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The Effect of Varying Dietary Starch and Fat Content on Serum Creatine Kinase Activity and Substrate Availability in Equine Polysaccharide Storage Myopathy

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The effect of dietary starch and fat content on serum creatine kinase (CK) activity and substrate availability was evaluated in 4 mares of Quarter Horse-related breeds with polysaccharide storage myopathy (PSSM). Four isocaloric diets ranging in digestible energy (DE) from 21.2% (diet A), 14.8% (B), 8.4% (C), to 3.9% (D) for starch, and 7.2% DE (diet A), 9.9% (B), to 12.7% DE (diet C and D) for fat were fed for 6-week periods (4 weeks with exercise) using a 4 × 4 Latin square design. Postprandial glucose and insulin responses were measured, and 4 hours postexercise, serum CK activity, glucose, insulin, free fatty acids (FFA), and β-hydroxybutyrate (β-HBA) were analyzed. Glycogen, glucose-6-phosphate, citrate synthase, 3-hydroxy-acyl-CoA dehydrogenase, lactate dehydrogenase as well as abnormal polysaccharide and lipid content were measured in middle gluteal muscle samples. Postprandial insulin and glucose response was higher for diet A versus D. Log CK activity was higher with diets A, B, and C versus D. Daily insulin was higher and FFA lower on diet A versus B, C, and D, whereas glucose varied only slightly with diet. Muscle oxidative capacity and lipid stores were low in PSSM horses and muscle glycogen and abnormal polysaccharide content high on both diets A and D. Individual variation occurred in the response of PSSM horses to diets differing in starch and fat content. However, for those horses with clinical manifestations of PSSM, a diet with <5% DE starch and >12% DE fat can reduce exertional rhabdomyolysis, potentially by increasing availability of FFA for muscle metabolism.

Key words: Exertional rhabdomyolysis; Glycogen; Horse; Insulin; Muscle.

Polysaccharide storage myopathy (PSSM) is a common, heritable form of exertional rhabdomyolysis (ER) in Quarter Horse-related breeds. It is characterized by excessive accumulation of glycogen, glucose-6-phosphate, and abnormal polysaccharide inclusions in skeletal muscle.[1–3] The hallmark feature used to establish a diagnosis of PSSM is the presence of periodic acid-Schiff (PAS)-positive inclusions that are resistant to amylase digestion in muscle biopsy specimens.[1,2] In addition to ER, clinical signs associated with PSSM in Quarter Horses include muscle stiffness, pain, shifting lameness, stretched-out stance, gait changes, muscle atrophy, and colic-like signs of pain.[1,2,4] PSSM is an unusual metabolic myopathy in that, unlike previously described glycogenoses, glycogen appears to be metabolized during exercise.[3] Previous studies suggest that PSSM horses exhibit increased insulin sensitivity and more rapid excursion of glucose from the bloodstream compared with clinically normal horses.[5,6]

For a number of years, the management of clinical signs of PSSM has been based on dietary modification and institution of an incremental training program.[7–11] Apparent success may be related to the fact that a low-starch, fat-supplemented diet will stabilize blood glucose and insulin concentrations and regular exercise will enhance glycogen metabolism. The specific effects of diet change and exercise, however, have never been scientifically evaluated. As a result, there is no consensus on the amount of fat in the diet required for beneficial effects or the form of fat to feed. Some veterinarians have recommended that the diet of horses with PSSM should contain as much as 25% of total daily calories as fat.[8,10] Owners' perceptions of the value of increasing fat and reducing soluble carbohydrate in the diet of PSSM horses have been evaluated in previous clinical studies.[9,12] In general, owners believe that this type of dietary modification decreases muscle stiffness and increases exercise tolerance in PSSM horses. However, it is difficult to objectively ascertain from these studies the best diet to feed PSSM horses because owners fed a wide variety of diets without any dietary analyses available. In addition, the amount of exercise received was not standardized and serum creatine kinase (CK) activity was not consistently monitored to provide an objective assessment of the degree of rhabdomyolysis with exercise.

The purpose of the present controlled exercise trial was to objectively determine the effect of 4 diets varying in starch and fat content on postprandial blood glucose and insulin concentrations, insulin sensitivity, as well as daily concentrations of blood glucose, insulin, and free fatty acids (FFA). In addition, postexercise serum CK activity as well as muscle glycogen and glucose-6-phosphate (G6P), were assessed on each diet. Furthermore, for diets A and D, the activities of 3-hydroxy-acyl-CoA dehydrogenase (HAD), citrate synthase (CS), and lactate dehydrogenase (LDH) activities were evaluated and histochemical stains were used to determine abnormal polysaccharide and lipid content of skeletal muscle fibers.

Materials and Methods

Horses

Four mares, a 3-year-old Appaloosa (horse 1), a 6-year-old Quarter Horse (horse 2), a 6-year-old Paint (horse 3), and a 6-year-old Appaloosa (horse 4) were used in this study. Horses weighed 568 ± 26.6 kg. Horse 1 was donated to the University of Minnesota because of repeated episodes of ER. Horse 2 was born at the University of Minnesota and has had repeated clinical episodes of ER with exercise.

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Table 1. Dietary composition and percentage of digestible energy (DE) per day in megacalories (MCal) provided by fat, starch, protein, and fiber for 4 diets, A–D.

	A	B	C	D
Total DE (Mcal)	25.1	21.7	23.7	23.2
% Starch	21.2	14.8	8.4	3.9
% Fat	7.2	9.9	12.7	12.7
% Protein	18.2	17.8	17.3	16.4
% Fiber	53.4	57.5	61.6	67.0

Horses 3 and 4 were identified in the University of Minnesota teaching herd as having PSSM based on biopsy. Horse 4 began the trial 6 weeks after the others because this horse was substituted for another PSSM horse that developed laminitis after 5 weeks of the trial.

A diagnosis of PSSM was based on the presence of abnormal PAS-positive inclusions resistant to amylase digestion in gluteal and semimembranous muscle biopsies. The horses were housed in an accredited facility and were cared for according to the principles outlined by the Institutional Animal Care and Use Committee.

Diet

Horses were rotated among 4 diets containing variable amounts of starch and fat (A, B, C, D) using a 4 × 4 Latin-square design for a period of 6 weeks on each diet. Each diet consisted of grass hay (from 1 pasture at the University of Minnesota) providing 60% of daily DE and a combination of concentrates providing 40% of daily DE. All diets were initially formulated to provide 21 megacalories (MCal) of DE/500 kg horse/d, including forage. Rations were analyzed at the end of the study period to determine the exact caloric density and concentrations of starch, fat, and protein. The actual caloric content and DE for starch, fat, and protein is shown in Table 1. Horses were weighed before the beginning of each 6-week trial to determine the exact amount to be fed.

The concentrate portion of the high-starch, low-fat diet A was composed of a molasses-supplemented grain[a] containing 45% corn, 45% oats, and 10% molasses (2.2 kg/500 kg horse/d). The concentrate portion of diet B was composed of less sweet feed than diet A (1.1 kg/500 kg horse/d) and a calcium-balanced rice bran[b] (0.8 kg/500 kg horse/d). The concentrate portion of diet C was composed of a small amount of sweet feed to keep diets isocaloric (0.1 kg/500 kg horse/d) and a calcium-balanced rice bran (1.6 kg/500 kg horse/d). The concentrate portion of the lowest starch, high-fat diet D was a commercial concentrate[c] containing soy hulls, rice bran, soybean, corn oil, wheat, and pellet binder (2.7 kg/500 kg horse/d). A protein (30% protein by weight) and vitamin and mineral supplement[d] (0.6 kg/500 kg horse/d) were added to diets A, B, C in order to provide the same amount of protein, vitamins, and minerals in each diet. It was not necessary to add the protein, vitamin, mineral supplement to diet D because it had a similar vitamin, mineral, and protein content to the fortified diets A, B, and C. A salt block was provided in each stall throughout the study. Forage was provided at 8.2 kg/500 kg horse/d. Horses were fed each day in 2 equal feedings, 10 hours apart. The morning meal was fed 1–3 hours before performing treadmill exercise.

Training Period

A 6-week training period was conducted before the beginning of the study. Each horse was randomly assigned to 1 of 4 diets. For the first 2 weeks, horses were rested and introduced to the diet. During weeks 3–6, a basal level of fitness was established and a standardized amount of daily exercise that each horse could tolerate was recorded. Horses were walked on the treadmill for 4 min and trotted for 2 min each day (Monday–Friday) and observed for signs of tucked up abdomen, muscle fasciculations in the flank, shifting lameness, and sweating. If horses were comfortable, 2-min intervals of walk (1.8 m/s) and trot (3.2 m/s) were continued for up to 30 min. Exercise was terminated if signs of discomfort were observed. This method established an individual exercise protocol over a 4-week period for each horse, which then was used as the exercise template for that horse during the subsequent diet trial (Table 2). Horse 4 readily adapted to the treadmill at a full 30 min of exercise and was assigned to the same block as had been assigned to the horse that developed laminitis.

Table 2. The weekly range in the amount of daily exercise (minutes) as well as the total amount of exercise that each horse completed on each diet.

Week	Horse 1	Horse 2	Horse 3	Horse 4
1	0	0	0	0
2	0	0	0	0
3	6–12	14–22	14–26	14–26
4	16–18	16–22	26–30	26–30
5	18–24	22–24	30	30
6	16–24	24–26	30	30
Total	328	402	526	526

Treadmill Exercise and Serum CK Activity

Horses were rested in a stall for the first 2 weeks of each 6-week trial before initiating treadmill exercise. Treadmill exercise was conducted for the next 4 weeks, Monday–Friday. Horses did not exercise on Monday of week 3 and week 6 because the glycemic response to a meal was measured on these days. Horses were exercised in the same order each day (horse 2, 3, 1, then 4) 1–3 hours after the morning meal. Each horse was subjected to the individual exercise protocol established during the adaptation period (Table 2). A blood sample was taken 4 hours after each exercise session. Serum was removed and 1 portion was submitted to a clinical chemistry laboratory for measurement of postexercise CK activity.

Blood Glucose and Insulin Response to a Meal

The blood glucose response to feeding the concentrate portion of the diet was measured after week 2 and week 6, preceded by 24 hours box stall rest and a 12-hour fast. Blood samples were obtained from a jugular catheter before and at 15, 30, 60, 120, 180, 240, and 300 min after one half of the daily concentrate portion of the diet was consumed. Blood glucose concentration was immediately measured by glucometer.[e] Plasma was removed and stored at −80°C for later analysis of insulin concentration (solid-phase radioimmunoassay).[13] Because glucose concentrations were identical at 4 and 6 weeks, insulin analysis was only performed for the week-6 samples.

Blood Glucose, Insulin, FFA, and β-Hydroxybutyrate 4 Hours Postexercise

Blood glucose concentration was measured 4 hours after exercise in venous blood samples by a glucometer. Plasma was removed from samples collected on Tuesdays and Thursdays and frozen at −80°C for later analysis of insulin via radioimmunoassay.[13] Samples obtained on Tuesdays 4 hours postexercise were also used for later analysis of FFA using an enzymatic colorimetric method.[f] Plasma samples obtained 4 hours after the last exercise session on each diet were used for measurements of β-HBA.[14]

Insulin Sensitivity Test

An insulin sensitivity test was performed after week 6 for horses on diets A and D.[15] Before this test, all horses were kept in a box stall for 24 hours and fasted for 12 hours. A blood sample was taken from

a jugular catheter before the experiment and 160 mL of Karo syrup was given PO. Ten minutes later, 0.30 U/kg of insulin was injected IV. Blood samples were then taken and glucose concentration measured immediately, and every 10 min for up to 40 min. If blood glucose concentration reached 40 mg/dL, horses were fed 1 kg of sweet feed and hay.

Muscle Biopsies

Middle gluteal muscle biopsies were obtained 8 hours after the last exercise session for week 6 from a standardized site.[16,17] One portion of muscle was oriented on cork to form approximately 2 × 2 cm^2 cross-section of muscle fibers. This sample was frozen in methylbutane chilled in liquid nitrogen. Ten-micrometer-thick sections were stained with hematoxylin and eosin, PAS, reduced nicotinamine adenine dinucleotide (NADH), and Oil Red O.[18] Five hundred contiguous fibers were analyzed from randomly-selected microscopic fields in each biopsy to determine the percentage of muscle fibers with abnormal polysaccharide in PAS stains and the percentage of lipid found in Oil Red O when horses were on diets A and D. The subjective intensity of NADH staining was used to estimate the oxidative capacity in muscle fibers when horses were on diets A and D.

Muscle Biochemistry

Each sample was frozen immediately using liquid nitrogen and stored at −80°C until analyzed. Glycogen was assayed fluorometrically in 10-mg samples of muscle that were boiled for 2 hours in 1 M HCl to produce glucose residues.[19] For G6P analysis, approximately 10-mg muscle samples were promptly weighed on a Cahn Microbalance and immediately submerged in 1.5 M perchloric acid. Samples were cold centrifuged for 10 min at 4.000 ×*g*, followed by addition of 1 M $KHCO_3$ to neutralize the supernatant. After further centrifugation, G6P was measured fluorometrically.[19] One to 2 mg of freeze-dried muscle that was free of blood, fat, and connective tissue was weighed and homogenized ultrasonically in a phosphate buffer (pH 7.3). CS, HAD, and LDH activities were then analyzed fluorimetrically.[20]

Statistical Analysis

Analysis of variance for repeated measures was used to determine the effect of diet and subject (horse) on glucose and insulin responses to diet, daily blood glucose, insulin, and β-HBA and FFA concentrations. Log of serum CK activity was examined by analysis of variance for repeated measures for effects of diet, horse, and time. A paired *t*-test was used to determine if there was a significant difference in sensitivity to insulin, CS, and HAD and LDH activities between diets A and D. Pearson correlation coefficients were determined for daily insulin, glucose, FFA, and log of serum CK activity. A 1-way ANOVA was used to determine if significant differences existed between muscle glycogen and G6P concentrations among diets. Values are expressed as means ± standard deviation of the mean with significance set at $P < .05$.

Results

Blood Glucose and Insulin Responses to a Meal

For an individual diet, glucose responses obtained at week 2 and week 6 were not significantly different from each other. Mean concentrations of glucose were as follows: For diet A, week 2, 123.2 ± 21.6 mg/dL, and week 6, 123.1 ± 21.6 mg/dL. For diet B, week 2, 118.0 ± 15.8 mg/dL, and week 6, 120.9 ± 19.8 mg/dL. For diet C, week 2, 118.5 ± 13.2 mg/dL, and week 6, 112.8 ± 14.6 mg/dL. For diet D, week 2, 116 ± 13.2 mg/dL, and week 6, 112.3 ± 4.9 mg/dL.

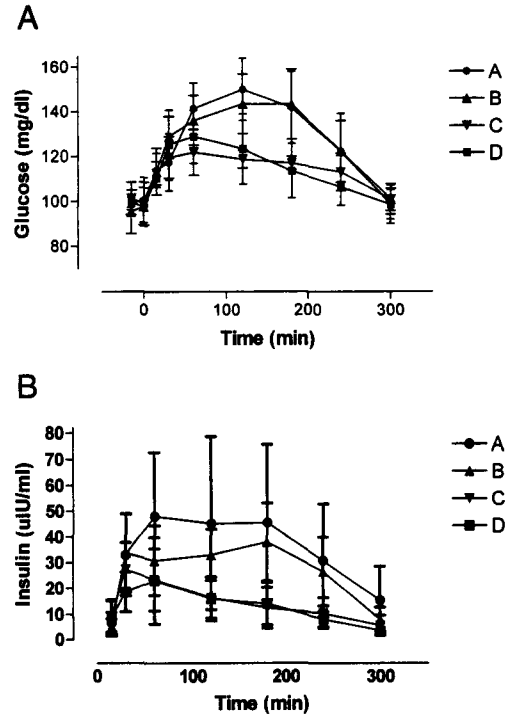

Fig 1. (**A**) Mean ± SD blood glucose concentrations before and after feeding 4 isocaloric diets that varied in their starch and fat content to 4 horses with polysaccharide storage myopathy (PSSM) at week 6 (see Table 1). Diet A had significantly higher postprandial blood glucose concentrations than diet D. (**B**) Mean ± SD plasma insulin concentrations before and after feeding 4 isocaloric diets that varied in their starch and fat content at week 6 to 4 horses with PSSM (see Table 1). Diets A and B were significantly higher in their insulin response than diets C and D.

No significant differences were found in fasting blood glucose concentration before feeding diets A, B, C, and D (Fig 1A). The blood glucose response to diet A was significantly higher than the response to diet D. There was no significant difference in blood glucose response comparing either diet A or diet D with diets B and C (Fig 1A). Horses on diets A and B had significantly higher insulin responses than those on diets C and D (Fig 1B). A significant effect was found on both glucose and insulin responses, with 1 horse (horse 2) that had a lower glucose and insulin response than the others. Mean postprandial blood glucose and insulin concentrations, respectively, in horse 2 were 112.8 ± 15.1 mg/dL and 9.4 ± 7.3 uIU/mL, compared with horse 1, 120.5 ± 19.0 mg/dL and 27.7 ± 24.1 uIU/mL; horse 3, 116.1 ± 15.5 mg/dL and 19.5 ± 16.6 uIU/mL; and horse 4, 123.6 ± 19.9 mg/dL and 19.5 ± 15.0 uIU/mL.

Blood Glucose, Insulin, FFA, and β-Hydroxybutyrate 4 Hours Postexercise

There was a significantly lower mean daily glucose concentration for diet B when compared with diets A, C, and D (Table 3). Daily insulin concentrations were significantly higher on diet A compared with B, C, and D (Table 3). Daily FFA concentrations were significantly lower on diet A compared with B, C, and D. Diet B had lower FFA concentrations than diet C (Table 3). A significant effect

Table 3. Daily glucose (G), insulin (I), and free fatty acid (FFA) concentrations and postexercise creatine kinase (CK), β-hydroxybutyrate (β-HBA), and log CK activity for diets in 4 horses with PSSM.[a]

	A	B	C	D
G (mg/dL)	96.9 ± 7.6[b]	92.7 ± 7.7[c]	99.1 ± 8.1[b]	98.8 ± 10.9[b]
I (μIU/mL)	14.05 ± 8.9[b]	9.8 ± 4.8[c]	10.2 ± 5.7[c]	9.1 ± 4.1[c]
FFA (μMol/L)	22 ± 4.0[b]	34 ± 8.0[c]	43 ± 8.0[d]	40 ± 16.0[cd]
β-HBA (mMol/L)	0.27 ± 0.08[b]	0.24 ± 0.12[b]	0.24 ± 0.16[b]	0.27 ± 0.16[b]
CK (U/L)	894 ± 1511[b]	1138 ± 1223[b]	743 ± 1005[b]	390 ± 325[b]
Log CK	2.72 ± 0.41[b]	2.74 ± 0.50[b]	2.65 ± 0.40[b]	2.52 ± 0.22[c]

[a] Data are expressed as means ± SD. Different superscripts in rows indicate differences, $P < .05$.

was found on blood glucose, insulin, and FFA concentrations. A significant negative correlation was found between daily FFA and daily insulin concentrations ($r = -.40$). No significant differences were found for plasma β-HBA concentrations among diets A, B, C, and D in blood samples taken 4 hours after the last exercise session (Table 3).

Insulin Sensitivity Test

There was no difference in blood glucose concentration after an IV injection of insulin between diets A and D (Fig 2A). Data for horses 1 and 2 revealed a rapid decline in blood glucose concentration, reaching 40 mg/dL between 15 and 20 min, whereas data for horses 3 and 4 revealed a slower rate of decline, with concentrations below 40 mg/dL after 30 min (Fig 2B).

Serum CK Activity

Mean serum CK activity was above the normal laboratory range (79–556 U/L) for all diets except diet D (Table 3). Log CK activity 4 hours after exercise was significantly higher when horses were fed diets A, B, and C when compared with diet D (Table 3). There was no significant difference in log CK activity after exercise among diets A, B, and C (Table 3). A significant effect was found on log CK activity, with 2 horses (horses 1 and 2) having significantly higher CK than horses 3 and 4 (Fig 3). Horses 1 and 2 had marked increases in log CK activity on diets A, B, and C compared with diet D. Horse 1 evidenced stiffness in 11/76 exercise sessions; 5 times when on diet A, 3 times when on diet B, and 3 times when on diet C. Because of this stiffness and discomfort, the amount of treadmill exercise was less than dictated by the protocol (328 min) when horse 1 was on diet C (284 min) and diet A (258 min). Horse 2 was able to complete the exercise protocol; however, her data revealed signs of stiffness in 4/76 exercise sessions (once on diet A, twice on diet B, and once on diet C). Neither horse 1 nor horse 2 showed signs of muscle stiffness on diet D. Horses 3 and 4 also did not reveal evidence of stiffness during the diet trial. There was no significant effect of time (days of exercise) on serum CK or log CK activity for diets A, B, C, or D (Fig 4). Horse 2 had a marked increase in CK on the final day of diet A from 1,847 U/L to 12,573 U/L, which resulted in a large increase in the mean CK activity for this day (Fig 4). After 3 days of rest, the CK activity in horse 2 was 3,040 U/L.

Muscle Histology

The mean percentage of fibers with abnormal polysaccharide accumulation was not significantly different between diets A and D. Out of 500 muscle cells analyzed per horse, the average percentage of fibers containing abnormal polysaccharide when horses were on diets A and D was 0.63 ± 0.96% and 0.55 ± 0.88%, respectively. Very few fibers contained intracellular lipid based on the Oil Red O staining with 2.85 ± 3.40% of fibers containing lipids on diet A and 7.65 ± 14.40% on diet D. The NADH-staining intensity was light for all PSSM horses and no subjective differences in staining intensity were noted between diets A and D.

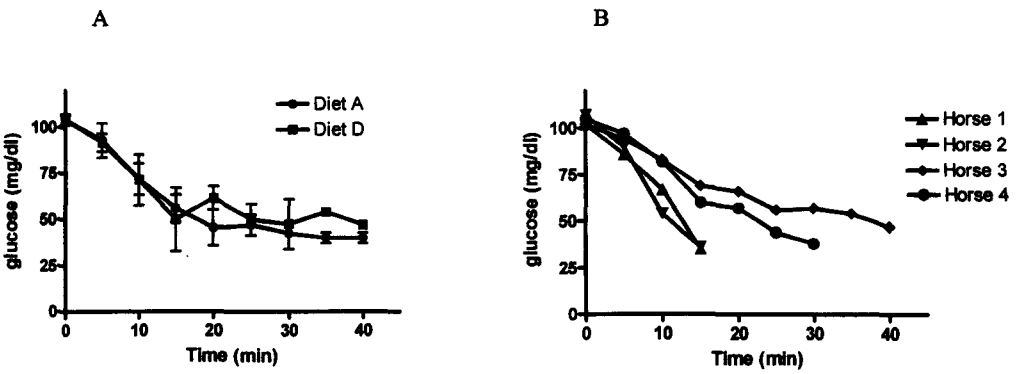

Fig 2. (A) Mean ± SD blood glucose concentrations before and after injection of 0.30 units of insulin IV for horses on diet A and diet D in 4 horses with polysaccharide storage myopathy (PSSM). No significant differences were found between diets. (**B**) Individual blood glucose concentrations for horses 1–4 before and after the insulin injection on diet D. Horses 1 and 2 had a more rapid decline in blood glucose than horses 3 and 4. Similar results were found for diet A (not shown).

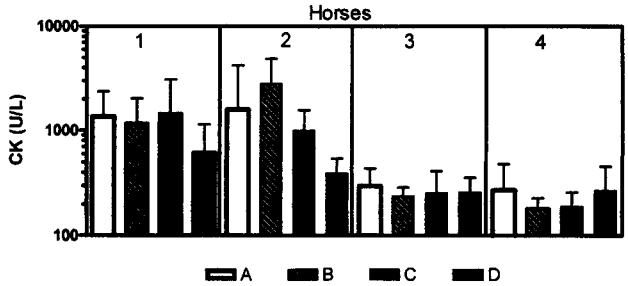

Fig 3. Mean ± SD postexercise serum creatine kinase (CK) activity over 4 weeks for horses 1–4 on each diet. Horses 1 and 2 had markedly higher serum CK activity than horses 3 and 4 when data was log transformed. For horses 1 and 2, the lowest CK activity occurred with diet D.

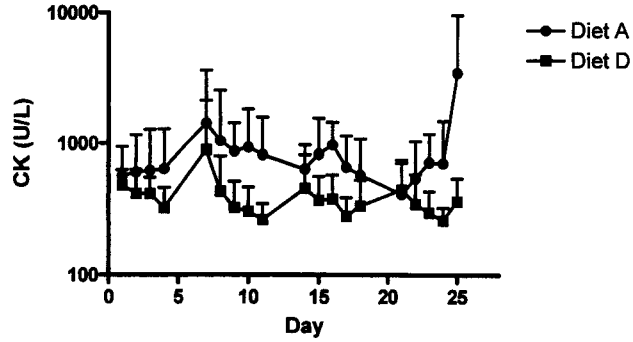

Fig 4. The daily mean ± SD postexercise serum creatine kinase (CK) activity on diets A and diet D. Log transformed CK activity was markedly higher on diet A than diet D. Horses began exercise on Tuesday and were then exercised Monday–Friday with no exercise on weekends. No marked effect of time on serum CK activity was found. One of 4 horses had a much higher CK activity on the last day on diet A resulting in a higher mean value and SD for this particular day.

Muscle Biochemistry

No significant differences in muscle glycogen and G6P concentrations were seen for the 4 diets and HAD and LDH activities did not differ between diets A and D (Table 4). Mean glycogen concentrations were 24 mmol/kg wet weight higher on diet A versus diet D; however, this was not significant. The mean muscle glycogen concentration was, for horse 1, 242 ± 51 mmol/kg; for horse 2, 229 ± 51 mmol/kg; for horse 3, 214 ± 14 mmol/kg; and for horse 4, 197 ± 29 mmol/kg. There was a tendency toward higher HAD activity for diet D compared with diet A ($P = .11$). A small but significantly lower CS activity was identified in PSSM horses on diet D versus A.

Discussion

The main objective of the present study was to determine the effect of varying the fat and starch content of the diet on serum CK activity in PSSM horses performing controlled exercise. The availability of sound horses that could exercise for approximately 30 min/d as well as the cost of conducting the trial over a 6-month period limited the number of horses that were included in the trial. In spite of this limitation, it was possible to demonstrate significantly lower serum CK responses to exercise in PSSM horses when consuming 4% of daily DE as starch and 13% of the daily DE as fat (diet D) as compared with diets with a starch content of >8% and an equivalent or lower fat content (Figs 3, 4; Table 3). In fact, when horses in this study consumed diet D, mean postexercise serum CK activities were within the normal range (Figs 3 and 4; Table 3).

There was significant individual variation among the 4 PSSM horses with regard to their response to the diets (Fig 3). Data from the 2 horses (1 and 2) that had the most severe clinical signs of PSSM, including muscle stiffness and reduced exercise tolerance, revealed the greatest improvement on diet D (Fig 3). These 2 horses also had the highest muscle glycogen concentrations and a fast rate of decline in blood glucose concentration in response to IV insulin compared with the other 2 PSSM horses (3 and 4) in the trial (Fig 2B). Data from horses 3 and 4 revealed minimal increases of serum CK postexercise even while consuming a high-starch diet and no clinical signs of muscle stiffness (Fig 3). It appears therefore that, in this small number of horses, those with the greatest insulin sensitivity and muscle glycogen concentrations were most predisposed to clinical episodes of ER. Thus, in designing an optimal diet for PSSM horses, the amount of starch an individual can tolerate appears to vary but the best response in clinically-affected PSSM horses could be expected from a diet with 4% of DE in starch and 13% of DE in fat. These results support owners' perceptions that a variety of diets that provide less starch and more fat will decrease clinical episodes of exertional rhabdomyolysis in horses with PSSM.[9,12]

One of the beneficial effects of a low-starch diet for PSSM horses may be that it decreases the amount of glucose taken up by skeletal muscle. In the current study, diet D produced relatively small increases in blood glucose and insulin concentrations postprandially compared with the higher starch diet A (Fig 1A, B). The lower starch content of diet D, as well as its potential slowing of intestinal glucose uptake by fat delaying gastric emptying, likely contributed to the lower postprandial glucose and insulin responses.[21–25] The postprandial glucose responses to the various diets in this study were measured by a handheld glucometer, which appeared to provide repeatable results as glucose curves were identical after 2 and 6 weeks on each diet. PSSM horses have a propensity to synthesize skeletal

Table 4. Muscle glycogen (GLY), glucose-6-phosphate (G6P), citrate synthase (CS), 3-OH-acyl-CoA dehydrogenase (HAD), and lactate dehydrogenase (LDH) activity in skeletal muscle of 4 horses with PSSM consuming four diets, A–D.[a]

	A	B	C	D
GLY	237 ± 48[b]	232 ± 58[b]	206 ± 32[b]	210 ± 16[b]
G6P	0.97 ± 0.16[b]	0.70 ± 0.30[b]	1.08 ± 0.58[b]	1.02 ± 0.42[b]
CS	8.1 ± 1.8[b]	nd	nd	5.4 ± 1.4[c]
HAD	13.4 ± 1.8[b]	nd	nd	19 ± 5.6[b]
LDH	1997 ± 280[b]	nd	nd	2184 ± 136[b]

Data are expressed as mean ± SD. Different superscripts in rows indicate differences, $P < .05$. Glycogen and G6P values are expressed as mmol/kg wet weight. CS, HAD, and LDH values are expressed as mmol/kg/min of dry weight. nd = not done.

muscle glycogen to achieve concentrations at least 1.5 times greater than those of normal horses.[1,3,21] This fact is in part related to higher insulin sensitivity in PSSM compared with healthy horses.[5,6,15] Over the 6 weeks of this trial, a significantly lower glycogen concentration was not observed in PSSM horses on diet D. This observation may have been because of the small number of horses in the trial because a trend toward higher mean glycogen concentrations on diets A and B was observed when compared with diets C and D (Table 4). A complete restoration of normal glycogen concentrations in PSSM horses may not have occurred because of the relatively short duration of the trial. Furthermore, PSSM horses may have an underlying metabolic defect that promotes glycogen synthesis to an extent that it cannot be completely inhibited by dietary management.

Diets C and D provided 13% of daily dietary fat in the form of rice bran for diet C and both rice bran and corn oil for diet D. Rice bran is 20% fat by weight and contains mainly oleic and linoleic acids.[26] Corn oil consists of 13% saturated FFA and 25% monounsaturated FFA with a high linoleic acid content.[27] Previous studies have revealed that normal horses adapt to a high-fat diet by an increase in lipoprotein lipase activity as well as an increase in plasma FFA concentrations.[28,29] In the present study, diet D resulted in higher daily plasma FFA concentrations and lower daily insulin concentrations compared with diet A, and insulin was negatively correlated to FFA concentrations (Table 3). Ketosis was not apparent on any diet. Increased lipoprotein lipase activity in horses on diet D and less inhibition of hormone-sensitive lipase in the face of low serum insulin concentration may have increased circulating FFA on diet D.[30] In humans, a diet of 65% DE as fat has been shown to increase lipid transporters in skeletal muscle and a similar effect may occur in horses, although high-fat diets in horses usually contain <25% DE as fat.[31] Availability of plasma FFA and possibly enhanced lipid transporters in skeletal muscle[31] on diet D may be especially important in PSSM horses because they have such low lipid stores and low oxidative capacity. The CS activity of PSSM horses, and Quarter Horses in general,[6] is low and is only about 25% of what is usually seen for untrained Standardbred trotters.[32]

In humans, high plasma FFA concentrations and intracellular lipids have been suggested to inhibit insulin signaling, leading to a reduction in insulin-stimulated muscle glucose transport that may be mediated by a decrease in the glucose transporter GLUT-4 translocation.[33] In the current study, we evaluated insulin sensitivity at rest with a single IV bolus of insulin and found no significant difference in the decline in blood glucose concentration between diets A and D (Fig 2A). This method, however, is likely not very sensitive and methods such as hyperinsulinemic euglycemic clamping would be necessary to determine if insulin resistance occurred in the horses on diet D.[6,15]

The mechanism of rhabdomyolysis with exercise in PSSM horses is not known, but it may be because of disruption in muscle energy metabolism.[9,34] High-starch diets and glycogen accumulation in skeletal muscle may exacerbate rhabdomyolysis.[35] One of the detrimental effects of diet A versus D may be that high insulin and glucose concentrations before exercise may augment exercise-induced glucose uptake by skeletal muscle, which may impair or alter substrate utilization in PSSM horses.[36–39] Insulin concentrations would be expected to be 2-fold higher on diet A versus diet D during daily exercise because horses were exercised between 60 and 180 min after feeding, when insulin and glucose concentrations peaked (Fig 1A, B). In contrast, by feeding diet D, glucose uptake into skeletal muscle may not be as exaggerated during exercise and high circulating FFA may favor oxidation of FFA over glycogen and glucose metabolism, providing a necessary balance to substrate flux in the muscle. FFA oxidation can increase the mitochondrial acetyl CoA/CoA ratio and can lead to a reduction in the supply of acetyl CoA from pyruvate as well as an increase in citrate, which ultimately inhibits phosphofructokinase activity.[40] A decrease in carbohydrate metabolism in favor of FFA metabolism would likely have a sparing effect on skeletal muscle glycogen in PSSM horses. A small decrease in muscle glycogen concentration was seen on diet D versus A, but with the small number of horses, this difference was not significant.

Training,[41] feeding high dietary fat,[42,43] and high circulating plasma FFA concentrations[44] enhance fat metabolism in skeletal muscle during submaximal exercise in normal horses. In humans, a high-fat diet increases gene expression for HAD in skeletal muscle with or without a notable increase in HAD protein activity.[31,45,46] Gene expression has not been studied in horses, and it has been difficult to establish an increase in total HAD protein activity in skeletal muscle with high-fat diets.[43,47,48] In agreement with other studies in horses, substantial changes in CS and HAD activity were not seen on a high-fat diet in PSSM horses, but a trend to higher HAD activity was noted. One of the benefits of daily exercise in PSSM horses may be to promote lipolysis, high plasma FFA concentrations, and enhanced ability to oxidize FFA in skeletal muscle. This study was not designed to separate the beneficial effects of training from those of a high-fat, low-starch diet, but previous studies suggest that these 2 management changes may be synergistic.[21]

In conclusion, variability exists in the degree of rhabdomyolysis seen after exercise in PSSM horses. Horses with clinical signs of exertional rhabdomyolysis appear to benefit from a high-fat, low-starch diet, which, by lowering plasma insulin concentrations and increasing plasma FFA concentrations, may promote FFA metabolism in skeletal muscle.

Footnotes

[a] Sweet Feed, Farmers Feed Mill, Inc, Lexington, KY
[b] Equi-Jewel Producers Rice Mill, Inc, Stuttgart, AR
[c] Re-Leve Farmers Feed Mill, Inc, Lexington, KY
[d] Stamm 30, Farmers Feed Mill, Inc, Lexington, KY
[e] Precision QID Abbott Laboratories, Abbott Park, IL
[f] Wako NEFA C test kit, Wako Diagnostics, Richmond, VA

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