The metabolic and clinical responses of Arabian horses with exertional rhabdomyolysis to a standardized field exercise test

by
Lauren V. Eyrich

A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Animal Sciences
(Honors Scholar)

Presented April 17, 2015
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Erica McKenzie

Exertional rhabdomyolysis (ER), or the occurrence of muscle damage with exercise, is prevalent among Arabian horses, the most common breed participating in endurance racing. The cause and pathogenesis of this disease has yet to be determined, and due to the potential for significant morbidity and even mortality associated with ER in the endurance discipline, this disorder represents a growing welfare concern. This study represented a preliminary investigation of this disease, during which 10 horses with ER and 9 healthy control horses were evaluated while performing a standardized exercise test. Heart rate monitoring and visual assessment for clinical signs of disease were performed continuously during exercise. Blood was collected prior to and after exercise to measure changes in packed cell volume, and total protein, glucose, lactate, and electrolyte concentrations in response to exercise. Serum creatine kinase activity was measured as a marker of acute muscle damage. Observed changes in monitored variables were minor, corresponded to exercise intensity, and did not differ between ER and control horses. These findings suggest that Arabian horses with ER have a normal response to limited-duration submaximal exercise, and are unlikely to have a muscular disorder that persistently affects critical metabolic functions of skeletal muscle.

Key Words: exertion, rhabdomyolysis, exercise, Arabian, endurance

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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Dedication

This thesis is dedicated to all the animals I've loved and lost throughout my life. From my childhood Labrador, Ozzy, who first showed me unconditional love, to my tuxedo Siamese, Herbie, who has taught me unwavering patience, every animal I've crossed paths with only pushed me further down the road I am on today. A career in veterinary medicine has always been my dream and it no doubt has been supported by these wonderful creatures with whom I have had the honor of sharing my life.

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Literature Review

Horses possess a number of remarkable adaptations that allow them to perform a variety of challenging athletic tasks. Many breeds have been specifically manipulated to optimize their capacity to perform unique athletic activities. For example, Thoroughbreds, Quarter Horses, and Standardbreds compete in high intensity races, Arabian horses complete long distance endurance events, Belgians and other draft breeds pull thousands of pounds, and Warmbloods compete in the technical sport of dressage. There is, of course, a large degree of crossover within the disciplines and virtually any breed can perform in a number of arenas. Regardless of the breed or the discipline, all horses share similar physiologic adaptations to exercise and a "normal" horse is therefore expected to display defined physiological responses to exercise challenges of different types and intensities.

During exercise in healthy horses, hundreds of muscles contract and extend throughout the horse's body. It is estimated that muscle accounts for approximately 50% of a horse's mature bodyweight compared to other mammals where it accounts for 30 to 40% of mature bodyweight[1].

A muscle cell is made up of myofibers, which are multinucleated cells[2]. Myofibers are arranged throughout the muscle parallel to one another to ensure maximum muscle contraction and relaxation during movement[2]. Along with myofibers, muscle tissue also contains fibroblasts, capillaries, adipocytes, and nerves[2]. Individual myofibers are surrounded by a membrane called the sarcolemma, which attaches to a layer of connective tissue called the endomysium, which supports each bundle of muscle fibers[2]. Each individual myofiber contains a contractile unit called a myofibril, which is responsible for both contraction and relaxation of muscles during exercise. A single myofibril is made up of rod-like structures called sarcomeres, which are long, filamentous fibers with a number of contractile proteins (actin, myosin, tropomyosin, and troponin)[2]. Surrounding the myofibrils are mitochondria, organelles responsible for producing energy rich adenosine triphosphate (ATP) used to power movement and exercise, among other processes[2].

In order for muscles to contract successfully during exercise, a specific pathway of excitation-contraction coupling is executed. This process commences with nerve impulses traveling through the central nervous system into the muscles. A single nerve impulse travels the length of the nerve from the central nervous system until it reaches the junction between the nerve terminus and the muscle cells[2]. Acetylcholine is released into the neuromuscular junction and binds to nicotinic receptors, causing a conformational change within the receptors to allow opening of ion channels in the sarcolemma[2]. Conductance of sodium and potassium ions causes an action potential to form at the end of the myofibril, which will lead to muscle cell contraction.

Once an action potential is formed, calcium is released into the sarcoplasm from the sarcoplasmic reticulum and binds troponin C, an integral regulatory protein complex, causing a conformational change which exposes the portion of the actin filament responsible for binding to myosin, called the G-actin monomer[2]. Binding of the G-actin monomer to myosin forms a cross-bridge and once this bridge forms, myosin ATP'ase cleaves ATP to form adenosine diphosphate (ADP) and adenosine monophosphate (AMP)[2]. This results in a secondary conformation change which shortens the length of the cross-bridge towards the center of the sarcomere. Subsequently, ADP and AMP detach from the myosin head, enabling this cycle to repeat. Throughout this process, the sarcomeres shorten in unison to complete the necessary degree of muscular shortening demanded by the central nervous system[2].

In order for the muscle cell to relax after contraction, calcium must be reabsorbed by the sarcoplasmic reticulum, and the membrane must repolarize. Calcium-magnesium ATP'ase pumps calcium back into the sarcoplasmic reticulum while sodium-potassium ATP'ase pumps sodium out of the cell and potassium into the cell[2]. These energy dependent changes in electrolyte gradients rely on the presence of available ATP, and lead to the restoration of troponin C, ultimately disrupting the interactions between myosin and actin filaments, causing muscle relaxation[2]. If ATP is unavailable, actin and myosin will remain engaged, causing muscles to remain contracted. This balanced coordination of contraction and relaxation of opposing muscles produces smooth and fluid movements during exercise.

The speed of cellular contraction and muscle fiber "type" is determined by the isoform of myosin ATP'ase enzyme that the muscle cell contains. Hence, muscle fibers are often categorized as "slow" or "fast" twitch based on their velocity of contraction[1,2]. Slow twitch fibers, also referred to as "Type I fibers", are highly oxidative and critical in aerobic exercise efforts[1,2]. Fast twitch, or Type II fibers, are less oxidative, contain more glycogen, and are capable of generating power through anaerobic processes[1,2].

Exertional Rhabdomyolysis

Exertional rhabdomyolysis (ER) is a general term that describes a variety of skeletal muscular disorders characterized by myocellular damage during periods of physical exertion or exercise. Most often muscle damage is reflected by necrosis or death of Type II muscle fibers and the release of the intracellular contents into circulation. These disorders are of great significance to the equine industry due to their prevalence and impact. Diseases associated with ER are often classified into two broad categories: underlying muscular defects that intermittently or repeatedly provoke clinical signs in response to exercise (chronic ER), and potentially reversible environmental or management triggers that provoke ER in horses with normal muscular systems (also referred to as sporadic or idiopathic ER)[2].

Horses that display repeated or chronic ER often have one of several underlying disorders which tend to be specific to particular breeds. The currently known and relatively well-defined causes of chronic ER include type 1 polysaccharide storage myopathy (PSSM 1), type II polysaccharide storage myopathy (PSSM 2), and recurrent exertional rhabdomyolysis (RER)[2]. Horses that do not fit within these disorders are commonly relegated into the category of idiopathic ER. Most commonly, PSSM 1 manifests in Quarter Horses and related breeds, PSSM 2 presents in Warmbloods, and RER affects Thoroughbreds and is suspected in Standardbred horses[2].

Common clinical signs of ER in horses occur during or shortly after exercise, or occasionally in anticipation of exercise, and include excessive sweating, stiffness, increased heart rate, trembling of the muscles (particularly in the hindquarters), and reluctance to move or to continue exercise[3,4]. Severely affected horses may become immobile or recumbent, with darkly colored orange, red, or brown urine as a result of

myoglobin released from damaged muscle tissue collecting in the urine[2]. Increased permeability or destruction of muscle cell membranes in horses with ER also leads to leakage of potassium, phosphorus, and the enzyme creatine kinase (CK) into the blood[2].

A number of diagnostic tools are used to identify ER in horses. Most commonly, the occurrence of rhabdomyolysis is supported through measurements of serum muscle enzyme activities including CK and aspartate transaminase (AST). Serum CK is considered to be a muscle specific enzyme and its activity typically peaks within four to six hours after a myocellular insult, returning to normal within 24 to 72 hours, unless ongoing or additional muscle damage occurs[1]. Aspartate transaminase is also an indicator of rhabdomyolysis; however it is also released from other damaged tissues, particularly the liver and erythrocytes. The serum half-life of AST is much longer than serum CK, and it typically peaks in activity 24 to 48 hours after muscle injury occurs, remaining elevated for several weeks after severe rhabdomyolysis[2,4].

Another diagnostic test commonly applied in the initial stages of evaluation of ER in horses is a submaximal exercise test, which is used to demonstrate an inappropriate response of muscle to exercise. Such a test typically consists of an initial blood sample for analysis of basal serum CK activity, followed by 15 minutes of walking and trotting exercise. During the exercise test, horses are monitored for any clinical signs of rhabdomyolysis, including reluctance to continue exercise, stiffness, labored breathing, and heavy sweating[2]. If clinical signs occur, the test is terminated, though subclinical rhabdomyolysis can occur in which the horse has an abnormal biochemical response to exercise (inappropriately elevated CK) without the presence of overt clinical signs. Blood is then obtained four to six hours after the conclusion of exercise to determine if there is an abnormal increase in serum CK activity, which is typically classified as a value two to four times higher than the pre-exercise value[2].

Type 1 Polysaccharide Storage Myopathy

Type 1 polysaccharide storage myopathy (PSSM 1) is a heritable, autosomal dominant form of ER that primarily affects Quarter Horses and related breeds including Appaloosas and Paints, as well as some draft breeds such as Percherons and Belgians[3,5]. Genomic studies indicate that affected horses are predisposed to disease due to a

mutation in the glycogen synthase 1 gene (*GYS1*), which results in a single arginine to histidine substitution[6,7]. Affected horses often display repeated or persistent signs of muscular disease of varying severity, with or without preceding exercise. These clinical signs include, but are not limited to, exercise intolerance, weakness, stiffness, gait abnormalities, back pain, muscle atrophy, sweating, and recumbency[2,3,8]. Affected individuals frequently develop elevations of serum CK, which may persist even in stall rested horses receiving no exercise[2].

It is estimated that the *GYS1* mutation is present in approximately 9% of the Quarter Horse population, though the presence of this mutation does not guarantee that clinical signs of ER will occur[5]. However, the mutation is present in approximately 80% of Quarter Horses that display clinical signs consistent with ER. Prior to genetic testing, the most common diagnostic procedure for identifying affected horses was a biopsy of the semimembranosus or middle gluteal muscle. Horses with PSSM 1 commonly demonstrate myocellular glycogen concentrations between 1.5 to 4 times higher than healthy horses[8,9]. This is likely due to increased insulin sensitivity and subsequent enhanced glucose uptake by muscle cells, leading to increased synthesis of muscle glycogen[2,9]. Histopathological analysis of biopsies from affected horses typically reveal accumulations of abnormal polysaccharides within scattered muscle fibers which fail to break down upon exposure to amylase[4,9]. In contrast, normally formed glycogen will break down upon exposure to amylase.

A small proportion of Quarter horses possess an autosomal dominant mutation of the skeletal muscle ryanodine receptor 1 gene (*RYR1*), which is associated with malignant hyperthermia[5]. Malignant hyperthermia typically presents in horses as a rapidly increasing body temperature accompanied by metabolic acidosis and rhabdomyolysis in response to specific anesthetic agents or muscle relaxant drugs[10]. Horses with PSSM 1 that concurrently possess both the *RYR1* and *GYS1* mutations have a tendency to display more severe clinical signs of ER[11].

In order to successfully manage horses with PSSM 1, research suggests that limiting the carbohydrate content of the ration (specifically soluble carbohydrates such as grain), as well as providing higher dietary fat can decrease the risk of clinical episodes

occurring. These ration changes, coupled with regular turn-out or exercise, can further decrease the likelihood of muscle stiffness and other clinical signs of disease[2,3,9].

Type II Polysaccharide Storage Myopathy

Type II polysaccharide storage myopathy (PSSM 2), affects several Warmblood breeds, including Dutch Warmbloods (most commonly affected), Swedish Warmbloods, Selle Francais, Hanovarians, Friesians, Irish Sport Horses, and Icelandic horses[4]. This disease is the most common myopathy of Warmblood horses and may have been introduced through the influence of draft horse blood lines, in which PSSM 1 is common[12]. Approximately 50% of muscle biopsies obtained from Warmblood horses with clinical signs of neuromuscular disease show changes consistent with PSSM 2[5]. The current guidelines for diagnosing PSSM 2 in a skeletal muscle biopsy include the presence of increased/abnormal amounts of amylase-sensitive, periodic acid Schiff (PAS)-positive material (i.e. glycogen) accumulating within the subsarcolemma[4,12]. Other histopathological changes include centrally located nuclei, subsarcolemmal vacuoles, regenerative fibers, and myocellular necrosis[12]. Inclusions observed in the myofibers of horses with PSSM 2 are similar to those that are often observed in individuals with PSSM 1, possibly explaining the link to draft horse breeds in Warmblood lineages. However, despite similarities between PSSM 1 and PSSM 2, horses with PSSM 2 lack the *GYS1* mutation affiliated with PSSM 1[4].

Warmbloods with PSSM 2 can display classic signs of ER with exercise. More commonly, however, affected individuals display reluctance to move forward or to perform in a collected frame. Horses with PSSM 2 can also have firm and painful hindquarters, muscle atrophy, and poor rounding of the back when jumping fences[4,5,12]. Individuals with PSSM 2 typically have normal serum CK and AST activities even when clinical signs are present[4,5].

Horses affected by PSSM 2 are currently managed similarly to horses with PSSM 1. It is recommended that the ration is changed to contain a greater proportion of fat and less carbohydrate, and turnout and regular exercise are encouraged to maintain fitness and mobility[12].

Recurrent Exertional Rhabdomyolysis

Recurrent exertional rhabdomyolysis (RER) is a form of ER primarily affecting Thoroughbreds and Standardbred horses, with an autosomal dominant pattern of inheritance[3,13,14]. In the presence of a genetic predisposition to RER, risk factors for the onset of clinical signs include young age, female gender, anxious temperament, and more than one day of rest prior to exercise when galloping is performed[3,14–16]. Horses fed a ration high in grain and those with concurrent lameness also display increased risk of developing signs of ER[3,15]. Clinical signs of RER include, but are not limited to, muscle cramping and stiffness, shifting limb lameness, sweating, and reluctance to move[2,3]. Clinical signs are typically accompanied by increases in serum muscle enzyme activities, and can be very intermittent in nature.

Compared to horses with PSSM, Thoroughbreds with RER have normal intramuscular glycogen concentrations that are often consistent with a high level of fitness. Affected individuals display non-specific changes on histologic analysis of muscle tissue, primarily consisting of an increased number of centrally located nuclei within muscle fibers[2]. Although a hereditary disorder underlies this condition, the causative mutation has not yet been identified. Therefore, the most common method used to diagnose an individual with RER is a combination of history, signalment, evaluation of muscle biopsy, and exercise testing with measurement of serum CK. Diagnosis can also be achieved through the use of the caffeine or halothane muscle contracture test[2]. However, this procedure requires a sample of intact external intercostal muscle, which is an invasive experimental technique not commonly used for diagnosis[2].

Effective management of RER can be achieved through minimizing stress and anxiety, modifying the ration to eliminate excess calories while providing energy primarily from fat sources, and by providing regular and frequent turnout or exercise[2,17].

Mitochondrial Myopathy

A single case report describes the diagnosis of an Arabian mare with a mitochondrial myopathy causing severe exercise intolerance without muscle necrosis[2,18]. Clinically, this individual displayed severe exercise intolerance and muscle stiffness, accompanied by extreme lactic acidosis but normal serum CK activity[2]. Histologically,

skeletal muscle tissue from this mare contained large accumulations of abnormal mitochondria[2,18]. Biochemical analysis determined that mitochondrial malformation caused abnormally low activity within the mitochondrial respiratory chain, preventing aerobic energy production pathways from functioning effectively during exercise[2]. Consequently, this mare relied entirely on anaerobic metabolism during mild exercise, resulting in signs consistent with extreme lactic acidosis and severe exercise intolerance[18].

Exertional Rhabdomyolysis in Arabian Horses

Arabian horses currently dominate the endurance racing discipline, which is growing in popularity around the globe[19]. Studies indicate that approximately 5% of horses participating in endurance racing events are eliminated during racing due to systemic metabolic disorders, including those related to ER[19,20]. Although Arabian horses often develop clinical signs consistent with ER during racing, and a high prevalence of ER has been demonstrated in this breed, no specific pathogenesis or underlying etiology has been determined. Anecdotally however, it has been suggested that this disorder might represent a unique and hereditary form of ER in Arabian horses.

Changes in Serum Biochemistry during Exercise

Exercise can provoke changes in a variety of hematologic and biochemical variables in horses which are dependent on both the intensity and duration of exercise. In general, brief, high intensity exercise produces more profound changes compared to brief, submaximal exercise, in which changes are generally minimal[1]. However, prolonged, submaximal exercise, especially if performed in excessive heat and humidity, can cause substantial changes to many laboratory variables, particularly if the horse becomes dehydrated.

Brief, high intensity exercise efforts typified by short periods of fast exercise have been studied in galloping Thoroughbred horses and trotting or pacing Standardbred horses[14,15]. High intensity racing events typically range between 1,000 to 4,200 meters in distance, during which very high speeds can be achieved[21]. The most common clinicopathologic changes observed during high intensity exercise in horses include a

substantial increase in packed cell volume (PCV) as a result of splenic contraction, mild to moderate hyperkalemia (increased serum potassium) associated with electrolyte flux from exercising muscle tissue, and low blood pH and high serum lactate concentrations, associated with the development of metabolic and respiratory acidosis during high intensity exercise efforts[22]. These alterations are often profound and transient, resolving within minutes of cessation of exercise.

Compared to high intensity exercise, submaximal exercise usually provokes minimal change in clinicopathologic variables in horses. However, prolonged submaximal exercise, such as endurance racing, can result in measurable changes over time. Endurance races typically range in distance from 80 to 160 km (50 to 100 miles) in length. While average speeds reached by endurance horses are nowhere near as high as those achieved by Thoroughbreds competing in galloping races, endurance horses can reach average speeds above 25 km/hr and maintain them over substantial distances[19]. Prolonged submaximal exercise can increase the metabolic rate by 10 to 20 fold[23], resulting in substantial heat generation, which must be dissipated primarily through evaporative processes (e.g. sweating).

Unlike humans, the equine sweat gland excretes a hypertonic solution of sodium, potassium, calcium, and chloride onto the surface of the skin[24]. This adaptation enables effective cooling, but creates the potential for substantial fluid and electrolyte loss during prolonged submaximal exercise, with up to 12 to 15 liters of sweat being generated per hour[24]. A number of studies evaluating biochemical changes in horses during single day endurance events have demonstrated predictable changes in serum electrolyte concentrations in competing endurance horses including decreases in serum sodium, potassium, chloride, and calcium[25–30]. Additionally, redistribution of fluid within the extracellular spaces results in increases in PCV and plasma total protein concentration (TP) which may be greater in horses with more substantial dehydration and exercise associated disease[31,32].

Prolonged submaximal exercise also commonly induces mild changes in serum CK activity. This enzyme is found primarily within the cytosol of myocytes, and therefore in the horse, serum activity reflects skeletal muscle mass and viability[2]. In healthy horses, brief submaximal exercise elicits minimal change, and serum CK should

remain within reference intervals[2]. Prolonged submaximal exercise such as endurance racing typically elicits mild to moderate increases in serum CK that may approach several thousand units per liter[30]. Horses performing high intensity galloping exercise display mild post-exercise increases in serum CK, which are typically 50% or less of the basal activity[33]. Given the relative specificity of this enzyme for skeletal muscle tissue, and its utility as an indicator of muscle damage, it is the most commonly measured marker of skeletal muscle damage in most species. Muscle disease is suspected when horses display persistent elevations in serum CK while rested from exercise, repeated elevations in response to exercise that are disproportional to the degree of effort, and if there are accompanying clinical signs of cramping, stiffness, discolored urine, excessive sweating, and/or an abnormally high heart rate during exercise[34].

Based on what is currently known about ER in various equine breeds and the physiologic and biochemical responses of normal horses to exercise of different intensities, the rationale of the planned study was to document the responses of Arabian horses with ER to a submaximal, standardized exercise test in order to provide preliminary information about the possible cause of disease in this breed.

Introduction

Exertional rhabdomyolysis (ER) in endurance racing horses is a growing concern as this sport continues to rise in popularity amongst members of the equestrian community. Exertional rhabdomyolysis represents both a medical concern for affected individuals, but also a welfare concern, as significant muscle damage and even death can occur if the disease is not recognized promptly and treated.

A recent study examined the prevalence of ER in endurance horses based on owner questionnaire and measurement of pre- and post-exercise serum CK activity in 101 horses participating in one of four 50 mile racing events[35]. This study reported that 4% of horses had evidence of significant muscle damage during the race based on abnormally high post-exercise serum CK activity (10,000 to >300,000 U/L; reference limit: 145-633 U/L), while an additional 12% of horses were reported by their owners to have previously displayed signs consistent with ER[35]. All affected horses were Arabian and none of the horses in the study possessed the *GYS1* mutation associated with PSSM 1[35]. To investigate this phenomenon further, gluteal muscle biopsies were collected from 27 Arabian horses, 11 of which had a history of ER. Histopathological assessment of biopsies from affected horses identified deviations in the structural alignment of myofibrils within the muscle cells and an absence of abnormal polysaccharide inclusions. Biochemical analysis revealed unremarkable intra-muscular glycogen concentrations (McKenzie, unpublished data). These findings confirm that PSSM 1 was not the cause of disease in Arabian horses with ER, and suggested that a novel muscular disorder might exist in this breed. This disorder is also suspected to be hereditary in nature, with anecdotal reports of similar signs between siblings and in the offspring of at least one affected dam (unpublished observations by Dr. McKenzie).

The study reported in this thesis describes the preliminary steps of investigating this potentially novel muscular disorder of the Arabian horse. A standardized, submaximal field exercise test was developed for horses with the disorder to perform alongside age, breed, and environmentally matched healthy control horses. During the exercise test, specific clinical and laboratory variables were concurrently monitored to identify differences between affected and healthy Arabian horses.

Materials and Methods

Horses: Ten horses with a history of ER and nine healthy control horses were evaluated during this study. Horses were paired as 1 control with 1 ER horse, with a single triplicate (1 control matched with 2 ER horses) to account for the uneven number of horses between groups. Pairing was based on age, property of origin, and selected, relevant management criteria (fitness level, training regimen, diet, and housing conditions). Healthy horses were defined as those that had participated in at least one year of endurance training or racing with no reported signs of ER. Affected horses were defined as those in which at least one prior episode of ER had been diagnosed by a veterinarian on the basis of consistent clinical signs. Seven of the ten affected horses had supporting elevations in serum CK and/or AST in conjunction with clinical signs (Table 1). At the time of the study, two pairs of horses were not undergoing forced exercise, but were turned out on a large pasture; one pair was undergoing less than 4 hours of exercise per week; 4 pairs and the triplicate were undergoing 6-8 hours of exercise per week; and one pair was undergoing more than 10 hours of exercise per week. In the ER affected group, there was 1 stallion, 4 geldings, and 5 mares with a mean age of 15.4 ± 5.6 years. Eight of these horses were purebred Arabians and two horses were half-Arabians. In the control group, there were 5 geldings and 4 mares with a mean age of 12.9 ± 6.1 years and all were purebred Arabians.

Exercise Test: After 24 to 48 hours of stall rest, each horse performed a 47 minute, ridden standardized exercise test consisting of alternating intervals of walk and trot, with one interval of canter, covering a distance between 4 to 4.5 miles (Figure 1). The exercise test was performed outside on flat ground for the 8 pairs and in an indoor arena for the triplicate. Individuals within each pair completed the exercise test simultaneously to ensure similar terrain was covered at a similar speed, and data regarding test duration, pace, course elevation, and distance traveled was captured for 6 pairs performing outside using a GPS enabled watch[i] worn by one rider in the pair.

Telemetric Electrocardiogram Monitoring: Prior to the test, each horse was fitted with a commercially available telemetric ECG recording system[ii], which recorded a full electrocardiogram during the exercise test. Small areas of hair (3 x 3 cm) were clipped in two sites at the left withers and in two sites behind the left girth, and patch electrodes were attached to the skin at those sites using tissue glue. Cables were secured to the saddle using tape to reduce trace artifact during exercise, and the recording box was secured to the saddle or martingale. Recording commenced immediately prior to the beginning of the exercise test and ceased immediately once exercise was completed.

Blood Sampling: Whole blood samples were collected via jugular venipuncture into EDTA and sodium heparin tubes prior to and immediately following the exercise test for analysis of packed cell volume (PCV), total protein (TP), glucose, lactate and electrolyte (sodium, potassium, chloride, ionized calcium) concentrations. Plasma CK activity was determined immediately before and three hours after exercise ceased. Heparinized samples were centrifuged immediately after collection and plasma was separated and stored in liquid nitrogen until analysis within 72 hours.

Packed cell volume was determined via the microhematocrit method and total protein by refractometry of EDTA blood and plasma respectively. Plasma glucose, sodium, potassium, chloride, ionized calcium, and lactate concentrations were determined by a bench top blood gas analyzer[iii] on thawed heparinized plasma samples. Plasma CK was determined on a commercially available chemistry analyzer[iv].

Hair Sampling: 20 to 40 hairs plucked from the mane of all horses were analyzed via PCR[v] for the *GYS1* and *RYR1* mutations that have been previously described[6,11].

Gluteal Muscle Biopsies: A needle biopsy of the middle gluteal muscle was obtained prior to and 3 hours after horses ceased the exercise test for measurement of muscle glycogen concentrations. The biopsy site was clipped and surgically sterilized, 2 cc of local anesthetic was injected subcutaneously, and a 2 cm stab incision was made through the skin. A 6 mm Bergstrom biopsy needle[vi] was introduced through the incision, and the

biopsy sample was obtained at a depth of approximately 6 cm, and 17 cm along a line running from the dorsal tuber coxa to the tail head. Biopsy sites alternated between the left and right gluteal muscle for the pre- and post-exercise samples on each horse. Glycogen concentration was assayed fluorometrically as glucose residues remaining after portions of muscle tissue weighing between 1 to 2mg were boiled for 2 hours in 1M HCl[36]. Remaining gluteal muscle tissue that was collected during this study is being assessed via novel histochemical techniques at the University of Minnesota Neuromuscular Diagnostics Laboratory.

Statistical Analysis: Plasma CK activity was not normally distributed and was subsequently log transformed prior to statistical analysis. Changes in blood and plasma variables, as well as muscle glycogen concentrations were compared between ER and control groups by a nested two factor factorial ANOVA. The simple effect comparisons of ER versus control for pre- and post-exercise variables, as well as pre- and post-exercise comparisons within each disease group were assessed with protected *t*-tests and planned contrasts within the ANOVA model. Heart rate during exercise was compared between ER and control groups by repeated measures ANOVA. The relationship between gender and disease status was assessed via contingency table and Fisher's Exact Test, and the relationship between age and disease status was assessed via independent *t*-test. Data is provided as mean ± SEM, unless otherwise indicated. Statistical significance was declared at $P < 0.05$ for all variables.

Results

Overall, no significant relationships were identified between disease status (control or ER) and the age or gender of the horses in the study. No horses displayed any suspicious or definitive clinical signs of ER during the study and all horses appeared to tolerate the standardized exercise test.

Exercise Test

GPS data collected from 6 pairs of horses indicated these horses completed an average distance of 4.37 ± 0.2 miles with an average elevation gain of 210 ± 239 feet during the testing period. The average pace of these 12 horses was 10.56 ± 0.04 min/mile. Complete cardiac tracings were obtained and analyzed on 14 of the 17 horses that exercised with the telemetric ECG system in place. Electrocardiogram data was negated for 1 ER and 2 control horses due to excessive motion artifact or premature detachment of the cables during exercise and this data was not considered. Individuals in both the control and ER groups showed significant changes in heart rates over time as exercise intensity changed ($P < 0.0001$). However, no significant difference was observed between groups when compared at the same time interval of exercise (Figure 2).

Blood Gas Analysis

No statistically significant changes were observed between ER and control horses in regard to plasma glucose, lactate or electrolyte concentrations, plasma TP concentrations, or PCV (Table 2). During exercise, a mild but significant decrease in plasma ionized calcium concentration occurred in both groups ($P = 0.03$ and $P = 0.05$ in control and ER horses, respectively), and a mild but significant increase in plasma sodium concentration also occurred during exercise ($P = 0.006$ and $P = 0.004$ in control and ER horses, respectively). Plasma chloride and potassium concentrations did not change significantly in either group during exercise (Table 2).

Significantly lower plasma glucose concentrations were observed in both ER and control horses immediately after exercise compared to before exercise ($P = 0.0007$ and $P = 0.0063$, respectively). However, no significant difference was observed between ER

and control horses in pre- or post-exercise values. While mild but significant increases were recorded for control horses in PCV and TP immediately after exercise ($P = 0.0013$ and $P = 0.04$, respectively), there was no significant difference in these variables before exercise compared to after exercise in ER horses ($P = 0.9$ for both variables).

Plasma Creatine Kinase Activity

Log transformed plasma CK was not significantly different between ER and control horses before or after exercise. Exercise induced significant increases in plasma CK in both groups of horses ($P = 0.003$ and $P = 0.04$ in ER and control horses, respectively; Figure 3). However, plasma CK before and after exercise was still within reference interval (145-633 U/L) for both ER and control horses, with the exception of two horses (1 ER horse and 1 control horse). These horses had serum CK values of 1,116 U/L (ER horse) and 1,028 U/L (control horse) 3 hours after exercise, which represented a 4.5 fold and a 1.5 fold increase from resting values, respectively.

Muscle Glycogen Concentrations

Muscle glycogen concentrations after exercise were no different from before exercise in all horses and no significant differences were observed in muscle glycogen concentration between ER and control horses before or after the exercise test ($P = 0.4$ and $P = 0.9$, respectively).

Discussion

In this study, no significant differences were identified between ER and control horses for several commonly measured biochemical variables, PCV, and heart rate before and immediately after performing a 47 minute standardized exercise test. Furthermore, plasma CK activity, the most commonly utilized indicator of muscle injury in horses with ER, was not different between control and affected horses when measured three hours after the test. Only one horse from the ER group demonstrated an abnormal response to the exercise test with a 4.5 fold increase in serum CK. All other horses undertaking the exercise test displayed a 1.5 fold or less increase in serum CK in response to exercise.

The standardized exercise test conducted in this study was relatively short compared to the length of typical endurance races, which often range from 25 to 100 miles in distance. It is possible therefore, that the test was insufficient in duration to induce ER in the affected individuals. However, the goal of the test was to induce subclinical rather than clinical muscle damage, and a protocol was necessary that all horses could perform and complete, regardless of their current level of fitness. Although a longer exercise test (2 hours) has been previously described for evaluating poor performance in endurance horses[37], historically, at least 5 of the individuals tested in the current study had previously displayed significant clinical signs of ER after only 4 to 5 miles of light exercise following a period of rest. A longer test raised the concern of inducing clinical disease and would have excluded some of the affected individuals that did not possess the fitness to perform a longer test. It is unclear if a longer standardized exercise test would more reliably contribute to the diagnosis of ER in Arabian horses suspected of having this disorder.

Based on anecdotal evidence collected from owners of horses with ER, clinical disease is frequently observed in endurance horses that achieve high levels of fitness, and tends to most commonly occur when a long racing event is followed by rest periods varying from as little as 3 days to 6 weeks in length. In these cases, reintroducing light exercise was observed to elicit severe clinical signs of ER within 10 to 20 minutes of exercise commencing. It is possible that the 24 to 48 hour rest period applied to horses in the current study might not have been long enough to promote development of

subclinical or clinical disease during the study, or perhaps that exercise management preceding the rest period also had some influence. Additionally, based on medical histories collected from owners, many of the horses assessed in this study had only displayed 1 to 3 clinical episodes of ER during their racing careers. Cumulatively, this information suggests that ER in Arabian horses is intermittent in nature, similar to RER in Thoroughbreds. Consequently, it is likely hard to initiate ER in affected horses in a single testing period.

Submaximal exercise tests are commonly utilized to provoke changes in serum CK in horses suspected of having muscular disorders. The exercise test used in this study was longer than typically applied on a clinical basis to horses with suspected muscle disease; however, it did not elicit measurable differences in serum CK activity between ER and control horses, with plasma CK remaining within reference interval for all but two horses. These two horses represented 1 ER and 1 control horse, both of which displayed mildly elevated plasma CK three hours after exercise (1,116 U/L and 1,028 U/L, respectively). However, only the ER horse demonstrated a change in plasma CK (a 4.5 fold increase from pre-exercise values) that fit the commonly used criteria for identifying inappropriate muscular response to exercise (more than a 2 fold increase from pre-exercise values). These findings indicate that either the test was of insufficient duration to elicit muscular damage in most horses with ER, or that other influences played a role, which might include the level of fitness of horses in the current study, an insufficient period of rest to prime horses before exercise testing, or the apparently intermittent nature of this currently uncharacterized disorder. It is quite probable that repeated evaluations of serum CK during training and racing might be a more successful method of identifying inappropriate muscular responses of potentially affected horses to exercise.

A challenge of the current study was also accurately phenotyping horses as control or affected subjects. Phenotyping was based on complete and detailed medical histories provided by the owners, previous muscle biopsy reports that identified non-specific abnormalities (an increased number of central nuclei in ER horses), and in 7 of 10 horses, pre-existing laboratory results demonstrating inappropriate increases in serum CK and/or AST. However, accurate phenotyping of ER in horses can be very challenging

to achieve because the disease can be subclinical, intermittent, or misdiagnosed. Also, this disorder appears to have a relatively late age of onset, with horses in the current study having their first reported episode of disease occurring between 7 to 15 years of age. Furthermore, reliable characteristic muscle biopsy findings for this specific disorder have yet to be been identified. Hence, it is possible that the control horse that developed a high post-exercise CK might actually have been affected with the disorder. This horse had been rested for four years and had an anxious temperament, a characteristic known to increase susceptibility to rhabdomyolysis in Thoroughbred horses with RER[3,15,16]. A decreased level of physical fitness related to prolonged pasture turnout could also have resulted in the exercise test exceeding muscular tolerance in this animal, creating mild muscle damage or basically, sporadic ER without an underlying predisposing disorder.

An inappropriate increase in heart rate is a common clinical sign of ER in horses, likely reflecting pain and discomfort associated with muscle cramping and damage. Hence, telemetric HR monitoring was performed during this study, and although average heart rates changed significantly ($P < 0.001$) over time for both disease groups during the exercise test, changes correlated clearly with the intensity of each interval of the test and did not differ between control and affected horses. Heart rate changes therefore reflected a normal physiologic response to exercise to maintain appropriate blood flow throughout tissues of the body, particularly the exercising musculature which receives up to 80% of cardiac output in the exercising horse[1]. These findings also indicate that the exercise test did not induce muscle damage and discomfort in ER horses, correlating with the negligible changes in plasma CK observed.

In the current study, plasma lactate concentrations remained within reference intervals, as expected for a submaximal exercise effort. Significant increases in plasma lactate during submaximal exercise would suggest mitochondrial dysfunction, which has been previously reported in one Arabian mare[18]. However, the normal clinical responses, exercise tolerance, and plasma lactate concentrations recorded during this study exclude this diagnosis in Arabian horses examined in the current study.

Despite a statistically significant decrease in plasma ionized calcium and an increase in plasma sodium in response to exercise, changes were very mild and values remained within the reference interval for both variables. Furthermore, these variables

were not different between ER and control horses, indicating that changes reflect a normal response to exercise.

Significantly lower serum glucose concentrations were observed immediately after exercise compared to before exercise in both ER and control groups, which is an expected physiologic response to exercise. Although the degree of change in blood glucose concentration during exercise varies depending on hormone fluctuations, as well as type and duration of exercise, glucose is the initial energy source used to generate ATP during exercise. Blood glucose concentration is typically expected to drop over time [1] with sustained exercise due to hormonal responses and gradual depletion of muscle and liver glycogen stores.

Analysis of muscle biopsies collected during this study found that muscle glycogen concentrations after exercise were no different than before exercise in both ER and control horses. This reflects the brief, submaximal nature of the exercise test. Muscle glycogen is typically used as an energy source during exercise exceeding 3 hours in duration[1,2]. The presence of normal muscle glycogen concentrations that were comparable to those measured in control horses also suggests that a glycogen regulating disorder is unlikely the cause of ER in Arabian horses. Results of further biopsy testing and analysis by the University of Minnesota Neuromuscular Diagnostics Laboratory are currently pending.

Conclusion

This study investigated the response of 10 Arabian horses with ER to a standardized field exercise test performed alongside 9 healthy age, breed, and management-matched control horses. Each horse completed a 47 minute ridden exercise test consisting of alternating walk and trot segments with one segment of canter. Blood samples were collected via jugular venipuncture prior to exercise, immediately after exercise, and 3 hours after exercise to evaluate a number of physiologic and metabolic variables, including serum CK activity, and lactate, blood glucose, total protein, and electrolyte concentrations. Electrocardiographic tracings were also recorded to evaluate heart rate responses, and hair samples were tested for the *GYS1* mutation associated with PSSM 1. Gluteal muscle biopsies were also collected prior to and after exercise to assess muscle glycogen concentrations and are currently undergoing advanced histochemical and molecular analyses to further define the possible cause of ER in Arabian horses.

Results derived from the exercise test identified no clinically significant differences between ER and control horses in regard to commonly evaluated metabolic and clinical responses to exercise. This suggests that a single submaximal exercise test likely has limited utility in identifying this disorder in affected horses, and that a constitutive metabolic myopathy is unlikely the cause of this disease. Therefore, more advanced molecular and histochemical techniques are now being pursued to determine if Arabian horses truly have a novel and unique disease. Furthermore, pedigree analysis of a large number of affected and healthy related horses will help to confirm or refute heritability of Arabian ER.

Table 1: Historical information and serum creatine kinase (CK) and aspartate transaminase (AST) activity in 10 Arabian horses with a history of exertional rhabdomyolysis.

Breed	Age (yrs)	Sex*	CK:AST (U/L)	Medical History
Arab	29	G	69 : 2,910 10 days after signs	One severe episode after a 50 mile race when 15 yrs old: poor HR recovery, discolored urine, reluctance to move.
Arab-cross	13	G		Two episodes, first as an 8 yr old in training: trembling, stiffness, cramping, sweating, poor HR recovery, reluctance to move.
Arab	17	F		Three episodes: trembling, stiffness, reluctance to move. Observed as a 17 yr old with light exercise following 6 weeks of rest after a 100 mile race, and again two days later. Full sister reported to have severe clinical ER at an elite 100 mile race.
Arab-cross	13	F		Clinical signs as a 10 yr old in training: trembling, stiffness, cramping, poor HR recovery, discolored urine, reluctant to move.
Arab	14	G	40,977 : 2,303 within 6 h of signs	At least 2 episodes, most severe when fit and performing well. First episode as 7 yr old beginning a training ride: stiffness, cramping, reluctance to move, high CK. Severe clinical signs and high CK as a 13 yr old with 30 min of light exercise after 2 weeks of rest following a 100 mile race.
Arab	18	G	21,612 : 2,552 within 12 h of signs	At least 2 episodes, first was subclinical as 15 yr old (CK 6,000 U/L after a 50 mile race). Severe clinical episode and high CK as a 17 yr old with 30 min of light exercise after 2 weeks of rest when very fit.
Arab	10	M	136 : 1,630 36 h after signs	Two episodes, first as a 9 yr old stall rested for 2 days before a race, clinical signs of ER within 30 min of race commencing. Second clinical episode shortly into a 100 mile race the same year, but finished the race.
Arab	12	F	1,991 : 4,066	Multiple clinical episodes most reliably induced by 10 min of light exercise after short periods of rest while very fit: excessive sweating, crab walking, firm gluteal muscles, parking out. First observed by new owner when horse was 9 yrs old. Excellent race results.
Arab	18	F	3,319 : 519 within 12 h of signs	Two episodes: 18 miles into a 50 mile race as an 11 yr old: mild tachycardia, dark urine, stiffness. One year later clinical signs and high CK 2 miles into exercise after rest. Dam had severe clinical episode during a 50 mile race as an 8 yr old (CK > 300,000 U/L)
Arab	10	F	120,000 : 32,242 36 h after signs	One episode diagnosed after racing: trembling, stiffness, cramping, poor HR recovery, discolored urine, reluctance to move from standing, high CK. Signs commenced about 8 miles into 30 mile race.

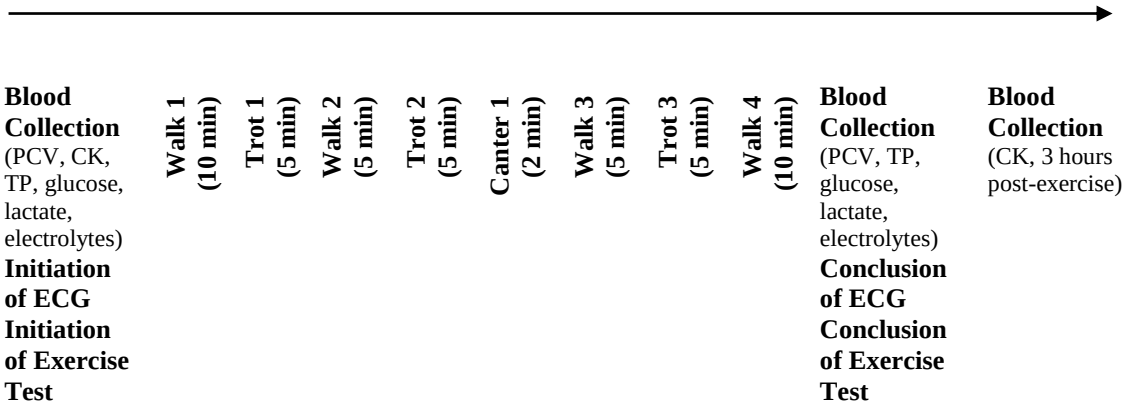
* G = gelding, F = mare, S = stallion

Table 2: Packed cell volume (PCV) and plasma concentrations of sodium, chloride, potassium, ionized calcium, total protein, glucose, and lactate before and immediately after completion of a standardized exercise test in horses with and without a history of exertional rhabdomyolysis.

Variable	Reference Interval	Pre-Exercise	Post-Exercise
PCV (%)			
Control	32 – 48	34 ± 1.2	36 ± 1.1*
ER		36 ± 1.1	36 ± 1.3
Sodium (mEq/L)			
Control	133 – 142	134 ± 0.5	135 ± 0.4*
ER		135 ± 0.5	136 ± 0.3*
Chloride (mEq/L)			
Control	94 – 105	102 ± 0.8	101 ± 0.9
ER		101 ± 1.0	101 ± 0.9
Potassium (mEq/L)			
Control	2.5 – 4.7	3.7 ± 0.2	3.8 ± 0.1
ER		3.7 ± 0.1	3.7 ± 0.1
Ionized Calcium (mg/dL)			
Control	5.0 – 6.0	5.8 ± 0.1	5.6 ± 0.1*
ER		5.9 ± 0.1	5.7 ± 0.1*
Total Protein (gm/dL)			
Control	5.9 – 7.6	6.5 ± 0.1	6.6 ± 0.1*
ER		6.4 ± 0.2	6.4 ± 0.2
Glucose (mg/dL)			
Control	79 – 109	111 ± 2.6	100 ± 2.2*
ER		114 ± 5.3	100 ± 3.8*
Lactate (mmol/L)			
Control	1.11 – 1.78	0.9 ± 0.1	0.7 ± 0.1*
ER		0.8 ± 0.1	0.7 ± 0.1

*Indicates significant difference between pre- and post-exercise values.

Figure 1: Linear timeline of events surrounding the standardized exercise test.



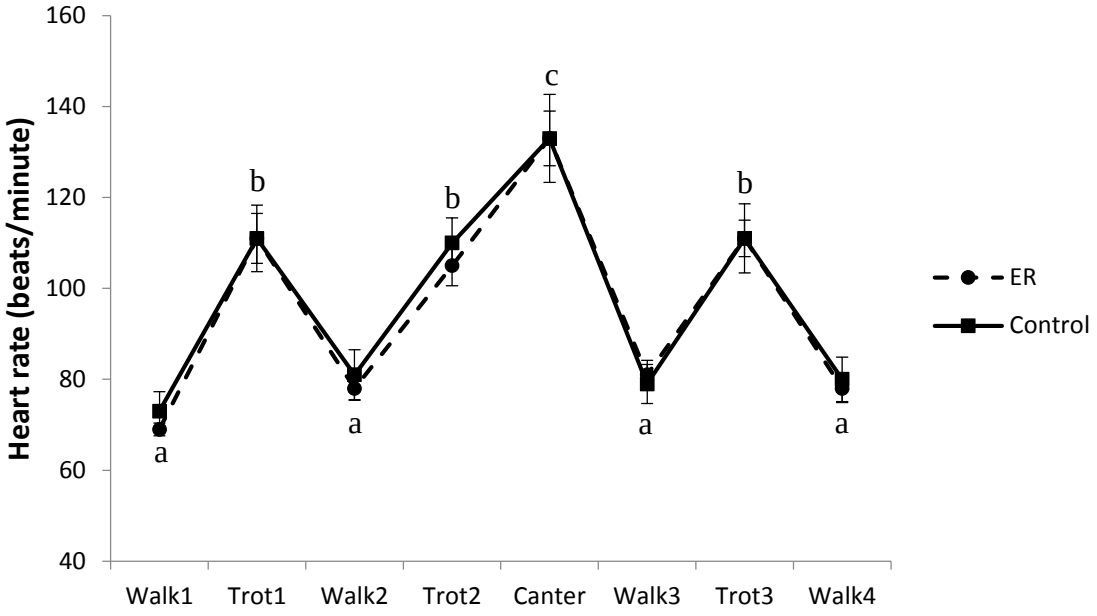
PCV: packed cell volume

CK: creatine kinase

TP: total protein

ECG: electrocardiogram

Figure 2: Average heart rate (beats per minute; mean ± SEM) of horses with (dashed line) and without (solid line) a history of exertional rhabdomyolysis performing a standardized exercise test.



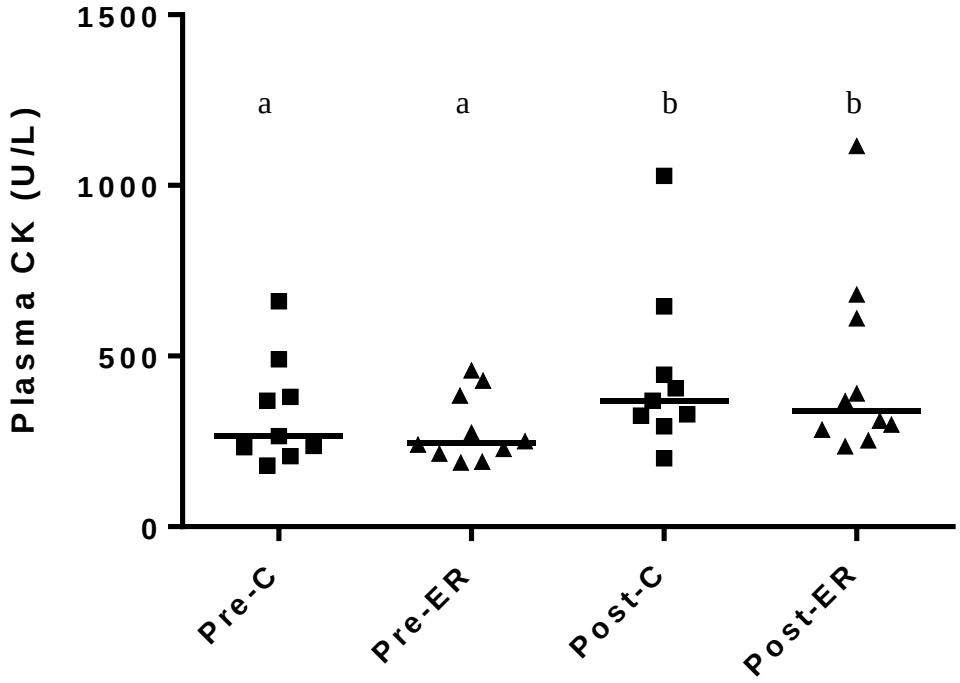
a, b, c: Different letters indicate significantly different ($P \leq 0.05$) HR between exercise intervals.

Technical difficulties caused partial or complete loss of ECG trace during exercise in 1 ER and 2 control horses. ECG monitoring was not performed in 1 ER and 1 control horse due to no available equipment.

Duration of each exercise interval: Walk 1 = 10 minutes, Trot 1, Walk 2, Trot 2, Walk 3, and Trot 3 = 5 minutes each, Canter = 2 minutes, Walk 4 = 10 minutes

Total duration of exercise test: 47 minutes

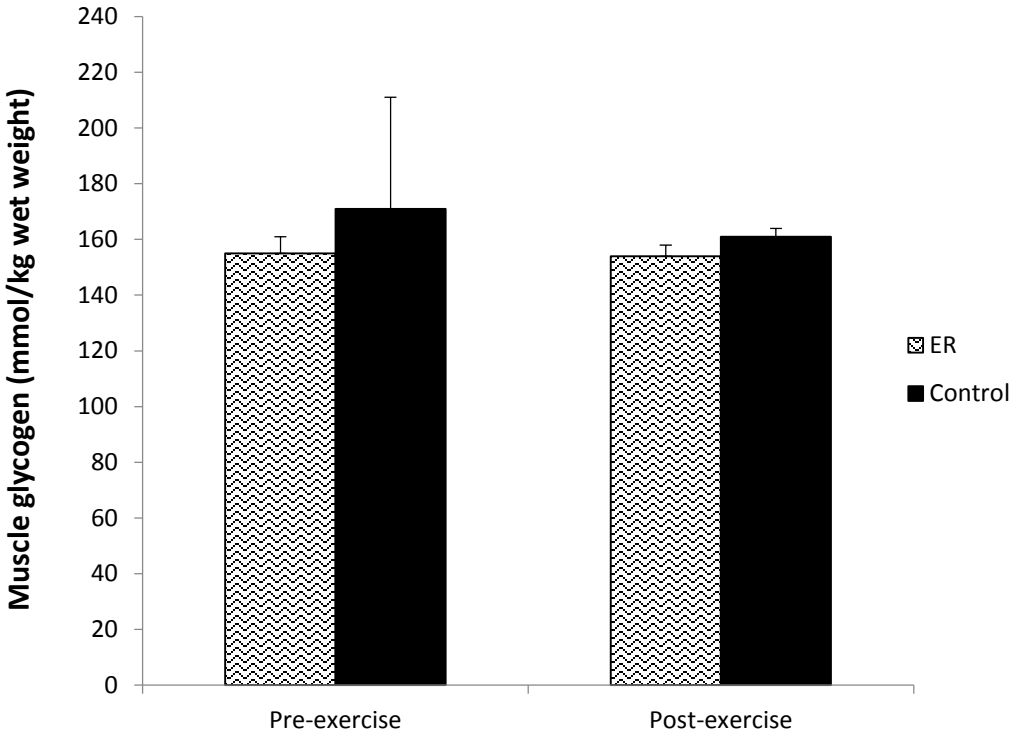
Figure 3: Scatter plot of plasma creatine kinase (CK) activity before (pre) and 3 hours after (post) performing a 47 minute standardized exercise test in horses with (n=10, C) and without (n = 9, ER) a history of ER.



a,b: Different letters indicate a significant ($P \leq 0.05$) difference in plasma CK after compared to before exercise.

Horizontal bars represents the median value.

Figure 4: Muscle glycogen concentration (mean ± SEM; mmol/kg wet weight) before and immediately after a standardized exercise test in horses with and without a history of exertional rhabdomyolysis.



Valid results were unable to be obtained for 2 ER horses and 1 control horse before exercise, and from 1 control horse after exercise.

Footnotes

i. Garmin Forerunner® 310XT, Garmin International, Inc., Olathe, KS.

ii. Televet 100 Telemetric ECG & Holter System, Engel Engineering Service GmbH, Heusenstamm, Germany.

iii. State Profile® pHOx® Ultra, Nova Biomedical, Waltham, MA.

iv. Hitachi 911, Roche-Boehringer Mannheim, Indianapolis, IN.

v. University of Minnesota Equine Center Neuromuscular Diagnostic Laboratory, Saint Paul, MN.

vi. Modified Bergstrom 6mm Muscle Biopsy Needle, Jørgen Kruuse A/S, Langeskov, Denmark.

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