to gastric ulcers, and thus frequent provision of hay with a moderate NSC and mixed alfalfa content may be indicated.

Low Starch, High Fat Concentrates

When RER Thoroughbreds are fed a moderate caloric intake (24 MCal/d) in the form of high starch concentrates (2.5 kg of corn/oats/wheat middlings/molasses) they show very little elevation in serum CK activity with exercise. Most Thoroughbred racehorses, however, are fed at least 5 kg of high starch concentrate/d at 30 MCal or more per horse per day, and, at this level of feeding, postexercise serum CK activity rises significantly. The discovery that substitution of fat for starch in a high caloric ration significantly reduces muscle damage in exercising RER horses was a major advance in nutritional management of RER. Practically, however, it was difficult to achieve the desired caloric intake of racehorses because the maximum amount of fat that finicky Thoroughbreds will happily consume is limited often to 600 mL for vegetable oil or 5 lb/d of balanced rice bran. Management of RER horses was significantly improved when a palatable means to provide the amount of fat required by fit
finicky RER Thoroughbreds was developed. A controlled trial using a specialized feed developed for RER (13% fat by weight and 9% NSC) determined that NSC should be no greater than 20% of daily DE and 20% to 25% of daily DE should be provided by fat for optimal management of RER horses fed 30 MCal or more per day. No beneficial effect on serum CK activity occurred when sodium bicarbonate (4.2% of the pellet) was added to a high starch pellet feed. The amount of fat in the diet of an RER horse depends on its caloric requirements. Over and above hay at 1.5% to 2% of body weight and 2.5 kg of grain, any additional calories should be supplied by fat.

The benefit of a fat diet does not appear to be a change in muscle metabolism. Pre- and postexercise muscle glycogen and lactate concentrations are the same in RER horses fed a low starch, high fat diet compared with a high starch. Rather, low starch, high fat diets in RER horses may decrease muscle damage by assuaging anxiety and excitability, which are tightly linked to developing rhabdomyolysis in susceptible horses. High fat, low starch diets fed to fit RER horses produce lower glucose, insulin and cortisol responses and led to a calmer demeanor and lower pre-exercise heart rates.

Neurohormonal changes may develop in response to high serum glucose, insulin, and cortisol concentrations, resulting in an anxious demeanor. While a calm demeanor is desired during training, some racehorse trainers feeding low starch, high fat feeds prefer to supplement with a titrated amount of grain 3 days prior to a race to potentially increase liver glycogen and increase a horse’s energy during the race.

Expectations

Studies in RER horses show that significant reductions or normalization of postexercise serum CK activity occurs within a week of commencing a low starch, high fat diet. Days off training in a stall are discouraged because postexercise CK activity is higher after 2 days of rest compared with values taken when performing consecutive days of the same amount of submaximal exercise.

Supplements

Horses require daily dietary supplementation with sodium and chloride either in the form of loose salt (30 to 50 g/d) or a salt block. Additional electrolyte supplementation is indicated in hot humid conditions. A myriad of supplements are sold that are purported to decrease lactic acid buildup in skeletal muscle of RER horses. These include sodium bicarbonate, B vitamins, branched chain amino acids, and dimethylglycine. Since lactic acidosis is no longer implicated as a cause for rhabdomyolysis, it is difficult to find a rationale for their use.

Medications

Low doses of tranquillizers, such as acepromazine, prior to exercise have been used in RER horses prone to excitement. A dose of 7 mg IV 20 minutes before exercise is reported to make horses more relaxed and manageable. Reserpine and fluphenazine, which have a longer duration of effect, have also been used for this purpose. Horses given fluphenazine may occasionally exhibit prolonged bizarre behavior. Use of tranquillizers may only be necessary when horses are in their initial phase of training and accommodation to a new environment. Horses obviously cannot compete on these medications, and withdrawal times must be observed.

Dantrium sodium acts to decrease release of calcium from the ryanodine receptor in skeletal muscle and is used to treat malignant hyperthermia. Studies of intact equine intercostal muscle from ER horses show that dantrolene lowers already elevated myoplasmic calcium concentrations. Controlled and field studies have also shown that oral dantrolene can significantly decrease signs of rhabdomyolysis in RER horses. One study indicated that horses must be fasted to achieve measurable blood levels of dantrolene, but more recent controlled trials suggest that absorption of dantrium is superior in fed versus fasted horses. Administration of 4 mg/kg of dantrium PO 1 hour before exercise to RER-susceptible horses fed high grain diets resulted in significantly lower serum CK activity 4 hours after exercise compared with placebo (Fig. 11). A dose of 800 mg of dantrium was given to Thoroughbred horses in the United Kingdom 1 hour prior to exercise and resulted in signifi-

![Fig. 11. Effect of oral administration of 4 mg/kg dantrolene 60 minutes before treadmill exercise in Thoroughbred horses with recurrent exertional rhabdomyolysis. Bars indicate mean values. Four-hour postexercise serum creatine kinase (CK) activity was significantly lower when dantrolene was administered.](image-url)
Phenytoin (7.7 to 11 mg/kg PO BID) has been reported to be effective in preventing rhabdomyolysis in horses with RER. Phenytoin acts on a number of ion channels within muscle and nerves, including sodium and calcium channels. Therapeutic levels vary, so oral doses are adjusted by monitoring serum levels to achieve therapeutic levels. Initial dosages start at 6 to 8 mg/kg orally twice a day for 3 to 5 days. If the horse is still experiencing rhabdomyolysis but is not drowsy, the dose can be increased by 1 mg/kg increments every 3 to 4 days. Phenytoin is a monoaminooxidase activator and can affect dosages of other medications. Unfortunately, long-term treatment with dantrolene or phenytoin is expensive, and these drugs must be withdrawn prior to competition.

Adjunct Therapies
Massage, myofascial release, mesotherapy, stretching, aqua-treadmills, and hot/cold therapy performed by experienced therapists may be of benefit in individual cases. Their use may promote relaxation and normal muscle tension and build muscle strength.

2. Malignant Hyperthermia
Malignant hyperthermia (MH) is a genetic disorder that occurs in Quarter Horses and Paint Horses as a result of a mutation in the ryanodine receptor 1 gene (RYR1). It was discovered by Dr. Monica Aleman, who investigated two research horses that developed classic clinical signs of MH when anesthesia was induced by inhalation of halothane by mask during a research project.

Pathogenesis
A point mutation in the RYR1 gene lowers the activation and heightens the deactivation threshold of the ryanodine receptor. When triggered, the abnormal ryanodine receptor remains open, causing a drastic efflux of calcium from the SR and an increase in myoplasmic calcium, producing a contracture. The process of reabsorbing excess myoplasmic calcium consumes large amounts of ATP and generates excessive heat. Myofibers are damaged by the depletion of ATP and possibly the high temperatures (Fig. 3).

Prevalence and Risk Factors
Malignant hyperthermia is a rare disorder in Quarter Horses and Paint Horses, affecting <1% of the breed. Halter and pleasure horse lines have the highest prevalence. Rhabdomyolysis may be induced by exercise and anesthesia but can be extremely intermittent in nature.

Genetics
An autosomal dominant mutation exists in exon 46 of the skeletal muscle RYR1 gene on ECA 10.

Clinical Signs
Clinical signs of hyperthermia (40.5°C, 104.9°F), hypercapnea (PaCO₂, 274 mm Hg) and acidosis (pH of 6.72) were reported in an experimental protocol approximately 60 minutes after anesthesia was induced by delivering halothane via a facemask without premedication. Death occurred from cardiopulmonary arrest, and profound rigor mortis was present almost immediately. Hematologic changes measured 2 minutes after death included hemocoagulation, hyperkalemia, hypercalcemia, hyperphosphatemia, hyperglycemia, and elevated creatinine. Serum CK activity was mildly elevated at 843 U/L, and myoglobin was 10× higher than the reference range in this horse.

Horses with the RYR1 mutation may intermittently show signs of ER and high body temperatures. Some MH-affected horses have died suddenly after an episode of ER. Horses with the MH mutation may also have the GYS1 mutation associated with type 1 PSSM. Such horses have more severe episodes of ER, higher serum CK activity after exercise, and a more moderated response to the diet and exercise regimes recommended for type 1 PSSM.

Diagnosis
Genetic testing is recommended in Quarter Horses and Paint Horses with difficult-to-manage forms of PSSM or a family history of postanesthetic complications. Testing is available through the Veterinary Diagnostic Laboratory at the University of Minnesota (http://www.vdl.umn.edu/vdl/ourservices/neuromuscular.html) and at the University of California, Davis. Muscle biopsy is not particularly useful for diagnosing MH. Biopsies may contain mild myopathic changes, including increased variation in fiber sizes, centrally located nuclei, fiber necrosis, glycogen depletion, and ringbinden fibers.

Treatment
The most successful outcome for a horse with suspected malignant hyperthermia would be pretreat-
ment with oral dantrolene (4 mg/kg) 30 to 60 minutes prior to anesthesia. There is, however, no cost-effective means to deliver dantrolene to horses intravenously once an episode has begun. Unfortunately, once a fulminant episode is underway, it is difficult to prevent cardiac arrest. The nature of ER with horses positive for MH is so intermittent that it is hard to justify premedication with dantrolene before exercise.

3. Polysaccharide Storage Myopathy
PSSM was first recognized as a specific myopathy in Quarter Horse–related breeds in 1992; however, there are individual cases of abnormal polysaccharide inclusion reported in equine muscle dating back to 1979. The remarkable feature of the first horses reported to have PSSM was 2-fold higher muscle glycogen concentrations than in normal horses (Fig. 12) and abnormal granular amylase-resistant inclusions in histological sections (Fig. 13). Similar biopsy findings were reported in Belgian and Percheron draft horses in 1997. Since that time, many hundreds of horses of a variety of breeds have been diagnosed with PSSM.

Terminology
Several acronyms are used for polysaccharide storage myopathy besides PSSM, including EPSM and EPSSM. Considerable controversy existed as to whether these acronyms encompassed one muscle condition. In 2008, a mutation in the glycogen synthase 1 gene was found to be highly associated with the presence of amylase-resistant polysaccharide in skeletal muscle from Quarter Horses with PSSM. Genetic testing of hundreds of horses previously diagnosed with PSSM by muscle biopsy revealed that the majority of cases of PSSM characterized by amylase-resistant polysaccharide in skeletal muscle had this genetic mutation. However, some cases previously diagnosed...
with PSSM by muscle biopsy, particularly those with excessive amylase-sensitive glycogen, did not possess the genetic mutation. This suggested that there are at least two forms of PSSM.167,168 For clarity, the form of PSSM caused by a glycogen synthase 1 (\textit{GYS1}) gene mutation is now termed type 1 (PSSM1), whereas the form of PSSM that is not caused by the \textit{GYS1} mutation and whose origin is yet unknown is now termed type 2 (PSSM2).168 PSSM1 probably is the same disorder described as “azoturia” or “Monday morning disease” in work horses in the 19th and 20th centuries.2–4

Type 1 PSSM

\textbf{Prevalence}

PSSM1 is estimated to have emerged approximately 1600 years ago when the great horse was being developed from European draft and light horse breeds to carry knights with heavy armor into battle.81 The highest prevalence of PSSM1 appears to occur in draft horses derived from Continental European breeds (Table 2).169 In fact, many Continental European drafts are homozygous for the dominant PSSM1 trait (90% prevalence of PSSM in Trekpaards, with 40% of tested Belgian Trekpaards homozygous). North American Belgians and Percherons also have a high prevalence of PSSM1, at 36% and 54% of horses affected.170 In contrast, the prevalence of PSSM is very low in United Kingdom–derived breeds such as Shires and Clydesdales,170 but PSSM1 is present in other UK horse breeds such as Irish Drafts, Cob, and Connemara.163,171 Numerous other breeds, more than 20, in fact, have the \textit{GYS1} mutation responsible for PSSM1.163,165,167,168,170

Prevalence estimates of PSSM1 in Quarter Horses range from 6% to 10% of the breed and 6% to 8% for American Paint and Appaloosa Horses.97,172 The highest frequency of PSSM1 occurs in halter Quarter Horses (28% affected) and the lowest frequency in racing Quarter Horses.172 The \textit{GYS1} mutation has been identified in approximately 72% of Quarter Horses diagnosed with PSSM by muscle biopsy and in 18% of Warmbloods of a variety of types diagnosed with PSSM by muscle biopsy.98 The prevalence of PSSM1 is very low to nonexistent in light horse breeds such as Arabians,170 Standardbreds,123 and Thoroughbreds170 (Table 2).

\textbf{Risk Factors}

Some horses with PSSM1 are asymptomatic, whereas other horses routinely show stiffness with exercise.99,104 In a family of Warmbloods sired by a stallion with the \textit{GYS1} mutation, the mutation resulted in a 7-fold higher risk of developing ER, with 34% of horses with the \textit{GYS1} mutation lacking clinical signs of PSSM1.104 The reason for variability in clinical signs of PSSM1 probably lies in the influence of diet, exercise, other environmental factors, and other genes on the expression of the disease.117,173,174 There is no significant temperament, body type, or sex predilection for PSSM1.8 The most common trigger for clinical signs of

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*Both randomized and nonrandomized samples.
PSSM1 is less than 20 minutes of exercise.\textsuperscript{104,162} Clinical signs occur most commonly at a walk and trot, particularly if the horse has been rested for several days prior to exercise or is unfit.\textsuperscript{104,104} Diets high in NSC also increase the risk of muscle pain and stiffness in PSSM1 horses.\textsuperscript{173,175} About 40\% of owners feel there is a seasonal incidence to the development of clinical signs, which some have attributed to quality of grass available at the time.\textsuperscript{162,171} Although exercise is by far the most common trigger for PSSM, Quarter and Paint Horse foals and weanlings may develop rhabdomyolysis in conjunction with a systemic illness.\textsuperscript{176}

**Genetics**

A small breeding trial of 4 horses combined with pedigree analysis of >20 PSSM Quarter Horses initially pointed to a recessive pattern of inheritance for PSSM.\textsuperscript{77,80} Conclusions were based on the fact that foals from PSSM × PSSM matings developed PSSM, and consanguinity was present in pedigrees. Further expansion of this breeding herd by outcrossing PSSM Quarter Horses to normal stallions indicated that a dominant rather than recessive pattern of inheritance was most likely because affected foals arose from mating PSSM affected to normal horses.\textsuperscript{81} Whole-genome analysis of Quarter Horses with PSSM1 identified an autosomal dominant single base-pair mutation in glycogen synthase 1 (\textit{GYS1}), which results in an arginine substitution for histidine at codon 309 in glycogen synthase.\textsuperscript{81} The effect of this amino acid substitution is a higher than normal activity of glycogen synthase both at basal states and when activated by G-6-P. This gain-of-function mutation in \textit{GYS1} is a novel genetic mutation that has never previously been proposed as a cause for myopathy or exertional rhabdomyolysis.

**Diagnosis**

A high degree of suspicion of a diagnosis of PSSM1 arises from clinical signs and persistent elevations in serum CK and AST activities (Fig. 10).\textsuperscript{103,104,177} If horses have normal serum muscle enzymes at rest, an exercise test consisting of a maximum of 15 minutes lunging at a walk and trot may help to determine if subclinical ER is present.\textsuperscript{109} Supportive evidence would include a minimum of a 3-fold elevation in CK activity 4 hours after exercise.

Muscle biopsy provides one means to diagnose type 1 PSSM.\textsuperscript{96,176,179} The distinctive features of PSSM1 in muscle biopsy samples are numerous subsarcolemmal vacuoles (Fig. 6B) and dense, crystalline periodic acid Schiff (PAS)-positive, amylase-resistant inclusions in fast-twitch fibers (Fig. 13, A and B). A diagnosis can be made irrespective of diet and proximity of sampling to recent episodes of rhabdomyolysis. A false-negative diagnosis of type 1 PSSM by muscle biopsy may occur if biopsy samples are small or if horses are less than 1 year of age.\textsuperscript{80}

The gold standard for diagnosis of PSSM1 is genetic testing for the \textit{GYS1} mutation performed on whole blood or hair root samples at the University of Minnesota in North America (http://www.vdl.umn.edu/vdl/ourservices/neuromuscular.html), in a genetic testing panel offered through the AQHA or in Europe by Laboklin (http://www.laboklin.co.uk/laboklin/GeneticDiseases.jsp). Other laboratories are not licensed to perform this test.

It is important to note that in draft horses of Continental European origin, the very high prevalence of PSSM in essence means that there is a high chance that any clinical sign could be falsely associated with the disease PSSM.\textsuperscript{99} Thus, clinical judgment is required to determine whether the clinical signs of the animal could reasonably be associated with a myopathic process.

**Acute Clinical Signs**

Horses usually show signs of PSSM1 at, on average, 5 years of age; however, this can range from 1 to 14 years of age.\textsuperscript{104,162} Acute clinical signs occur when horses are calm and include tucking up of the abdomen, fasciculations in the flank, muscle stiffness, sweating, reluctance to move forward, and overt firm muscle contractures.\textsuperscript{162} The hindquarters are frequently most affected, but back muscles, abdomen, and forelimb muscles may also be involved. Signs of pain can last for more than 2 hours, and about 10\% of cases become recumbent.\textsuperscript{162} Less common signs of PSSM in Quarter Horses include gait abnormalities, mild colic, and muscle wasting. During an acute episode of ER, horses with PSSM1 often have markedly elevated serum CK activity of >35,000 U/L, and myoglobinuria may be present. Severe colic-like pain after exercise and myoglobinuric renal failure are less common presenting complaints.\textsuperscript{177,180}

**Chronic Clinical Signs**

\textit{Light breeds:} Chronic signs of PSSM1 in riding horses include a lack of energy when under saddle, reluctance to move forward, stopping and stretching out as if to urinate, and a sour attitude toward exercise.\textsuperscript{103,162} Horses may have a combination of low-grade reluctance to exercise, poor performance, and repeated episodes of ER.\textsuperscript{164} PSSM1 may also present in dressage and show jumpers as chronic back pain, failure to round over fences, and fasciculations or pain on palpation of lumbar muscles.\textsuperscript{162} The range of severity of clinical signs of PSSM1 can be wide, with complete incapacitation in rare cases. Serum CK activities are often elevated in unmanaged horses, even when horses are rested.\textsuperscript{177} The median CK and AST activity for all PSSM1 Quarter Horses with muscle biopsies submitted to the University of Minnesota was 2,809 and 1,792 U/L, respectively.

A small number of Quarter Horses and Paint Horses have both the \textit{GYS1} mutation and the genetic mutation for malignant hyperthermia.
This results in particularly severe signs of ER and a limited response to diet and exercise changes. During an episode of ER, excessively high body temperatures may develop, and sudden death can occur in horses with the RYR1 mutation. This combination occurs most often in halter and pleasure horses.

**Draft Horse and Draft Crosses**

The average age of draft horses diagnosed with PSSM is about 8 years. Many draft horses with PSSM are asymptomatic. Signs of severe rhabdomyolysis and myoglobinuria may occur in horses fed high grain diets, exercised irregularly with little turnout or horses that undergo general anesthesia. Rhabdomyolysis can be so severe that it leads to recumbency and death. Other signs of PSSM in draft horses include progressive weakness and muscle loss resulting in difficulty rising in horses with normal serum CK activity. Pronounced weakness is more prevalent in homozygotes for the GYS1 mutation. Gait abnormalities, such as excessive limb flexion, fasciculations, and trembling are also reported in draft horses. Although the condition “shivers” was previously attributed to PSSM, a recent study found no causal association between these two conditions. The median serum CK and AST activities in draft horses from which biopsies were sent to the University of Minnesota was 459 and 537 U/L, respectively.

**Pathogenesis**

**Exercise**

The vast majority of horses that develop rhabdomyolysis with PSSM do so with aerobic exercise when blood lactate concentrations are unchanged from pre-exercise samples at 1.0 mmol/L. While elevated serum CK activity occurs with near-maximal exercise on occasion, the degree of elevation is often less than with submaximal exercise. The primary clinical sign with maximal exercise is exercise intolerance, and it is interesting to observe that very few racing Quarter Horses have the GYS1 mutation. A deficit in the ability to generate adequate ATP to power muscle contraction at high speeds may account for selection away from the GYS1 mutation in racing horses.

**Energy Depletion**

Aerobic metabolism is stimulated when exercise decreases the ratio of ATP:ADP. However, if aerobic or anaerobic metabolism cannot effectively restore this ratio, the myokinase reaction produces ATP and AMP from 2 ADP. AMP stimulates AMP deaminase, leading to an increase in IMP concentrations. In normal horses, IMP is only generated during maximal exercise or with marked metabolic stress. One of the critical studies performed in PSSM1 horses determined that a deficit in energy metabolism represented by high myofiber IMP occurred after light exercise in individual muscle fibers of horses with PSSM in association with high serum CK activity (Fig. 14). This finding was a key indicator that PSSM1 was due to a defect in muscle energy metabolism.

**Glycogenolysis/Glycolysis**

In the majority of human and animal studies, glycogen storage disorders arise from defective enzymes in glycogenolysis or glycolysis. As such, much of the initial research into the basis for PSSM initially focused on identifying a defect in enzymes involved in glycogen or glucose metabolism. The first studies used an in vitro model of anaerobic glycolysis and glycogenolysis. Muscle lactate production from a variety of glycolytic substrates was lower in PSSM1 horses than in controls; however, there was clearly flux through glycolysis in PSSM1 horses. PFK deficiency was an initial candidate because clinically and histologically it resembles PSSM1. However, maximal PFK activity as well as PFK activity in the presence of allosteric modifiers were normal in horses with PSSM1. In addition, activities of muscle enzymes such as phosphorylase, phosphorylase b kinase, enolase, phosphoglycerate mutase, phosphoglycerate kinase, and lactate dehydrogenase in PSSM Quarter Horses were all found to be similar to that in controls.

Another in vivo mechanism used to screen for defects in glycogenolysis/glycolysis is to determine if lactate production is impaired during anaerobic exercise. Quarter Horses with PSSM were reluctant...
to perform anaerobic exercise, but at their maximum speed, they depleted glycogen by 25% (Fig. 12) and generated higher concentrations of lactic acid than normal horses performing the same exercise test. Thus, combined, this research indicated that a glycogenolytic/glycolytic defect was improbable in PSSM1. Since glycogen degradation was unimpaired with maximal exercise, research to uncover the basis for excessive glycogen storage in PSSM1 shifted to potential novel defects that enhanced glucose uptake or glycogen synthesis.

**Glucose Uptake Into Muscle Cells**

Glucose transport occurs via GLUT1, which is constitutively present in the cell membrane, and GLUT4, which translocates to the muscle cell membrane in response to insulin secretion and exercise. Oral and intravenous glucose tolerance testing showed that blood glucose clearance occurred 1.5 times faster in PSSM1 Quarter Horses compared with control horses despite their lower insulin concentrations. Enhanced insulin sensitivity in PSSM1 Quarter Horses was confirmed by euglycemic hyperinsulinemic clamping. This enhanced sensitivity to insulin was breed-specific, however, as Belgian Draft horses with PSSM did not show similar enhanced insulin sensitivity. Enhanced insulin sensitivity and glucose uptake did not appear to be responsible for ER in PSSM horses, as dexamethasone treatment suppressed insulin sensitivity without any effect on serum CK responses to exercise. When the Quarter Horse data was reevaluated once their genotypes were discovered, those horses with both GYS1 and RYR1 mutations had the greatest increase in insulin sensitivity. This suggests a possible role of the RYR1 gene in enhancing the insulin sensitivity of PSSM horses. The content of GLUT1 or GLUT4 in muscles from horses with PSSM1 was evaluated by Western blot, and no differences were found compared with controls.

An increase in PSSM1 skeletal muscle glucose uptake in response to exercise was suggested by the significantly higher glucose concentrations in muscle cells of PSSM horses after light exercise, which was not present in either basal resting conditions or in control horses after exercise. Once inside the muscle cell, glucose is phosphorylated to form glucose-6-phosphate (G-6-P) by hexokinase. No difference in hexokinase activity was identified in PSSM versus control horse muscle; however, variably high G-6-P concentrations have been found in PSSM skeletal muscle.

**Glycogen Synthesis**

The finding that PSSM horses rapidly resynthesize glycogen even when postexercise concentrations become as low as pre-exercise concentrations in healthy horses suggested that glycogen synthesis was abnormally regulated in PSSM horses (Fig. 12). Glycogen synthesis begins with a protein (glycogenin) and grows to form at least 12 tiers of branched glucose residues forming a highly folded β-glycogen particle. Glucose residues are added to glycogen via UDP glucose through α,1,4 linkages catalyzed by the enzyme glycogen synthase and stimulated by insulin and G-6-P (Fig. 15). Branching enzyme, which is not tightly regulated, adds branch points to the growing glycogen molecule with α,1,6 linkages (Fig. 15). Together with branching enzyme, GS forms a low-molecular-weight (400 kDa) glycogen granule termed proglycogen that grows larger (up to 10,000 kDa) to form macroglycogen. Horses with PSSM1 were found to have high activities of glycogen synthase (in basal and G6P-stimulated states), high branching enzyme activity, and an increase in both pro- and macro-glycogen concentrations. The effect of the autosomal dominant mutation in the GYS1 gene encoding glycogen synthase is a unique gain of function. The higher-than-normal activity in both basal states and when activated by insulin and G-6-P appears to have a novel link to the regulation of energy generation in muscle fibers during exercise under certain dietary conditions that increase insulin and muscle G-6-P concentrations.

**Abnormal Polysaccharide Formation**

The iodine absorption spectra of muscle samples from PSSM1 horses indicated that the abnormal polysaccharide in PSSM1 horse muscle fibers has a less branched structure than normal glycogen. This is confirmed by ultrastructural studies that indicate abnormal polysaccharide consists of both beta glycogen particles and filamentous material. The formation of a less branched abnormal polysaccharide is probably related to chronic enhanced activity of glycogen synthase enzyme in the presence of elevated G-6-P and insulin concentrations without the same relative activation of branching enzyme. Over years, this leads to a less highly branched polysaccharide, which becomes resistant to amylase digestion. When abnormal polysaccharide accumulates in large amounts in muscle cells, it is associated with autophagic rimmed vacuoles and is tagged for degradation by ubiquitin. Foals and yearlings with the GYS1 mutation rarely have abnormal polysaccharide in skeletal muscle, yet they have rhabdomyolysis. Thus, abnormal polysaccharide itself is an indicator of PSSM1 but not the direct cause for rhabdomyolysis.

**Diet and GYS1**

A high NSC diet increases the propensity to develop muscle pain with aerobic exercise in PSSM1 horses. A high NSC versus low NSC, high fat diet results in higher muscle lactate and citrate concentrations, lower G-6-P, and similar muscle pyruvate concentrations with aerobic exercise in PSSM1 horses. This suggests that the enhanced glycogen synthase activity in PSSM horses may impair oxidative metabolism of substrates such as pyruvate and fatty acids. While the exact mechanism for this is not known, it may involve a link between
glycogen synthase or glycogen concentrations and metabolic switches and nutrient sensors. One of the key sensors and regulators of substrate flux in skeletal muscles is AMP kinase. When energy supply is deemed to be insufficient (\( \frac{[\text{ATP}]}{[\text{ADP}]})\), 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. We speculate that excessive stimulation of glycogen synthesis resulting from the \( \text{GYS1} \) mutation (stimulated further by insulin) is interpreted by nutrient sensors in the cell as an indication not to activate glycogenolysis and lipolysis. A potential scenario in PSSM1 horses on high NSC diets could be that nutrient switches do not fully activate enzymes such as pyruvate dehydrogenase during exercise, limiting adequate acetyl CoA for oxidative metabolism (Fig. 5).

Although acetyl CoA could also be supplied by fatty acid oxidation, PSSM horses on high NSC diets have low plasma nonesterified FFA concentrations, possibly due to suppression of lipolysis by high insulin. A further factor reducing fatty acid oxidation in PSSM horses on high NSC diets could be that high muscle citrate concentrations direct acetyl CoA away from the citric acid cycle toward malonyl CoA, the committed step for fatty acid synthesis. In addition, accumulation of malonyl CoA causes inhibition of carnitine palmitoyltransferase, the key enzyme necessary to transport long-chain fatty acids into the mitochondria for \( \beta \)-oxidation. Thus, PSSM horses fed high NSC diets may be unable to generate sufficient acetyl CoA from either carbohydrate or fat metabolism to fuel muscle contraction during submaximal exercise. The common occurrence of clinical signs during the first 20 minutes of exercise may be be-

Fig. 15. Glycogen synthesis begins with a protein (glycogenin) and grows to form at least 12 tiers of branched glucose residues forming a highly folded \( \beta \) glycogen particle. Glucose residues are added to glycogen via UDP glucose through 1,4 linkages catalyzed by the enzyme glycogen synthase and stimulated by insulin and G-6-P. Branching enzyme adds branch points to the growing glycogen molecule with 1,6 linkages. Source: Wikipedia.
cause at this stage of exercise, muscles rely heavily on glycogen/glucose for energy. Low dietary starch and fat supplementation decrease muscle citrate concentrations and increase plasma FFA concentrations, which may well facilitate muscle fat metabolism in PSSM1 horses.\textsuperscript{175}

Type 2 PSSM
There is much less known about type 2 PSSM because as it turns out, previous research on PSSM has largely involved horses with type 1 PSSM. Current knowledge of type 2 PSSM is based on retrospective evaluation of cases diagnosed with PSSM by muscle biopsy\textsuperscript{103} that are now known to be free of the \textit{GYS1} mutation and a few years of prospective clinical cases.

\textbf{Prevalence}
Approximately 28\% of cases of PSSM diagnosed by muscle biopsy in Quarter Horses have PSSM2, as they do not have the \textit{GYS1} mutation.\textsuperscript{167} Type 2 PSSM seems to be common in both high-performance Quarter Horse types such as barrel racing, reining, and cutting horses as well as pleasure and halter horses. In the United Kingdom, approximately 35\% of PSSM cases diagnosed by muscle biopsy have PSSM2.\textsuperscript{163} It also occurs in Paint, Appaloosa, and Morgan Horses. About 80\% of cases of PSSM diagnosed by biopsy in Warmbloods have PSSM2.\textsuperscript{167} Breeds affected include Dutch Warmbloods, Selle Français, Hanoverians, Friesians, Westfalian, Canadian Warmblood, Irish Sport Horse, Gerdlander, Hussien, and Icelandic Horses. Many other light breeds have also been diagnosed with PSSM2, including Standardbreds and Thoroughbreds. Type 2 PSSM also occurs in Arabians; however, in my experience, this breed is distinct in that it often has amylase-resistant rather than amylase-sensitive polysaccharide but is negative for the \textit{GYS1} mutation.

\textbf{Pathogenesis}
The cause of PSSM2 is currently unknown. Muscle glycogen concentrations are not as high as those found in type 1 PSSM, and glycogenolytic enzyme defects have not been identified in the few cases in which enzymes were measured (Valberg, unpublished data). To date, the only reported abnormality in muscle ultrastructure is an increase in normal beta glycogen particles.\textsuperscript{179} It may well be that there is a group of conditions currently captured under the term type 2 PSSM that have separate etiologies but share common findings of glycogen accumulation and poor performance. A heritable predisposition is suspected in Quarter Horses, but yet to be proven.

\textbf{Acute Clinical Signs}
Horses with PSSM2 do not necessarily have the same calm temperament as horses with PSSM1. In adults, acute clinical signs of rhabdomyolysis are similar between type 1 and type 2 PSSM.\textsuperscript{163,167,168} Muscle atrophy after rhabdomyolysis is a common complaint in Quarter Horses with PSSM2. There are more Quarter Horses less than 1 year of age reported with PSSM2 than PSSM1, and these foals may present with an inability to rise or a stiff hind limb gait.\textsuperscript{168}

\textbf{Chronic Clinical Signs}
Chronic signs of PSSM2 are often most closely related to poor performance rather than recurrent ER and elevations in serum CK activity. An undiagnosed gait abnormality, sore muscles, and drop in energy level and willingness to perform after 5 to 10 minutes of exercise are common complaints in horses with PSSM2. Warmbloods with PSSM2 may also have painful, firmed back and hindquarter muscles, reluctance to collect, canter, and engage the hindquarters, poor rounding over fences, gait abnormalities, and slow onset of atrophy. The mean age of onset of clinical signs in Warmbloods is between 8 and 11 years of age, with the median CK and AST activity being 323 and 331 U/L, respectively, according to Neuromuscular Diagnostic Laboratory records.

\textbf{Diagnosis}
Type 2 PSSM must be diagnosed by muscle biopsy where increased or abnormal PAS-positive material that is usually amylase-sensitive is apparent, particularly in subsarcolemmal locations (Fig. 13, C and D).\textsuperscript{179} Determination of what constitutes an abnormal amount of amylase-sensitive glycogen is subjective; the specificity of the diagnosis may be low, and false-positive diagnoses may occur. Consideration should be made for state of training, as highly trained horses have increased glycogen storages as a normal response to training. Further, glycogen is commonly found near capillaries in normal horses and PAS-positive sarcoplasmic masses can be found in 60\% of healthy horses, so these should not be diagnostic criteria for PSSM2.\textsuperscript{199} Formalin-fixed sections show a greater deposition of subsarcolemmal glycogen and precipitation of glycogen in one area of a fiber even in healthy horses, and these should not be considered diagnostic criteria for PSSM2.\textsuperscript{178} Other histopathologic features that may be present with PSSM2 include centrally located nuclei, subsarcolemmal vacuoles, muscle necrosis, macrophage infiltration of myofibers, regenerative fibers, and fiber atrophy.\textsuperscript{179} Some laboratories grade polysaccharide accumulation as mild, moderate, and severe, where mild accumulation represents a category that has a higher chance of being a false-positive diagnosis.\textsuperscript{98,163} Mild PSSM cases in particular should receive a full physical examination to ensure that there are not other underlying causes for performance problems.
Owners must be aware that any horse diagnosed with PSSM will always have an underlying predilection for muscle soreness. The best that can be done is to manage horses in the most appropriate fashion to minimize clinical signs. With adherence to both diet and exercise recommendations provided below, at least 70% of horses show notable improvement in clinical signs and many return to acceptable levels of performance.\textsuperscript{103,117,162} There is, however, a wide range in the severity of clinical signs shown by horses with PSSM; those horses with severe or recurrent clinical signs will require more stringent adherence to diet and exercise recommendations to regain muscle function.

Rest
PSSM horses that are confined for days to weeks after an episode of rhabdomyolysis often have persistently elevated serum CK activity.\textsuperscript{80} In contrast, PSSM horses kept on pasture with little grain supplementation often show few clinical signs of rhabdomyolysis and have normal serum CK activity.\textsuperscript{80} As a result, a common recommendation for horses with PSSM is to limit stall confinement to less than 48 hours after an episode of rhabdomyolysis and then provide turnout in paddocks of gradually increasing size. Providing horses with as much free exercise as possible on pasture appears to be beneficial in the long term. If pasture is lush, a grazing muzzle may be needed.\textsuperscript{164} After an acute episode, excitable horses may require tranquilization prior to turnout to avoid excessive galloping. Hand-walking horses initially recovering from an episode of PSSM for more than 5 to 10 minutes at a time may trigger another episode of rhabdomyolysis.

Exercise
The beneficial response to low starch, fat supplemented diets only occurs if it is instituted in conjunction with a regular incremental exercise program.\textsuperscript{173} Regular daily exercise in PSSM horses over a 3-week period has been shown to produce a dramatic decrease in serum CK responses to exercise (Fig. 16), whereas stall confinement often causes elevations in serum CK activity after exercise.\textsuperscript{80,173} One common adaptation to daily training is an increase in oxidative capacity in skeletal muscle. The oxidative capacity of skeletal muscle in PSSM Quarter Horses was found to be very low, based on markers such as citrate synthase activity and $\beta$-fatty acid oxidative marker HAD.\textsuperscript{173} The activity of these enzymes, however, was equally low in control Quarter Horses. Whether the beneficial effect of daily exercise on PSSM horses is a result of improved oxidative enzyme capacities or enhanced substrate flux or both has not been elucidated.

Exercise Regimes
Important principles to follow when starting exercise programs in PSSM horses include (1) provide adequate time for adaptation to a new diet prior to commencing exercise, (2) recognize that the duration of exercise, not the intensity, is of primary importance, (3) ensure that the program is gradually introduced and consistently performed, and (4) minimize any days without some form of exercise. If horses have experienced a moderate to marked episode of rhabdomyolysis recently, 2 weeks of turnout and diet change are often beneficial prior to recommencing exercise. Exercise should be very relaxed, and the horse should achieve a long, low frame without collection. For many horses, this is most readily done in a round pen or on a lunge-line but can be done under saddle if needed. Successive daily addition of 2-minute intervals of walk and trot beginning with only 4 minutes of exercise and working up to 30 minutes after 3 weeks is often recommended.\textsuperscript{105,177} If horses had minor elevations in 4-hour postexercise CK with the 15-minute exercise test, horses may begin at 15 minutes of exercise. Owners often do not recognize that walking the horse for 10 minutes or more initially can trigger muscle soreness in PSSM horses. Advancing the horse too quickly often results in an episode of rhabdomyolysis and repeated frustration for the owner. Work can usually begin under saddle after 3 weeks of ground work and can gradually be increased by adding 2-minute intervals of collection or canter to the initial relaxed warm-up period at a walk and trot. Unless a horse shows an episode of overt rhabdomyolysis during the initial first 4 weeks of exercise, reevaluating serum CK activity is not helpful for the first month. This is because it is very common to have subclinical elevations in CK activity when exercise is re-introduced, and a return to normal levels often requires 4 to 6 weeks of gradual exercise.\textsuperscript{177} Keeping horses with PSSM fit seems
Dietary change appears to improve clinical signs of muscle pain, stiffness, and exercise tolerance in draft horses, Warmbloods, Quarter Horses, and other breeds.103,104,117,162 This type of diet improves clinical signs of muscle pain, stiffness, and exercise tolerance in draft horses, Warmbloods, Quarter Horses, and other breeds.103,104,117,162 Selection of Forage

 owners report for-and stimulation of-glycogen synthesis and glucose uptake in muscles cells, less substrate available for-and stimulation of-glycogen synthesis and normalization of substrate flux. Owners report that this type of diet improves clinical signs of muscle pain, stiffness, and exercise tolerance in draft horses, Warmbloods, Quarter Horses, and other breeds.103,104,117,162 Dietary change appears to have lesser impact on alleviating gait changes such as shivers.103

Caloric Balance

the horse's caloric requirements at an ideal body weight are the most important considerations in designing the diet for PSSM (Table 3). This is because many horses with PSSM are easy keepers and may be overweight at the time of diagnosis. Adding excessive calories in the form of fat to an obese horse may produce metabolic syndrome and is contraindicated. If necessary, caloric intake should be reduced by using a grazing muzzle during turnout, feeding hay with a low NSC content at 1% to 1.5% of body weight, providing a low-calorie ration balancer, and gradually introducing daily exercise. Rather than provide dietary fat to an overweight horse, fasting for 6 hours before exercise can be used to elevate plasma FFAs prior to exercise and alleviate any restrictions in energy metabolism in muscle.

Selection of Forage

Quarter Horses have been shown to develop a significant increase in serum insulin concentrations in response to consuming hay with an NSC of 17%, whereas insulin concentrations are fairly stable when fed hay with 12% or 4% NSC content.175 Because insulin stimulates the already overactive enzyme glycogen synthase in the muscle of PSSM1 horses, selecting hay with 12% or less NSC is advisable. The degree to which the NSC content of hay should be restricted below 12% NSC depends on the caloric requirements of the horse. Feeding a low NSC hay of 4% provides room to add an adequate amount of fat to the diet of easy keepers without exceeding the daily caloric requirement and inducing excessive weight gain. For example, a 500-kg horse on a routine of light exercise generally requires 18 MCal/d of DE. Fed at 2% of body weight, a 12% NSC mixed grass hay almost meets their daily caloric requirement by providing 17.4 MCal/d. Thus with a 12% NSC hay, there is only room for 0.6 MCal of fat per day (72 mL of vegetable oil) to achieve 18 MCal of energy. In contrast, a 4% NSC Blue Grama hay would provide 13.5 MCal/d, which would allow a reasonable addition of 4.5 MCal of fat per day (538 mL of vegetable oil).

Selection of Fat Source

High fat diets increase plasma FFA concentrations and thus the availability of fats for oxidation in skeletal muscle.173 Long-chain fat diets also appear to increase glycogenolytic/glycolytic and oxidative flux in PSSM1 muscle as shown by higher G-6-P, lower lactate, and higher pyruvate concentrations in muscle of PSSM1 horses fed and trained on a commercial high fat diet compared with sweet feed.175 Thus, one means to overcome limitations in delivery of oxidative substrates in PSSM1 horses is to provide ample long-chain fat in the diet. The major sources of dietary fat for horses are vegetable-based, including vegetable oils and rice bran or animal based fat (tallow, lard, fish oil). Vegetable oils are highly unsaturated, very digestible (90% to 100%), and very energy-dense. Suitable forms include soybean, corn, safflower, canola, flaxseed, linseed, peanut, and coconut. Controlled research

Table 3. Feeding Recommendations for an Average-Sized 500-kg Horse With Exertional Rhabdomyolysis Due to Recurrent Exertional Rhabdomyolysis or Polysaccharide Storage Myopathy

<table>
<thead>
<tr>
<th>RER</th>
<th>PSSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise intensity</td>
<td>Light</td>
</tr>
<tr>
<td>Caloric intake</td>
<td>20</td>
</tr>
<tr>
<td>Percentage of daily digestible energy as NSC</td>
<td>15–20</td>
</tr>
<tr>
<td>Percentage of daily digestible energy as fat</td>
<td>15</td>
</tr>
<tr>
<td>Selenium, mg/d</td>
<td>2.2</td>
</tr>
<tr>
<td>Vitamin E, mg/d</td>
<td>1,400</td>
</tr>
<tr>
<td>Daily amount, kg, of 12% NSC, 10% fat concentrate†</td>
<td>2.5</td>
</tr>
<tr>
<td>Grass hay, kg, with &lt;12% NSC by weight</td>
<td>7.5–10</td>
</tr>
<tr>
<td>Sodium chloride, g</td>
<td>34</td>
</tr>
</tbody>
</table>

RER indicates recurrent exertional rhabdomyolysis; PSSM, polysaccharide storage myopathy.

†Concentrate for horses with exertional myopathies

*Natural vitamin is preferable to synthetic vitamin E due to better absorption.

References

173-175,200,201 Quarter Horses have been shown to develop a significant increase in serum insulin concentrations.173,200,201 Quarter Horses naturally produce metabolic syndrome and is contraindicated. If necessary, caloric intake should be restricted below 12% NSC depends on the caloric requirements of the horse. Feeding a low NSC hay of 4% provides room to add an adequate amount of fat to the diet of easy keepers without exceeding the daily caloric requirement and inducing excessive weight gain. For example, a 500-kg horse on a routine of light exercise generally requires 18 MCal/d of DE. Fed at 2% of body weight, a 12% NSC mixed grass hay almost meets their daily caloric requirement by providing 17.4 MCal/d. Thus with a 12% NSC hay, there is only room for 0.6 MCal of fat per day (72 mL of vegetable oil) to achieve 18 MCal of energy. In contrast, a 4% NSC Blue Grama hay would provide 13.5 MCal/d, which would allow a reasonable addition of 4.5 MCal of fat per day (538 mL of vegetable oil).
studies in exercising PSSM horses have shown a decrease in muscle pain and serum CK in response to the addition of corn oil\textsuperscript{117,162,175} and also soybean oil and rice bran\textsuperscript{a} (Fig. 16).\textsuperscript{173,175} The amount of oil added in these trials constituted at least 13\% of daily DE. Some veterinarians have advocated as much as 25\% of DE be fed in the form of fat to PSSM horses.\textsuperscript{118} As discussed above, the principle consideration here should be whether this provides excessive calories and additional weight gain because feeding 13\% DE as fat may well be effective in reducing muscle pain.

Limited research has been performed on the form of oil to feed PSSM horses. An odd carbon 7 chain fat (triheptanoin) fed to PSSM horses had a detrimental effect on muscle pain, exercise tolerance, and serum CK activity, whereas in the same study, long-chain fats fed in the form of corn oil or a rice bran/soy oil supplemented feed had a beneficial effect on lowering serum CK activity.\textsuperscript{175} Whether there is any direct beneficial effect on skeletal muscle of providing energy in the form of Omega 3 versus Omega 6 fatty acids has yet to be determined. Corn oil, sunflower oil, and safflower oil are high in Omega-6 and lower in Omega-3, whereas soybean and canola oils are moderately high in Omega-6 and Omega-3, and flax seed (linseed) and fish oils contain more Omega-3 than Omega-6. It is usually cost-prohibitive to provide sufficient energy to a PSSM horse each day in the form of dense Omega 3 fat supplements. Soybean and canola oils provide a relatively affordable alternative with moderately high Omega-6, or a mix of these oils and flax or fish oil can be provided. Due to the potential additional oxidant stress of fats, vitamin E (1000 t 5000 U/d) should be fed to horses receiving high oil diets.

Low Starch, High Fat Concentrates
While oils are energy-dense and inexpensive, they have the disadvantages of being messy, unpalatable to some horses, prone to rancidity in warm temperatures, and difficult to feed in large amounts. As such, a number of concentrates have been developed that contain fats. The important principle to be met by such feeds is that the starch and sugar components are low enough and fat supplementation high enough to ensure that in the total diet, the calories supplied by NSC comprise no more than 10\% to 15\% of the daily DE and the calories supplied by fat comprise about 12\% to 15\% of daily DE.\textsuperscript{142,173} Common fat sources used in such concentrates include, in addition to the oils mentioned above, stabilized rice bran or animal fats. Rice bran and its products are palatable to most horses, have a moderate NSC content, contain 25\% by weight, contain 20\% fat by weight as well as vitamin E, and are naturally high in phosphorus.\textsuperscript{142} The NSC component of rice bran can vary if the manufacturing process is not careful to exclude the white rice grain. Commercial rice bran products occur as powder or an extruded pellet and are considerably more stable in warm temperatures compared with animal fat and vegetable oils. A number of well-balanced low starch, high fat commercial diets are suitable for horses with PSSM.\textsuperscript{142,173} Some commercial feeds meet the recommended nutritional needs of PSSM horses in one pelleted ration. These feeds typically contain at least 10\% to 15\% by weight and less than 20\% NSC by weight. Some feed companies offer similar nutritional content by blending two or more manufactured feeds or by supplementing with oils or rice bran. At present, the NSC content of equine feed products is not consistently listed on the feed tag, and consultation with the feed manufacturer is necessary to obtain this information. Nutritional support is available through most feed manufacturers in designing an appropriate diet. There is a great deal of variation in individual tolerance to dietary starch; however, horses with more severe clinical signs of PSSM appear to require the greatest restriction in starch intake.

Expectations
The beneficial effect of low starch, high fat diets require that horses are trained daily to enhance enzymes involved in fat and glucose metabolism. It is important to note that a horse diagnosed with PSSM will always have an underlying predilection for muscle soreness, and the best that can be done is to manage horses to minimize clinical signs. With adherence to both the diet and exercise recommendations 70\% to 75\% of Quarter Horses and Warmbloods show notable improvement in clinical signs, and many return to acceptable levels of performance.\textsuperscript{165,118,162,164} There is, however, a wide range in the severity of clinical signs shown by horses with PSSM; those horses with severe or recurrent clinical signs will require more stringent adherence to diet and exercise recommendations to regain muscle function. PSSM horses that also have the mutation for malignant hyperthermia do not respond as well to diet and exercise recommendations and may continue to develop ER with the possibility of a fatal episode.\textsuperscript{174}

Supplements
There are no specific supplements that have been shown to benefit horses with PSSM. Chromium, which is reported to increase glucose absorption, may be contraindicated in PSSM horses. Carnitine is a dietary supplement that may replenish muscle carnitine stores if depleted and assist in transport of fat into the mitochondria. A deficiency of plasma carnitine has not been identified in PSSM horses. Carnitine is a dietary supplement that may replenish muscle carnitine stores if depleted and assist in transport of fat into the mitochondria. A deficiency of plasma carnitine has not been identified in PSSM1 horses (Valberg, unpublished data; PSSM1 plasma-free carnitine; PSSM1 range, 10.24 to 27.11 \textmu M; normal range, 13.20 to 25.63 \textmu M).

VI. Mitochondrial Myopathies
Disorders or aerobic metabolism reported to date in horses have not been characterized by rhabdomyolysis but rather most profoundly by exercise intoler-
ance or weakness. The severe acidosis arising with aerobic enzyme deficiencies may prevent horses from exercising to the point where muscle damage develops in muscle fibers from an energy deficit.

Congential Mitochondrial Myopathy

Clinical Signs
A deficiency of mitochondrial respiratory chain function has been identified in a 3-year-old Arabian filly that presented with profound exercise intolerance.20 After 5 minutes of trotting, the horse would consistently become stiff, sweat profusely, and be reluctant to move for about 60 minutes after exercise. No respiratory or cardiovascular causes for poor performance were identified in this filly. Over the course of years, the filly developed significant muscle atrophy.

Exercise Testing
Serum CK activity 4 hours after exercise was within normal limits. Venous pH and bicarbonate concentrations were normal before exercise and dropped abnormally after 6 minutes of trotting (pH 7.24; normal, 7.39; and bicarbonate, 19 mEq/L; normal, 24 mEq/L). During a treadmill exercise test, the horse also showed a drastically reduced maximum oxygen consumption (maximal VO\(_2\), 0.5 mL/kg/s; normal, 2.5 mL/kg/s) and a marked plasma lactic acidosis at 7 m/s (20 mmol/L; normal <1 mmol/L).

Pathogenesis
Analysis of muscle samples from this filly was indicative of a deficiency of Complex I [nicotinamide adenine dinucleotide (NADH):ubiquinone reductase] in the mitochondrial respiratory chain.20 The respiratory chain in the inner mitochondrial membrane has five multisubunit enzyme complexes that serve to translocate protons from the matrix to the intermembrane space and synthesize ATP. The inability of muscle to utilize oxygen resulted in an almost complete reliance on anaerobic energy metabolism regardless of the speed of exercise. The severe lactic acidosis appeared to induce fatigue.

Diagnosis
An increase in blood lactate concentrations at aerobic speeds such as a walk and trot in otherwise healthy horses suggests a mitochondrial myopathy. Further support for the diagnosis is provided by frozen sections of muscle biopsies of locomotor muscles that show an increase or abnormal pattern of mitochondrial staining (Fig. 17B). Electron microscopy may reveal that the mitochondria are abnormally shaped and/or have bizarre cristae formations. Biochemical analysis of oxidative en-
zymes is needed to identify a deficiency in oxidative enzymes or respiratory chain malfunction.

Treatment

There is no known treatment for congenital mitochondrial myopathies.

Acquired Mitochondrial Abnormality and Vitamin E Deficiency

Clinical Signs

An acquired disorder affecting mitochondria has recently been reported in horses with low muscle vitamin E concentrations. Horses show a decrease in performance due to exercise intolerance, generalized locomotor muscle wasting, muscle fasciculations, and weakness. The distinction between this disorder and equine motor neuron disease is the presence of myopathic rather than neuropathic features in sacrocaudalis dorsalis muscle biopsies and the remarkable response to treatment with vitamin E.

Exercise Testing

Exercise testing beyond a neurologic examination has not been reported for acquired cases associated with low vitamin E. This is because horses often present with such severe weakness that it is not clinically practical to perform.

Pathogenesis

The observed generalized weakness in the horses with vitamin E–deficient myopathy is suggested to be due to a reversible manifestation of skeletal muscle mitochondrial oxidative stress associated with vitamin E deficiency. Vitamin E–deficient myopathy may be an entity unto itself or a predecessor to development of equine motor neuron disease.

Diagnosis

Acquired vitamin E–deficient myopathy is diagnosed by confirming low serum or muscle vitamin E concentrations and identifying a moth-eaten staining pattern in frozen biopsies of the sacrocaudalis dorsalis muscle (Fig. 17C). Mitochondrial stains of muscle such as the gluteus medius are normal, whereas highly oxidative muscles such as the sacrocaudalis dorsalis muscle biopsies and the remarkable response to treatment with vitamin E.

Treatment

Acquired vitamin E–deficient myopathy is highly responsive to treatment with natural vitamin E (5000 IU/d per 500-kg horse) and a very gradual reintroduction to exercise once strength returns.

Conclusions

The past 30 years have offered exciting discoveries of several new equine-specific muscle diseases, many of which manifest exclusively with exercise.

I hope within this review that I have paid tribute to the many clinician scientists past and present whose fascination with muscle disorders provided the building blocks for these advances and inspired other young scientists to take up the call. As our knowledge of equine exercise physiology, muscle histopathology, biochemistry, and genetics continues to expand and young clinician scientists are drawn to the field, other new discoveries are likely imminent.

I received amazing mentoring during my PhD in Uppsala, Sweden, by Birgitta Essen-Gustavssson and Sune Persson, who taught me the discipline and the thrill of science. Drs. Arne Lindholm, Gary Carlson, and John Madigan shaped my vision of the clinician scientists, melding clinical medicine with basic sciences for the betterment of the horse. Dr. Cardinet III shared with me his insights into what muscles look like from the inside looking outward and how to run a diagnostic service with the highest standards. I have had many essential collaborators over the years that I am indebted to. Dr. John Baird and Dr. Billi DiMauro have been staunch supporters and invaluable resources for more than 20 years. I have a huge debt of gratitude to my friend and colleague Jim Mickelson, who has been at the calm and reasoned core of all of the muscle work performed at the University of Minnesota and to my ultra-organized technician Michelle Lucio. I have been inspired and encouraged by my many academic daughters and sons whose sharp minds, diligent graduate work, and humor have made coming to work fun. My family has been my rock through stormy times; to John, you make life worth living; to my son Niels, you are the light of my life; and to my daughter Annika, I miss you every single day.

Conflict of interest statement: Drs. Valberg, Mickelson, and McCue own the license for PSSM testing and receive sales income from its use. Their financial and business interests have been reviewed and managed by the University in accordance with its conflict of interest policies. The University of Minnesota has licensed the sale of Re-Leve and Dr. Valberg receives royalties from Kentucky Equine Research.

References and Footnote


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*Re-Leve, Kentucky Equine Research, Versailles, KY 40383.*