Effect of ration and exercise on plasma creatine kinase activity and lactate concentration in Thoroughbred horses with recurrent exertional rhabdomyolysis

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Objective—To determine the effects of 3 rations (low grain, fat, high grain) on plasma creatine kinase (CK) activity and lactate concentration in Thoroughbred horses with recurrent exertional rhabdomyolysis (RER).

Animals—5 Thoroughbreds with RER and 3 healthy Thoroughbreds (control horses).

Procedures—Rations were formulated to meet (low-grain and fat rations) or exceed (high-grain ration) daily energy requirements. Each ration was fed to horses in a crossover design for 3 weeks. Horses were exercised on a treadmill Monday through Friday; maximum speed on Monday and Friday was 11 m/s (6% slope), on Tuesday and Thursday was 9 m/s, and on Wednesday was 4.5 m/s. Plasma CK activity and lactate concentration were determined before and after exercise.

Results—Horses with RER fed the high-grain ration had significantly greater CK activity and change in CK activity 4 hours after exercise, compared with those fed the low-grain ration. Horses with RER exercised at the trot or canter had significantly greater increases in CK activity, compared with those exercised at the gallop. Plasma lactate concentrations after exercise were similar in control and affected horses. Lactate concentration and CK activity were not correlated in horses with RER.

Conclusions and Clinical Relevance—Rations high in grain and formulated to exceed daily energy requirements may increase episodes of rhabdomyolysis in Thoroughbred horses susceptible to RER. (*Am J Vet Res* 2000;61:1390–1395)

Creatine kinase (CK) is an enzyme found in high concentrations in skeletal, smooth, and cardiac muscles.[1] Creatine kinase is important in energy production within muscle cells, because it catalyzes the conversion of creatine phosphate and ADP to creatine and ATP.[2] Creatine kinase can leak into the blood when membrane permeability is altered and when muscle cells die.[1] In general, plasma or serum CK activity peaks within 4 to 6 hours after exercise and reflects the degree of muscle damage. The half-life of CK in the blood of horses is approximately 90 minutes.[3] Therefore, CK is a useful marker for determining magnitude and duration of muscle damage.[4,5]

In healthy horses, sled dogs, and human beings, physical exertion may cause increased serum or plasma CK activity that may be 2- to 4-fold higher than values before exercise and exceed the resting reference range.[6-9] The magnitude of the increase is dependent on exercise intensity and duration and is primarily influenced by an animal's fitness, but age, sex, and diet also may play a role.[10-14] Persistent or intermittent increases of greater than 10 times the reference range are suggestive of cellular death or underlying myopathy.[3,9] In horses with chronic exertional rhabdomyolysis, episodes of rhabdomyolysis may be clinical or subclinical. Horses with clinical episodes of rhabdomyolysis typically have large increases in serum CK activity accompanied by altered gait attributable to muscle stiffness or cramping. Horses with subclinical episodes do not have evidence of muscle stiffness but may have peak increases in serum CK activity as high as 30,000 U/L.[15]

Detection of intermittent large increases in serum or plasma CK and **aspartate transaminase (AST)** activity after exercise in horses can help clinicians diagnose the syndrome of exercise-associated muscle necrosis, commonly referred to as exertional rhabdomyolysis or tying-up.[16] Chronic exertional rhabdomyolysis is the most common myopathy in horses. Many factors have been implicated in the pathogenesis of exercise-induced rhabdomyolysis, including exercise intensity, daily ration, and underlying myopathy.[17,18] Two specific myopathies that result in chronic exertional rhabdomyolysis in horses are polysaccharide storage myopathy and **recurrent exertional rhabdomyolysis (RER)**.[18] Polysaccharide storage myopathy is a glycogen storage disorder of Quarter Horses associated with enhanced glucose clearance and insulin sensitivity.[19] Recurrent exertional rhabdomyolysis may occur in as many as 5% of racing Thoroughbreds and is an important source of economic loss.[20-22] The etiopathogenesis of RER in Thoroughbreds is unknown, although recent evidence suggests that it is a heritable stress-related disorder of muscle contractility.[23-25]

Results of research by Carlstrom[26] implicated a high-carbohydrate ration and periods of stall rest in the development of chronic exertional rhabdomyolysis. Carlstrom hypothesized that glycogen loading of the muscle developed as a result of horses being fed a high-carbohydrate ration. Subsequent exercise led to lactic

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acidosis and rhabdomyolysis. Therefore, a common recommendation for horses with chronic rhabdomyolysis, regardless of breed, has been to decrease the total soluble-carbohydrate portion of the ration or replace calories supplied as carbohydrate with fat.[27,28] A ration low in carbohydrate can decrease plasma CK activity in horses with polysaccharide storage myopathy.[28] However, to our knowledge, controlled studies examining the effect of ration and exercise on induction of lactic acidosis and rhabdomyolysis have not been performed in horses with RER. Therefore, the purpose of the study reported here was to compare the effect of 2 isocaloric diets (low grain, fat) and 1 high-caloric diet (high grain) on plasma lactate concentration and CK activity in Thoroughbred horses with RER.

Materials and Methods

Horses—Five female Thoroughbred horses (4, 5, 6, 10, and 13 years old) with RER and 3 healthy Thoroughbreds (2 mares, 4 and 13 years old; 1 gelding, 12 years old; control horses) were used in the study. Horses ranged from 477 to 580 kg in body weight (mean, 516 kg). All horses were housed in an accredited facility and were cared for in accordance with principles outlined by the National Institute of Health.[29]

Recurrent exertional rhabdomyolysis was diagnosed in the 5 affected horses on the basis of history (ie, episodes of exercise-induced muscle cramping, signs of pain, and stiffness) and increased serum CK activity. Histochemical analysis of muscle biopsy specimens from all horses with RER revealed increased numbers of central nuclei in type-2A and -2B fibers and lack of abnormal polysaccharide when specimens were stained with periodic acid Schiff (PAS) stain.[19] In addition, in vitro contracture responses of external intercostal muscle bundles from horses with RER to stimulation by potassium, caffeine, and halothane were abnormal.[23] Control horses did not have a history of rhabdomyolysis. Results of histochemical analysis and contracture tests on muscle biopsy specimens from control horses were within the expected range. To establish a comparable degree of fitness among horses, all horses were exercised on a treadmill 4 to 5 days/wk for 30 min/d at a walk (1.89 m/s), trot (4.00 m/s), canter (7.00 m/s), or gallop (11.00 m/s) for at least 6 weeks prior to the study.

Ration composition—Horses were fed 5.7 kg of alfalfa-timothy hay cubes/d and a protein, vitamin, and mineral supplement (0.7 kg/d). Horses were fed 1 of 3 additional rations in the same order for 3 weeks (21 days), using a crossover design, with the first ration assigned such that all 3 rations were fed initially to at least 2 horses. Two rations were designed to meet maintenance energy requirements for horses performing 30 minutes of exercise/d. These rations consisted of either molasses-supplemented grain (2.5 kg/d; low-grain ration) or the isocaloric equivalent of a calcium-balanced rice bran[a] (2.3 kg/d; fat ration). The third ration was a high-caloric ration and consisted of molasses-supplemented grain (4.6 kg/d; high-grain ration). The same grain was used in the low- and high-grain rations; grain contained 37% corn, 32% wheat middlings, 15% oats, 5.4% soy hulls, 4% soybean meal, 3.5% molasses, 1% limestone, 0.5% vitamin-mineral premix, and flavoring.

The maintenance daily caloric intake for each horse (in Mcal) was calculated according to the following formula[30]:

$$\text{Mcal/d} = ([5.9 + 0.13] \times \text{body weight in kg})/4.18$$

Fat and low-grain rations were formulated to provide 25% more calories than maintenance to meet the estimated mean caloric needs of horses performing 30 minutes of exercise/d (ie, 21.4 Mcal/d). High-grain ration was formulated to provide 70% more calories than maintenance (ie, 28.8 Mcal/d). Samples of feed were analyzed to verify composition (**Appendix**).

Exercise regimen—Horses performed identical exercise protocols Monday through Friday for each of the 3 weeks (15 bouts of exercise) during which the rations were consumed. On Mondays and Fridays, horses exercised on a treadmill in accordance with a gallop protocol, on Tuesdays and Thursdays with a canter protocol, and on Wednesdays with a trot protocol. The warm-up for the gallop and canter protocols consisted of walking (speed, 1.8 m/s) for 4 minutes, trotting (4.5 m/s) for 5 minutes, walking for 2 minutes, trotting for 5 minutes, and walking for 2 minutes. On Mondays and Fridays, this warm-up was followed by a 2-minute gallop (11 m/s) on a 6% slope. On Tuesdays and Thursdays, warm-up was followed by three 2-minute canters (9 m/s) interspersed with 2 minutes of walking. The trot protocol (Wednesdays) consisted of walking for 4 minutes followed by trotting for 30 minutes. On the third Friday, horses performed a standardized exercise test.[31] The day after this test, rations were switched. Horses remained in their stalls and were not exercised on Saturdays and Sundays.

Determination of plasma lactate concentration and CK activity—Venous blood samples were collected into heparin-coated vacuum tubes each day of exercise while horses stood on the treadmill immediately before exercise began and within 15 seconds after exercise was completed. A third blood sample was collected 4 hours after the completion of exercise. Plasma for determination of lactate concentration was stored at −20 C. Lactate concentration was determined in samples collected before and immediately after exercise, using an automated analyzer.[b] Plasma CK activity was measured daily in samples collected before and 4 hours after exercise, using an automated analyzer.[c]

Statistical analyses—Data were analyzed, using a commercially available software program.[d] A general linear model procedure was used to analyze plasma lactate concentration, CK activity before and after exercise, and change in CK activity. Independent variables included group (RER, control), ration (low-grain, high-grain, fat), exercise intensity (gallop, canter, trot), and their interactions. Results were reported as least-square means ± SEM. Significance was set at $P \leq 0.05$.

Results

One control horse was only able to complete the portion of the study for consumption of the high-grain ration because of lameness unrelated to rhabdomyolysis, and 1 horse with RER completed only the portions for consumption of low-grain and fat rations because of mechanical problems with the treadmill. Including data from all horses, when horses were fed the low-grain or fat rations, they lost 1.2 ± 1.6% or 1.1 ± 1.6% of their body weight, respectively. Horses gained 2.4 ± 0.3% of their body weight while consuming the high-grain ration. Body weights did not differ significantly among groups or among horses when fed the various rations.

Plasma CK activity before exercise—Ration or exercise intensity did not affect plasma CK activity before exercise in control horses. We also did not detect a significant difference in CK activity between horses with RER (319 ± 25 U/L) and control horses

(256 ± 37 U/L). However, when horses with RER were fed the high-grain ration, they had significantly (P = 0.01) higher mean plasma CK activity before exercise, compared with the same horses when fed the fat ration (389 ± 44 U/L vs 226 ± 43 U/L).

Plasma CK activity after exercise—Ration or exercise intensity did not affect CK activity determined 4 hours after exercise in control horses. When horses with RER were fed the high-grain ration, they had significantly (P = 0.03) higher mean plasma CK activity after exercise than the same horses when fed the low-grain ration, and they had a slightly, but not significantly (P = 0.09), higher plasma CK activity after exercise than when fed the fat ration (high grain, 839 ± 109 U/L; low grain, 383 ± 106 U/L; fat 585 ± 107 U/L). Similar results were found for change in CK activity (CK activity before exercise subtracted from CK activity after exercise; **Table 1**). When values for ration and exercise intensity were combined, mean plasma CK activity 4 hours after exercise and mean change in CK activity were not significantly different between horses with RER (after exercise, 602 ± 62 U/L; change, 310 ± 62 U/L) and control horses (after exercise, 482 ± 91 U/L; change, 176 ± 91 U/L).

Exercise intensity and duration did affect plasma CK activity after exercise and change in CK activity in horses with RER. When these horses were exercised for 34 minutes in accordance with the trot protocol (ie, 4 minutes of walking followed by 30 minutes of trotting), CK activity after exercise was significantly (P = 0.04) greater, compared with the same horses exercised for 18 minutes in accordance with the gallop protocol (731 ± 126 U/L vs 406 ± 97 U/L). Horses with RER that were exercised for 30 minutes in accordance with the canter protocol had a slightly, but not significantly (P = 0.06), higher plasma CK activity than the same horses exercised for 18 minutes in accordance with the gallop protocol (671 ± 96 U/L vs 406 ± 97 U/L). Similarly, horses with RER exercised at the trot or canter had significantly greater changes in CK activity than horses exercised at the gallop (Table 1).

When examining the combined effects of ration and exercise intensity and duration on CK activity in horses with RER, the highest CK activity after exercise (1,226 ± 168 U/L) and the greatest change in CK activity (944 ± 168 U/L) were detected in horses when fed the high-grain ration and exercised at the canter. The second highest CK activity (913 ± 223 U/L) and change in CK activity (627 ± 223 U/L) were detected in horses when fed the high-grain ration and exercised at the trot.

Table 1—Change in plasma creatine kinase (CK) activity (U/L; CK activity after exercise—CK activity before exercise) determined for 3 healthy Thoroughbreds (control horses) and 5 Thoroughbreds with recurrent exertional rhabdomyolysis (RER)

Variable	Control horses	Horses with RER
Low-grain ration	113 ± 168	95 ± 106*
Fat ration	146 ± 165	273 ± 106
Gallop exercise	149 ± 146	106 ± 97†
Canter exercise	129 ± 144	384 ± 96
Trot exercise	250 ± 180	439 ± 126

Data reported as least-squares mean ± SEM.
*Significantly (P = 0.002) different from value determined for horses with RER when fed the high-grain diet. †Significantly (P = 0.04) different from value determined for horses with RER exercised at the canter or trot.

Table 2—Plasma lactate concentration (mM) determined immediately after exercise in 3 healthy Thoroughbred horses (control horses) and 5 Thoroughbreds with RER

Exercise	Control horses	Horses with RER
Gallop*	11.6 ± 0.5[a]	8.7 ± 0.3[b]
Canter†	1.2 ± 0.5[c]	0.9 ± 0.3[c]
Trot‡	1.1 ± 0.6[c]	0.5 ± 0.4[c]

Data reported as least-squares mean ± SEM.
*Peak speed during exercise, 11 m/sec; total time of exercise, 18 minutes. †Peak speed during exercise, 9 m/sec; total time of exercise, 28 minutes. ‡Peak speed during exercise, 4.5 m/sec; total time of exercise, 34 minutes. [a-c]Values with different superscript letters differ significantly (P < 0.001).

We did not detect a significant difference in change in CK activity on Mondays, compared with values obtained on Fridays. Mean change in CK activity for control horses on Mondays was 129 ± 143 U/L and on Fridays was 178 ± 149 U/L. For horses with RER, mean change in CK activity was 119 ± 36 U/L on Mondays and 84 ± 49 U/L on Fridays.

Plasma lactate concentration—Ration or exercise intensity did not affect plasma lactate concentrations determined before exercise in affected or control horses. In both groups of horses, mean lactate concentration determined immediately after horses were exercised at the gallop were significantly higher, compared with values determined after horses were exercised at the trot or canter (**Table 2**). Moreover, lactate concentration was significantly higher in control horses exercised at the gallop, compared with horses with RER exercised at the same speed. For all exercise and ration protocols combined, mean lactate concentration after exercise was significantly higher in control horses, compared with horses with RER (5.4 ± 0.6 mM vs 3.5 ± 0.3 mM). Ration did not affect lactate concentration determined immediately after exercise in either group.

Creatine kinase activity and plasma lactate concentration—Plasma CK activity determined 4 hours after exercise and plasma lactate concentration determined immediately after exercise in horses with RER were not correlated (r = 0.01).

Behavior—We did not detect muscle stiffness (eg, shortened stride length, reluctance or refusal to move) in any horse before, during, or after exercise. Subjectively, horses appeared more nervous and excitable (eg, more reactive in their stalls, difficult for 1 person to catch, and difficult to lead from the barn to the treadmill) while consuming the high-grain ration, compared with when they were consuming the other 2 rations.

Discussion

Feeding a high-grain ration designed to exceed daily caloric requirements for 3 weeks to 5 fit Thoroughbred horses with RER resulted in an increase in plasma CK activity after periods of exercise. It has been proposed that exertional rhabdomyolysis develops after exercise following a period of rest in horses fed a full ration. This purportedly leads to accumula-

tion of glycogen in muscle (ie, glycogen loading or supercompensation). With exercise, glycogen breakdown results in production of excessive amounts of lactate. Subsequently, the accumulation of lactate leads to muscle necrosis.[32,33]

Glycogen loading has been documented in human beings and dogs but not in horses.[34-37] In human beings, glycogen loading is accomplished by consumption of high-carbohydrate meals alone or by consumption of low-carbohydrate meals combined with intense exercise for several days, which results in glycogen depletion, followed by rest and consumption of high-carbohydrate meals, which results in glycogen loading. Such protocols lead to a 41 or 75% increase in muscle glycogen concentrations, respectively, but have not been associated with rhabdomyolysis.[36-40] In trained horses, rations high in grain result in modest increases in muscle glycogen content (approx 20%), compared with isocaloric rations that are high in protein or fat.[41] In horses, glycogen loading has not been documented despite the use of glycogen-depletion protocols.[34,35] Muscle glycogen concentrations in the horses included in the study reported here were not significantly increased when the high-grain ration was fed, even though the total daily caloric intake exceeded the caloric content of the low-grain and fat rations.[31] Therefore, glycogen loading may not be associated with RER in Thoroughbred horses.

Carlstrom[26] proposed lactic acidosis as a cause for muscle necrosis in draft horses with clinical signs of rhabdomyolysis. However, results of a more recent study[42] indicate that the plasma concentrations of lactate reported by Carlstrom are similar to or less than those found in clinically normal horses performing high-intensity exercise without apparent adverse effects. We did not find a correlation between plasma lactate concentration and CK activity in horses with RER. In addition, plasma lactate concentration immediately after exercise in horses with RER was not significantly different from the concentration in our small control group. Therefore, it seems unlikely that lactic acidosis is the primary cause for rhabdomyolysis in Thoroughbred horses with RER. The protocol used in this study dictated that all horses exercised at the same speed rather than the same relative exercise intensity, such as a percentage of maximum oxygen uptake. This may have imposed slightly differing degrees of effort for specific horses, particularly at the higher speeds. The lack of a large difference in plasma lactate concentration after exercise between affected and control horses does not support the hypothesis that lactic acidosis causes rhabdomyolysis. Furthermore, in another study of Thoroughbreds performing a near-maximal standardized exercise test designed to achieve a heart rate of 200 beats/min,[31] muscle lactate concentrations were comparable between control horses and horses with RER. These findings are similar to those of other studies,[43,44] indicating that lactate production and muscle and plasma lactate concentrations after exercise in Standardbred horses susceptible to exertional rhabdomyolysis were not higher than in healthy control horses.

Results of recent studies[23,45,e] in which investigators evaluated in vitro contracture responses in intact isolated muscle bundles and isolated sarcoplasmic reticulum membranes indicate that skeletal muscle from horses with RER has an abnormality in muscle contractility that is similar, but not identical, to that found in swine with malignant hyperthermia.[23,45,e] Evidence also suggests that RER, similar to malignant hyperthermia, may be initiated by environmental stress.[20,44] Therefore, ration and exercise intensity may impact the expression of RER in susceptible horses by influencing behavior responses to stress. For example, horses with a nervous temperament and management factors that increase excitement, such as feeding rations high in grain and galloping at a moderate pace, have been found to be associated with episodes of exertional rhabdomyolysis in Thoroughbred racehorses.[20]

Subjectively, the horses in the study reported here appeared more nervous and excitable while consuming the high-grain ration, compared with the low-grain and fat rations. When consuming the high-grain ration, all horses were more reactive in their stalls, difficult for 1 person to catch, and difficult to lead from the barn to the treadmill. When fed the fat ration, the same horses appeared more docile and willing to exercise. These subjective observations are similar to results of 2 studies[41,46] in which horses fed rations high in fat were more tractable than horses fed control rations and horses fed a high-grain ration were more excitable than horses fed a low-grain ration. Similarly, human beings fed rations that exceeded daily caloric requirements were found to have greater nonexercise activity (eg, fidgeting, spontaneous muscle contraction, and maintenance of posture).[47] To date, the mechanisms by which environmental and mental stress trigger episodes of rhabdomyolysis or malignant hyperthermia remain unknown.

Thoroughbreds in the study reported here were more likely to have high plasma CK activity when exercised at the trot or intervals of walk, trot, and canter, compared with exercising at the gallop. One explanation for this may be that development of rhabdomyolysis increases with exercise of prolonged duration. In a study in which investigators evaluated Standardbred horses with chronic exertional rhabdomyolysis,[15] increased CK activity was detected after 15 to 30 minutes of trotting, whereas little change in CK activity was detected after 12 minutes of high-intensity exercise. A further explanation for the effect of exercise intensity on plasma CK activity may be its effect on excitability. Exercise at a slow or moderate pace may have been frustrating for our fit horses. Evidence for this included behavioral changes such as leaning on the front bar of the treadmill or playing (ie, bucking, kicking out) on the treadmill in an effort to go faster.

Our results suggest that exercise protocols consisting of short bouts of intense exercise and feeding rations low in grain or high in fat, formulated to meet but not exceed daily caloric energy requirements, may be practical management tools to control episodes of rhabdomyolysis in Thoroughbred horses susceptible to RER. However, additional studies, using larger groups of horses, are required to elucidate the exact effect of caloric density, dietary energy source, and exercise

intensity or duration on rhabdomyolysis in susceptible Thoroughbreds.

[a]Equi-Jewel Producers Rice Mill, Inc, Kentucky Equine Research, Inc, Versailles, Ky.
[b]YSI 2500 L STAT lactate analyzer, Yellow Springs Instrument Co, Yellow Springs, Ohio.
[c]Beckman CX4 analyzer, Beckman ICS, Brea, Calif.
[d]SAS/STAT7 user's guide [software]. Version 6 edition. Cary, NC: SAS Institute, Inc, 1990.
[e]Ward TL, Valberg SJ, Gallant EM, et al. Skeletal muscle membrane Ca^{2+} regulation in Thoroughbred horses with exertional rhabdomyolysis (abstr). *J Vet Intern Med* 1998;12:220.

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Appendix

Composition of rations fed to 5 Thoroughbred horses with recurrent exertional rhabdomyolysis (RER) and 3 healthy Thoroughbreds

	Low-grain ration			High-grain ration			Fat ration		
Fraction	As fed (%)	Intake (g/d)	Energy (% total)	As fed (%)	Intake (g/d)	Energy (% total)	As fed (%)	Intake (g/d)	Energy (% total)
NSC	33.6	2,986	47	38.6	4,244	53	24.8	2,158	34
Fat	2.6	235	8	2.8	303	8	7.1	619	20
NDF	30.4	2,705	18	27.6	3,036	16	31.2	2,714	18
Protein	14.9	1,326	27	13.6	1,496	23	16.3	1,421	28

NSC = Nonstructural carbohydrates. NDF = Nondetergent fiber.