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Clinical, histopathological and metabolic responses following exercise in Arabian horses with a history of exertional rhabdomyolysis

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ABSTRACT

A previous report suggests a substantial incidence of exertional rhabdomyolysis (ER) in Arabian horses performing endurance racing. This study compared formalin histopathology and clinical and metabolic responses to a standardised field exercise test (SET) between Arabians with and without ER. Arabian horses with ($n = 10$; age 15.4 ± 5.6 years) and without ($n = 9$; 12.9 ± 6.1 years) prior ER were stall-rested for 24–48 h, after which paired ER and control horses were fitted with a telemetric ECG and performed a 47 min submaximal SET. Plasma glucose, lactate, electrolyte and total protein concentrations and packed cell volume were measured before and immediately after exercise. Blood and percutaneous gluteal muscle samples were also obtained before and 3 h after exercise for measurement of plasma creatine kinase (CK) activity and muscle glycogen concentration, respectively. Histopathologic analysis of formalin-fixed pre-exercise muscle sections was performed. Data were analyzed by ANOVA and non-parametric tests ($P < 0.05$).

No horses displayed clinical signs of ER during exercise, and plasma CK increased similarly in ER and control Arabians. Muscle glycogen, heart rate, and remaining plasma variables did not differ between horses with ER and control horses. Horses with ER had more internalised nuclei in mature myofibers, more aggregates of cytoplasmic glycogen and desmin, and higher myopathic scores than control horses. Although many horses with ER had histopathologic evidence of chronic myopathy, muscle glycogen concentrations and metabolic exercise responses were normal. Results did not support a consistent metabolic myopathy or a glycogen storage disorder in Arabians with ER.

Introduction

Exertional rhabdomyolysis (ER) encompasses muscular disorders with subclinical or clinical evidence of muscle necrosis associated with athletic activity (Valberg, 2012). Clinically affected horses classically demonstrate excessive sweating, tachycardia, stiffness, and reluctance to continue during or after exercise. Subclinical disease has also been reported, with individual horses appearing clinically unremarkable despite biochemical evidence of substantial muscle necrosis (Wilberger et al., 2015).

Previous reports have established a strong tendency for specific ER disorders to present in particular breeds (Valberg et al., 1999; McCue et al., 2010). Thoroughbred and Standardbred horses have a disorder, recurrent exertional rhabdomyolysis (RER), attributed to abnormal skeletal muscle calcium regulation, and for which a heritable basis is suspected, but causative mutations have not been identified (MacLeay et al., 1999; Isgren et al., 2010). In many other breeds, including Quarter horses and Belgian draft horses, ER is usually attributable to type 1 polysaccharide storage myopathy (PSSM), a disorder of skeletal muscle glycogen metabolism arising from a missense, gain of function mutation of the glycogen synthase 1 gene (*GYS1*; McCue et al., 2008; McCue et al., 2010). The *GYS1* mutation has not been identified in Arabian horses (Wilberger et al., 2015). However, PSSM has been previously diagnosed in Arabians based on the presence of increased amylase-resistant or amylase-sensitive glycogen (Valentine et al., 2000).

A recent study assessed the prevalence of ER in 101 horses (including 88 Arabian and Arabian-cross horses) participating in 80 km endurance races, utilising owner questionnaire and measurement of pre- and post-race serum creatine kinase (CK) activity (Wilberger et al., 2015). Four percent of horses demonstrated abnormally high serum CK activity after racing, with or without accompanying clinical signs of ER, and another 12% of horses were reported by owners to have previously demonstrated signs of ER, suggesting a relatively high prevalence of ER in this breed. All horses with biochemical or historical evidence of ER were Arabian or Arabian-cross, and none

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tested positive for the *GYS1* mutation associated with type 1 PSSM (McCue et al., 2008; Wilberger et al., 2015).

The aim of this study was to determine if Arabian horses with a history of ER had evidence of a consistent metabolic myopathy, by comparing muscle histopathology and clinical and metabolic responses to a standardised field exercise test between affected horses and healthy, age- and environment-matched Arabians.

Materials and methods

Horses

All procedures were approved by the Oregon State University Institutional Animal Care and Use Committee (Protocol # 4480; Approved 1 September, 2013), and all clients provided informed consent. Horses for the study were recruited by contacting a large group of active endurance riders in the Pacific Northwestern USA. Nineteen Arabian or Arabian-cross horses from six different farms were evaluated. Ten horses (age 15.4 ± 5.6 years) were defined as ER-susceptible based on clinical episodes of stiffness and reluctance to move that were associated with exercise and diagnosed by a veterinarian as consistent with ER on at least one occasion (Table 1). None of these horses had displayed clinical signs of ER within 6 months to a number of years prior to the study. Serum creatine kinase (CK) and/or aspartate aminotransferase (AST) activity was historically measured within 1–10 days of a reported clinical episode in seven of these horses, with abnormal increases in one or both variables recorded in all of these horses (Table 1). In 6/10 horses, a period of rest reportedly preceded episodes of ER. Nine control horses (12.9 ± 6.1 years) were selected such that a horse with ER and a control horse were located on the same property and had similar feeding and exercise routines. Control horses had all participated in endurance activities for a minimum of 3 years with no prior evidence of ER. Pre-existing serum CK/AST measurements were only available for one control horse which participated in a previous study; values were within reference intervals after completing an 80 km (50 mile) race. At the time of the current study, all horses were receiving a ration based on grass hay or pasture grass, with minor additional supplementation typically consisting of small amounts of beet pulp, rice bran and 1–2 lb of commercial concentrate products per day.

The exercise history for pairs of horses with ER and control horses was as follows: two pairs were turned out on a large pasture (400 acres/162 hectares) and did not participate in forced exercise; one pair performed <4 h exercise per week; four pairs and one triplicate (two horses with ER, one control horse) performed 6–8 h of exercise per week, and one pair exercised for >10 h per week.

Standardised exercise test (SET)

An exercise test was devised that all horses could complete regardless of their current fitness. Horses were stall rested for 24–48 h prior. The 47 min SET was performed on flat ground with all horses ridden under saddle, over which it was estimated they would cover 6.4–7.2 km (4.0–4.5 miles). The SET commenced with 10 min of walking followed by 5 min trot, 5 min walk, 5 min trot, 2 min canter, 5 min walk, and 5 min trot before concluding with 10 min of walking. Eight horses with ER and eight control horses were paired by property of origin and each pair completed the SET simultaneously outdoors. In one case, two horses with ER were matched with one control horse from the same property, and the SET was performed in a covered arena by one pair and subsequently by the remaining horse with ER with one of the same riders. Distance, pace and elevation data were collected using a GPS enabled wristwatch (Forerunner 310XT, Garmin International) worn by of one of the riders in six pairs performing the SET outside. Mean heart rate (HR) data from horses during each interval of the exercise test was collected on seven pairs of horses performing outside, and on the three horses performing in the arena, using a commercially available telemetric ECG system (Televet 100, Engel Engineering Services).

Blood sampling

Venous blood was collected into EDTA and sodium heparin immediately before and after the SET for measurement of packed cell volume (PCV) and plasma total protein, glucose, lactate and electrolytes (sodium, potassium, chloride, and ionised calcium). Plasma CK was measured before and 3 h after exercise. Heparinised samples were centrifuged immediately and plasma removed and frozen in liquid nitrogen until analyzed within 72 h. PCV and total protein concentrations were determined using microhematocrit determination and refractometry of EDTA samples, respectively. Plasma glucose, lactate and electrolytes were measured using a commercially available bench top blood gas analyzer, and plasma CK was determined using a commercially available chemistry analyzer.

Muscle biopsies

A needle biopsy of the middle gluteal muscle was obtained prior to and 3 h after horses completed the SET for histologic analysis (pre-exercise samples only) and measurement of muscle glycogen concentration. A 3 h window was chosen to accommodate ideal timing for planned studies of gene expression following exercise, and because this short delay was unlikely to have any significant impact on

Table 1
Descriptive and historical information for 10 horses with a history of exertional rhabdomyolysis (ER).

Breed	Age (yrs.)	Gender	CK:AST (U/L)	History
Arabian	29	G	69:2910 measured 10 days after signs	One severe episode after an 80 km (50 mile) race when 15 years old: poor HR recovery, discolored urine, reluctance to move. Previously won an elite 160 km (100 mile) race.
Arabian × Saddlebred	13	G	ND	Two episodes, first as an 8 year old in training: trembling, stiffness, cramping, sweating, poor HR recovery, reluctance to move.
Arabian	17	M	ND	Three episodes: trembling, stiffness, cramping, reluctance to move. Observed as a 17 year old with light exercise following 6 weeks of rest after a 160 km (100 mile) race, and again 2 days later. Full sister reported to have severe clinical ER at an elite 160 km (100 mile) race.
Arabian × Saddlebred	13	M	ND	Clinical signs as a 10 year old during training: trembling, stiffness, cramping, poor HR recovery, discolored urine, reluctance to move.
Arabian	14	G	40,977:2303 measured within 6 h of signs	Clinical signs as a 10 year old during training: trembling, stiffness, cramping, poor HR recovery, discolored urine, reluctance to move.
Arabian	18	G	21,612:2552 measured within 12 h of signs	At least two episodes; first was subclinical as 15 year old (CK 6000 U/L after an 80 km [50 mile] race). Severe clinical episode and high CK as a 17 year old with 30 min of light exercise after 2 weeks of rest when very fit.
Arabian	10	S	136:1630 Measured 36 h after signs	Two episodes, first at 9 years old when stall rested for 2 days before a race, clinical signs of ER within 30 min of race commencing. Second clinical episode shortly into a 160 km (100 mile) race the same year (finished the race).
Arabian	12	M	1991:4066 obtained during training season with many episodes	Multiple clinical episodes 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 years old. Excellent race results.
Arabian	18	M	3319:519 Measured within 12 h of signs	Two episodes, 30 km (18 miles) into a race as an 11 year old: mild tachycardia, dark urine, stiffness. One year later clinical signs and high CK 3 km (2 miles) into exercise after rest. Dam had severe clinical episode during an 80 km (50 mile) race as an 8 year old (CK > 300,000 U/L)
Arabian	10	M	120,000: 32,242 Measured 36 h after signs	One episode diagnosed after racing: trembling, stiffness, cramping, poor HR recovery, discolored urine, reluctance to move from standing, commencing about 13 km (8 miles) into race.

M, Mare; G, Gelding; S, Stallion; CK, Creatine kinase; AST, Aspartate aminotransferase; ND, Measurement of serum CK and/or AST not performed.