Serum creatine kinase response to exercise during dexamethasone-induced insulin resistance in Quarter Horses with polysaccharide storage myopathy

Anna M. Firshman, BVSc, PhD; Stephanie J. Valberg, DVM, PhD; Tami L. Karges, BSc; Luke E. Benedict, MD; Erin J. Annandale, MS; Elizabeth R. Seaquist, MD

Objective—To determine effects of dexamethasone on insulin sensitivity, serum creatine kinase (CK) activity 4 hours after exercise, and muscle glycogen concentration in Quarter Horses with polysaccharide storage myopathy (PSSM).

Animals—4 adult Quarter Horses with PSSM.

Procedure—A 2 × 2 crossover design was used with dexamethasone (0.08 mg/kg) or saline (0.9% NaCl) solution administered IV every 48 hours. Horses were exercised on a treadmill daily for 3 wk/treatment with a 2-week washout period between treatments. Serum CK activity was measured daily 4 hours after exercise. At the end of each treatment period, serum cortisol concentrations were measured, a hyperinsulinemic euglycemic clamp (HEC) technique was performed, and muscle glycogen content was determined.

Results—Mean ± SEM serum cortisol concentration was significantly lower after 48 hours for the dexamethasone treatment (0.38 ± 0.08 mg/dL), compared with the saline treatment (4.15 ± 0.40 mg/dL). Dexamethasone significantly decreased the rate of glucose infusion necessary to maintain euglycemia during the HEC technique, compared with the saline treatment. Muscle glycogen concentrations and mean CK activity after exercise were not altered by dexamethasone treatment, compared with the saline treatment.

Conclusions and Clinical Relevance—Dexamethasone significantly reduced whole-body insulin-stimulated glucose uptake in Quarter Horses with PSSM after a 3-week period but did not diminish serum CK response to exercise or muscle glycogen concentrations in these 4 horses. Therefore, a decrease in glucose uptake for 3 weeks did not appear to alleviate exertional rhabdomyolysis in these horses. It is possible that long-term treatment may yield other results. (*Am J Vet Res* 2005;66:1718–1723)

Received October 21, 2004.
Accepted February 25, 2005.
From the Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN 55108 (Valberg, Karges, Annandale); and the Department of Medicine, College of Medicine, University of Minnesota, Minneapolis, MN 55108 (Benedict, Seaquist). Dr. Firshman's present address is Department of Clinical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331.
Supported by the University of Minnesota Equine Center with funds provided by the Minnesota Racing Commission, University of Minnesota Agricultural Experiment Station, and private donors.
Presented in part at the American College of Veterinary Internal Medicine Forum, Minneapolis, June 2004.
The authors thank Dr. Anthony Tobias for assistance with statistical analysis.
Address correspondence to Dr. Firshman.

Polysaccharide storage myopathy (PSSM) is a hereditary glycogen storage disorder[1,2] in Quarter Horses characterized by the accumulation of glycogen, glucose-6-phosphate, and abnormal polysaccharide inclusions in skeletal muscle.[1,3] Studies[3-5] in Quarter Horses with PSSM reveal that glycogen accumulation appears to be attributable to increased synthesis of glycogen, rather than a deficiency of glycolytic or glycogenolytic enzymes and decreased glycogen utilization. Enhanced glycogen synthesis in Quarter Horses with PSSM may be facilitated by increased insulin-stimulated glucose uptake into skeletal muscle. Faster excursion of glucose from the bloodstream in Quarter Horses with PSSM, compared with that for control horses, has been documented[5,6] during oral and IV glucose tolerance testing. Furthermore, results of testing by use of a **hyperinsulinemic euglycemic clamp (HEC)** technique, which is a more rigorous assessment of insulin sensitivity, confirm that glucose clearance in Quarter Horses with PSSM is remarkably enhanced during steady-state hyperinsulinemia.[7] The HEC technique provides supramaximal steady-state insulin concentrations during which the rate of glucose infusion required to maintain euglycemia serves as a measure of the insulin sensitivity of muscle and adipose tissues. Its advantage over oral and IV glucose tolerance testing is that it overrides an increase in endogenous insulin secretion and thereby prevents fluctuations in glucose homeostasis. It also can be used to study other disorders of glucose metabolism in horses, such as hyperlipemia and hyperadrenocorticism.[8]

Insulin stimulates uptake of glucose from the blood into skeletal muscle and adipose tissue via translocation of an intracellular pool of **glucose transporter protein (GLUT)**4 into the cell membrane.[9] Quarter Horses with PSSM have similar skeletal muscle GLUT4 and insulin receptor content, compared with values for control horses.[7] Alterations in the insulin-signaling cascade or regulation of GLUT4 translocation are possible explanations for enhanced cellular glucose uptake and glycogen synthesis in Quarter Horses with PSSM.

The primary clinical sign in most Quarter Horses with PSSM is exercise-associated rhabdomyolysis in adult horses as well as severe, potentially fatal, rhabdomyolysis not associated with exercise in young Quarter Horses.[1,10] A longitudinal study[2] of young Quarter Horses with PSSM documents that high serum **creatine kinase (CK)** activity with forced exercise and enhanced insulin sensitivity are already evident by 6 months of age. Young Quarter Horses with PSSM develop muscle stiffness and cramping prior

to the accumulation of abnormal polysaccharide concentrations within skeletal muscle.[2] The etiopathogenesis of rhabdomyolysis in PSSM-affected horses remains unknown. Furthermore, there is no clear explanation that would link enhanced glucose uptake and glycogen synthesis in skeletal muscle of PSSM-affected horses with the subsequent development of rhabdomyolysis during exercise. It is possible that enhanced glucose transport and accumulation of glucose-6-phosphate disrupt the regulation of energy metabolism within skeletal muscle.[7]

We proposed to determine whether there was a direct relationship between insulin sensitivity and rhabdomyolysis by inducing insulin resistance in Quarter Horses with PSSM and evaluating the subsequent effect on muscle necrosis during exercise. Dexamethasone can induce insulin resistance and impair glucose tolerance,[11] in addition to its potent anti-inflammatory and immunosuppressive properties.[12] A single dose of dexamethasone (0.03 mg/kg) can cause measurable impairment of glucose tolerance in human subjects,[13] and 5 days of administration of higher doses (2.9 mg/kg/d) can noticeably decrease GLUT4 translocation in rat skeletal muscle and redistribute glucose away from glycogen synthesis toward glycolysis.[11] We hypothesized that if rhabdomyolysis and enhanced glucose transport were linked, insulin-stimulated glucose uptake could be reduced and rhabdomyolysis minimized by administering dexamethasone to Quarter Horses with PSSM. The specific objectives of the study reported here were to determine whether dexamethasone decreases insulin sensitivity in Quarter Horses with PSSM, compared with results after administration of an inert control substance, and whether dexamethasone decreases serum CK activity at 4 hours after exercise, compared with values after exercise for a control treatment. The results could be immediately applicable in the treatment of Quarter Horses with PSSM that have rhabdomyolysis.

Materials and Methods

Animals—Five adult Quarter Horses were available for use in the study. A diagnosis of PSSM was established for each horse on the basis of a history of clinical signs of exertional rhabdomyolysis during treadmill exercise testing; increased CK activity 4 hours after exercise; and amylase-resistant, periodic acid-Schiff–positive inclusions found in repeated skeletal muscle biopsy specimens. One horse developed acute onset of abnormal neurologic signs during the second week of the experiments and was withdrawn from the study. The remaining 4 horses consisted of 1 gelding and 3 mares between 2 and 7 years of age (mean ± SEM, 4.0 ± 1.2 years). Throughout the study, horses were fed 10 kg of grass hay daily and a combination of 1.25 kg of sweet feed and 0.9 kg of rice bran daily. This diet can cause moderate increases in CK activity without inducing signs of painful muscle stiffness.[14] The horses were housed and cared for in an accredited facility, and the study was conducted in accordance with principles outlined by the University of Minnesota Institutional Animal Care and Use Committee.

Study design—Horses were trained by use of a treadmill for 3 weeks to ensure fitness. After the 3-week training period, horses were included in a study conducted as a 2 × 2 crossover design. Two horses were assigned to receive dexamethasone injections for 3 weeks, whereas the other 2 horses were assigned to receive injections of a control solution for 3 weeks. After the 3-week treatment period, horses were allowed a 2-week washout period. Then, treatments were reversed, and horses received the other treatment for 3 weeks. Daily exercise on a treadmill to maintain fitness was continued during the treatment periods as well as throughout the washout period.

The dexamethasone treatment consisted of administration of dexamethasone[a] (0.08 mg/kg, IV, q 48 h for 3 weeks). The control treatment consisted of administration of an equal volume of saline (0.9% NaCl) solution, IV, every 48 hours for 3 weeks. Thus, there were 11 injections/treatment. The dosage of dexamethasone was extrapolated from a study[15] in adult horses that revealed marked increases in blood glucose and insulin concentrations during and for 5 days after treatment with dexamethasone in oil (63 mg/d, IM, q 24 h for 4 days). We used a lower dosage to decrease the chance of horses developing laminitis when fed a grain diet.

At the end of each treatment period, testing was conducted by use of the HEC technique. Initiation of the injections was staggered for the 2 treatments because only 2 tests with the HEC technique could be performed during 1 day.

Serum cortisol concentrations[b] were also determined at the end of each treatment period on samples obtained in the morning 48 hours after the last injection. Cortisol concentrations were measured to ensure that the dose of dexamethasone was sufficient to inhibit endogenous cortisol secretion. In addition, to ensure that a sufficient amount of time was allowed for the washout period between treatments, serum cortisol concentrations were determined on all horses at the end of the washout period.

Testing by use of the HEC technique—Testing by use of the HEC technique[7] was performed at the end of each treatment period. Horses were allowed to rest for 1 day after the final day of each treatment period. Then, 14-gauge catheters were inserted in each jugular vein of each horse, and horses were not allowed food for 12 hours. Horses were kept in stocks and groomed by an attendant to maintain a calm state during testing conducted by use of the HEC technique.

Three baseline blood glucose concentrations were determined by use of a handheld glucose meter[c] on jugular venous blood samples obtained at 10-minute intervals from 1 of the catheters. To assure validity of the glucose meter, 99 plasma samples obtained during other modified insulin tolerance tests were assayed by use of an automated chemistry analyzer and compared with values obtained for whole-blood samples assayed by use of the glucose meter. A strong correlation (r^2, 0.91) between blood glucose concentrations measured by use of the handheld glucose meter and corresponding plasma concentrations indicated that the handheld glucose meter provided accurate measurements. Insulin[d] was infused by use of a pump[e] at a constant rate of 3 mU/kg/min into the second jugular catheter. Prior to infusion, insulin was mixed in 500 mL of saline solution and 2 mL of homologous blood. The infusion was maintained for 3 hours. Blood glucose concentrations were monitored at 5-minute intervals, and a 50% dextrose solution was infused via the same catheter that was used for infusion of insulin. The glucose solution was infused by use of a pump[e] at a rate that maintained blood glucose concentration at 100 ± 5 mg/dL.

The first 60 minutes of testing by use of the HEC technique was considered an equilibration period. To assess steady-state conditions for blood glucose, space correction was calculated at 10-minute intervals from 60 to 180 minutes for consecutive measurements of glucose concentration by use of the following equation[8]:

$$\text{Space correction} = (G1 - G2) \times 0.019,$$

where G1 is the first blood glucose concentration and G2 is the subsequent glucose concentration. The rate of glucose infusion during testing conducted by use of the HEC tech-

nique was calculated. The rate of glucose infusion during testing by use of the HEC technique corrected for changes in glucose space[8] was compared between the control and dexamethasone treatments within each horse.

Plasma samples were obtained at 20-minute intervals by centrifugation of blood samples immediately after collection. Plasma was harvested and stored at –80°C for subsequent measurement of insulin concentrations by use of a radioimmunoassay.[16,f] Insulin clearance during 60 to 180 minutes of testing by use of the HEC technique was also calculated on the basis of serum insulin concentration and insulin dose[17] and compared between control and dexamethasone treatment within each horse.

Muscle glycogen concentrations—Biopsy specimens were obtained from the gluteus medius muscle of each horse before each test conducted by use of the HEC technique. Specimens were obtained by use of a 6-mm-diameter modified Bergstrom biopsy needle at a standardized site. A 1-cm incision was made in the skin at a point 14 cm on a line extending from the top of the tuber coxae to the tail head. The biopsy needle was inserted through the incision to obtain a muscle specimen (200 mg) at a depth of 8 cm. Muscle samples were immersed in liquid nitrogen immediately after collection and stored at –80°C until processing. Approximately 10 to 20 mg of muscle tissue (wet weight) was boiled for 2 hours in 1M HCl, and glucose residues were analyzed fluorometrically to measure glycogen concentration in accordance with a method described elsewhere.[18]

Exercise testing and serum CK activity—Each horse exercised on a treadmill daily from Monday through Friday for 3 weeks during the treatment periods. An individualized exercise regimen was selected for each horse during the 3-week acclimation period (to attain fitness of each horse); this regimen was continued for both treatment periods and the intervening washout period. Duration of exercise was a maximum of 30 minutes; a shorter duration was used when horses developed muscle stiffness before they had completed 30 minutes of exercise during the training period. The exercise regimen consisted of walking (1.8 m/s) for 4 minutes, followed by alternating periods of trotting (3.3 m/s) for 2 minutes and walking (1.8 m/s) for 2 minutes. Horses A, B, C, and D completed a total of 30, 12, 24, and 24 minutes of exercise, respectively. Blood samples were obtained 4 hours after exercise for analysis of serum CK activity.

Statistical analyses—Values were expressed as mean ± SEM. Daily serum CK activity 4 hours after exercise for both treatments was also expressed as median values. A cortisol concentration < 1 µg/dL on the morning 48 hours after the last dexamethasone injection was assumed to indicate dexamethasone-induced suppression of endogenous cortisol secretion.

Statistical comparisons were performed on raw and logarithmically transformed values for CK activity. Results for daily serum CK activity and rate of glucose administration during 30-minute time periods of testing by use of the HEC technique as well as blood glucose and serum insulin concentrations during testing by use of the HEC technique were compared by use of an ANOVA with repeated measures and blocking for effects of each horse. A paired *t* test was used to compare the mean rate of glucose administration between 60 and 180 minutes of testing by use of the HEC technique that was corrected for changes in glucose space, mean insulin clearance during 60 to 180 minutes of testing by use of the HEC technique, and muscle glycogen concentrations after dexamethasone or control treatments. Statistical analyses were conducted by use of computer software programs.[g,h] For all analyses, values of $P \leq 0.05$ were considered significant.

Results

Cortisol concentration—Forty-eight hours after completion of the treatments, mean ± SEM serum cortisol concentrations were lower for the dexamethasone treatment (0.38 ± 0.08 µg/dL), compared with concentrations for the control treatment (4.15 ± 0.3 µg/dL). At the end of the 2-week washout period, serum cortisol concentrations for the 2 horses initially administered the dexamethasone treatment were 4.4 and 4.0 µg/dL, respectively, whereas the serum cortisol concentrations for the 2 horses initially administered the control treatment were 2.6 and 2.0 µg/dL, respectively.

Results for testing by use of the HEC technique—Mean ± SEM baseline blood glucose concentrations in

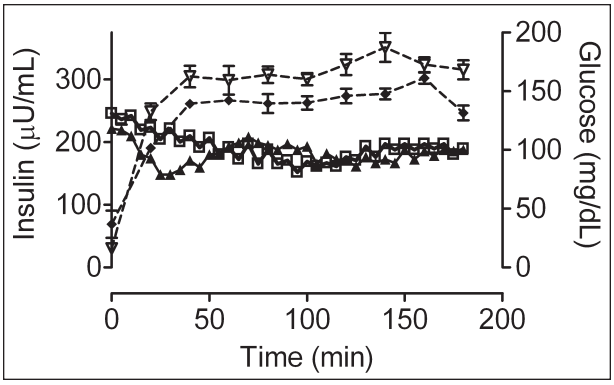

Figure 1—Mean ± SEM serum insulin concentrations (dotted lines) and whole-blood glucose concentrations (solid lines) during testing conducted by use of a hyperinsulinemic euglycemic clamp (HEC) technique for 4 Quarter Horses with polysaccharide storage myopathy (PSSM) after 3 weeks of treatment with dexamethasone (0.08 mg/kg, IV, q 48 h; open symbols) or an equivalent volume of saline (0.9% NaCl) solution (IV, q 48 h; solid symbols). Insulin infusion and onset of testing conducted by use of the HEC technique were designated as time 0. The first 60 minutes was considered an equilibration period. The insulin concentrations were higher but not significantly ($P = 0.07$) different when horses were treated with dexamethasone, compared with concentrations when horses received the control treatment.

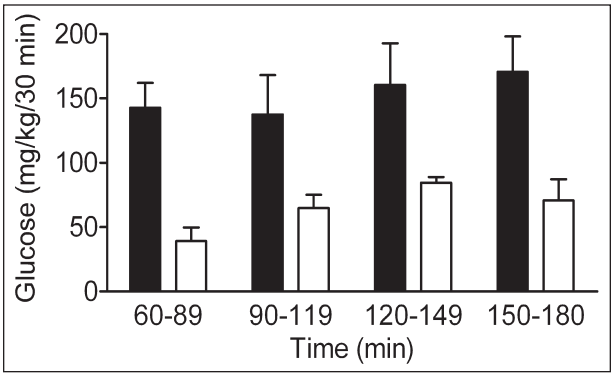

Figure 2—Mean ± SEM rate of glucose infusion required to maintain euglycemia during 30-minute time periods of testing conducted by use of the HEC technique in 4 Quarter Horses with PSSM after 3 weeks of treatment with dexamethasone (white bars) or an equivalent volume of saline solution (black bars). Insulin infusion and onset of testing conducted by use of the HEC technique were designated as time 0. The first 60 minutes was considered an equilibration period. Horses treated with dexamethasone required significantly ($P = 0.04$) lower rates of glucose infusion to maintain euglycemia, compared with glucose infusion rates when horses were treated by injection of saline solution.

Table 1—Mean ± SEM values for serum creatine kinase (CK) activity in serum samples obtained 4 hours after exercise, mean muscle glycogen concentration, and mean ± SEM corrected glucose infusion rate in 4 Quarter Horses with polysaccharide storage myopathy administered dexamethasone or an equivalent volume of saline (0.9% NaCl) solution (control), IV, at 48-hour intervals for 3 weeks.

	Serum CK activity (U/L)*		Glycogen concentration (mmol/kg of muscle)†		Glucose infusion rate (mg/kg/min)‡	
Horse	Dexamethasone	Control	Dexamethasone	Control	Dexamethasone	Control
1	667 ± 126 (228–1,910)	329 ± 37 (222–721)	148	193	2.7 ± 0.41	5.3 ± 0.7
2	744 ± 199 (216–2,690)	6,605 ± 3,100 (894–38,700)	228	208	2.6 ± 0.76	11.5 ± 2.8
3	2,933 ± 430 (504–7,380)	6,066 ± 2,767 (914–44,100)	188	165	3.2 ± 0.96	8.2 ± 2.1
4	11,199 ± 1,940 (2,810–24,060)	2,306 ± 444 (704–6,150)	141	168	3.4 ± 1.02	6.1 ± 1.4
All 4 horses	3,900 ± 743	3,920 ± 1,110	176 ± 20	184 ± 10	2.98 ± 0.40	7.69 ± 0.97

Values in parentheses represent the range.
*Reference range for serum CK activity is 79 to 556 U/L. †Weight of muscle tissue was on a wet-weight basis. ‡Represents mean corrected value determined during testing conducted by use of a hyperinsulinemic euglycemic clamp technique.

samples obtained from the horses after a 12-hour period of food withholding before onset of testing conducted by use of the HEC technique were higher but not significantly (P = 0.07) different for the horses when treated with dexamethasone (127.1 ± 5.1 mg/dL), compared with concentrations when the horses received the control treatment (115.5 ± 3.5 mg/dL). Mean ± SEM baseline serum insulin concentrations in samples obtained from the horses after a 12-hour period of food withholding before onset of testing conducted by use of the HEC technique were higher but not significantly (P = 0.11) different for the horses when treated with dexamethasone (43.8 ± 13.1 µU/mL), compared with concentrations when the horses received the control treatment (17.3 ± 11.9 µU/mL). Blood glucose concentrations during 60 to 180 minutes of testing conducted by use of the HEC technique were not significantly (P = 0.22) different between horses when treated with dexamethasone (96.3 ± 1.2 mg/dL) or the control injections (98.1 ± 1.0 mg/dL). Insulin concentrations obtained during the same time period were higher but not significantly (P = 0.07) different in horses when treated with dexamethasone, compared with concentrations when horses received the control treatment (**Figure 1**). Clearance of insulin was not significantly (P = 0.23) different between horses when treated with dexamethasone (0.01 ± 0.0007 mL/kg/min) and the control injections (0.01 ± 0.002 mL/kg/min). Rate of glucose infusion during testing by use of the HEC technique was significantly (P = 0.04) higher (1.9 to 3.6 times as high) when horses received the control treatment, compared with the rate of infusion when horses received the dexamethasone treatment (**Figure 2**). Mean rate of glucose administration between 60 and 180 minutes of testing by use of the HEC technique (corrected for changes in glucose space) was 2.98 ± 0.40 mg/kg/min for horses when treated with dexamethasone, which was significantly lower (P < 0.001) than the rate for horses when treated with the control injections (7.69 ± 0.99 mg/kg/min). Ratio of the mean glucose infusion rate to mean insulin concentration between 60 and 180 minutes of testing by use of the HEC technique was 0.85 and 2.30 for horses when administered dexamethasone and the control solution, respectively.

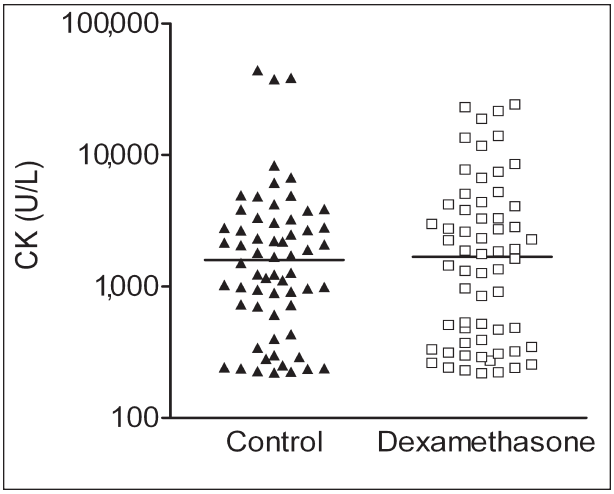

Figure 3—Daily serum creatine kinase (CK) activity 4 hours after exercise in 4 Quarter Horses with PSSM during 3 weeks of treatment with dexamethasone or an equivalent volume of saline solution (control). Each symbol represents results for 1 day. Median values for each group are indicated (horizontal bar). Reference range for serum CK activity is 79 to 556 U/L. The CK activity did not differ significantly (P = 0.99) between dexamethasone and control treatments.

Muscle glycogen concentrations—Resting muscle glycogen concentrations were not significantly altered after alternate-day injections of dexamethasone for 3 weeks (**Table 1**).

Serum CK activity—Mean CK activity and logarithm of CK activity in samples obtained 4 hours after exercise were not significantly (P = 0.995 and P = 0.96, respectively) different between the dexamethasone and control treatments (**Figure 3**; Table 1). There was no significant (P = 0.32) effect of day of treatment on serum CK activity.

We detected differences in CK activity among horses. Two horses had a decrease in CK activity after exercise when treated with dexamethasone, compared with CK activity after exercise when treated with the control injections, whereas the other 2 horses had an increase in CK activity after exercise when treated with dexamethasone, compared with CK activity after exercise when treated with the control injections (Table 1).

Discussion

To our knowledge, the study reported here is the first in which investigators used PSSM-affected horses and documented, by use of an HEC technique, the ability of dexamethasone to induce insulin resistance. In dexamethasone-treated horses, insulin and blood glucose concentrations were higher at rest. A 2-fold higher rate of glucose infusion was required to maintain euglycemia during testing by use of the HEC technique for control-treated horses, compared with the infusion rate for dexamethasone-treated horses. Dexamethasone induces substantial insulin resistance in other species by several mechanisms.[19] Glucocorticoids oppose the actions of insulin in the liver by promoting gluconeogenesis[19] and act peripherally in muscle and adipose tissue to inhibit glucose uptake[20] and decrease glycogen synthesis.[21] Corticosteroids reduce glucose uptake in rat skeletal muscle by decreasing insulin-stimulated phosphorylation of tyrosine kinase without affecting the number or affinity of insulin receptors for insulin.[22] In conjunction with impaired phosphorylation of insulin receptors,[22] dexamethasone causes a large reduction in insulin-stimulated translocation of GLUT4 in the soleus muscle fibers of rats.[11]

Quarter Horses with PSSM have enhanced insulin sensitivity, as documented by use of the HEC technique.[7] Although the precise mechanism involved in enhanced insulin sensitivity is unknown in horses with PSSM, it is clear from the study reported here that dexamethasone is capable of reducing the remarkably high insulin sensitivity characteristic of PSSM. The rate of glucose infusion during testing by use of the HEC technique for Quarter Horses with PSSM treated with dexamethasone (2.98 ± 0.40 mg/kg/min [corrected] and 2.7 ± 0.10 mg/kg/min [uncorrected]) in our study was similar to that required to maintain normoglycemia in healthy horses (2.03 ± 0.27 mg/kg/min [uncorrected]) evaluated by use of the same conditions in another study.[7] Quarter Horses with PSSM have similar GLUT4 content in skeletal muscle to that found in healthy horses.[7] Although not investigated in the study reported here, decreased GLUT4 translocation during testing by use of the HEC technique could potentially explain the reason that Quarter Horses with PSSM treated with dexamethasone required less glucose to maintain eu-glycemia, compared with the amount needed for the control treatment.[11]

During the 3-week period, dexamethasone treatment in Quarter Horses with PSSM may have been expected to decrease muscle glycogen synthesis through suppression of glucose uptake by muscles. However, a study[23] in muscles of rainbow trout revealed that the effect of dexamethasone on muscle glycogen is dependent on existing glycogen concentrations. When muscle glycogen is depleted, dexamethasone acts to increase synthesis, but when there are typical glycogen concentrations, dexamethasone may promote glycogenolysis.[23] To the authors' knowledge, there is no information on the effects of dexamethasone on muscle glycogen concentration in healthy horses. There was no pattern for a decrease in muscle glycogen concentrations in exercising Quarter Horses with PSSM when the horses were treated with dexamethasone. It is possible that a longer period of treatment with dexamethasone or inclusion of more horses in the study may have enabled us to detect a reduction in muscle glycogen concentration. However, a further explanation for the lack of effect of dexamethasone on muscle glycogen concentration may be that the underlying defect that drives glycogen synthesis in skeletal muscles of PSSM-affected horses is not solely dependent on enhanced glucose uptake to promote glycogen synthesis.

No beneficial effect of dexamethasone treatment on CK activity after exercise was found in Quarter Horses with PSSM. There was only a small number of horses evaluated in our study, so it is possible that a minor effect could have been missed. However, analysis revealed adequate power to detect a significant difference if it truly existed ($\alpha = 0.05$). In addition, mean serum CK activities were increased above the reference range (79 to 556 U/L) in all PSSM-affected horses receiving dexamethasone treatment (Figure 3). Thus, analysis of results of the study reported here indicates that dexamethasone is capable of reducing the remarkable insulin sensitivity in Quarter Horses with PSSM to that of clinically normal horses, as reported in another study.[7] However, dexamethasone is not capable of reducing serum CK activity. The results do not support a direct link between enhanced whole-body insulin-stimulated glucose uptake and the development of rhabdomyolysis in Quarter Horses with PSSM.

In horses with PSSM, exertional rhabdomyolysis can be alleviated by providing a low-starch, high-fat diet.[14,24,25] It has been speculated[14] that this type of diet may be beneficial because it decreases daily insulin concentrations, decreases glucose uptake of muscles, and increases plasma concentrations of free fatty acids for energy metabolism of muscles. Analysis of the results of the study reported here suggests that changes in energy metabolism associated with this type of diet may be of more importance in preventing rhabdomyolysis than just decreasing insulin-stimulated glucose uptake into skeletal muscle. The link between enhanced glucose uptake, energy metabolism of muscles, and rhabdomyolysis in horses with PSSM may lie in newly discovered energy regulatory pathways that affect glucose uptake as well as ATP synthesis from glycolytic, mitochondrial, and fat metabolism.[26] Furthermore, analysis of the results of our study indicated that there does not appear to be a beneficial effect of treating PSSM-affected Quarter Horses with a potent anti-inflammatory (ie, dexamethasone) before exercise.

a. Dexamethasone sodium phosphate injection, Burns Veterinary Supply Inc, Westbury, NY.
b. Immulite, cortisol chemiluminescent assay, Diagnostic Products Corp, Los Angeles, Calif.
c. Precision QID glucose monitoring system, Abbott Laboratories Inc, Bedford, Mass.
d. Novolin R, human insulin injection, recombinant DNA origin USP, Novo Nordisk Pharmaceutical Industries, Clayton, NC.
e. IVACP pump, Islington Voluntary Action Council, London, UK.
f. Coat-a-count insulin, Diagnostic Products Corp, Los Angeles, Calif.
g. GraphPad Prism, version 4.00, GraphPad Software Inc, San Diego, Calif.
h. NCSS 2004 statistical analysis system, NCSS, Kaysville, Utah.

References

1. Valberg SJ, Cardinet GH, Carlson GP, et al. Polysaccharide storage myopathy associated with recurrent exertional rhabdomyolysis in horses. *Neuromuscul Disord* 1992;2:351–359.

2. De La Corte FD, Valberg SJ, MacLeay JM, et al. Developmental onset of polysaccharide storage myopathy in 4 Quarter Horse foals. *J Vet Intern Med* 2002;16:581–587.

3. Valberg SJ, Mickelson JR, Gallant EM, et al. Exertional rhabdomyolysis in Quarter Horses and Thoroughbreds: one syndrome, multiple aetiologies. *Equine Vet J Suppl* 1999;30:533–538.

4. Valberg SJ, Townsend D, Mickelson JR. Skeletal muscle glycolytic capacity and phosphofructokinase regulation in horses with polysaccharide storage myopathy. *Am J Vet Res* 1998;59:782–785.

5. De La Corte FD, Valberg SJ, MacLeay JM, et al. Glucose uptake in horses with polysaccharide storage myopathy. *Am J Vet Res* 1999;60:458–462.

6. De La Corte FD, Valberg SJ, MacLeay JM, et al. The effect of feeding a fat supplement to horses with polysaccharide storage myopathy. *World Equine Vet Rev* 1999;4:12–18.

7. Annandale EJ, Valberg SJ, Mickelson JR, et al. Insulin sensitivity and skeletal muscle glucose transport in horses with equine polysaccharide storage myopathy. *Neuromuscul Disord* 2004;14:666–674.

8. Rijnen KE, van der Kolk JH. Determination of reference range values indicative of glucose metabolism and insulin resistance by use of glucose clamp techniques in horses and ponies. *Am J Vet Res* 2003;64:1260–1264.

9. Gould GW, Seatter MJ. Introduction to the facultative glucose transporter family. In: Gould GW, Seatter MJ, eds. *Facilitative glucose transporters*. New York: Chapman & Hall, 1997;1–37.

10. Byrne E, Cohen N, Jones SM, et al. Rhabdomyolysis in two foals with polysaccharide storage myopathy. *Compend Contin Educ Pract Vet* 2000;22:503–507.

11. Dimitriadis G, Leighton B, Parry-Billings M, et al. Effects of glucocorticoid excess on the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle. *Biochem J* 1997;321:707–712.

12. Toutain PL, Brandon RA, de Pomyers H, et al. Dexamethasone and prednisolone in the horse: pharmacokinetics and action on the adrenal gland. *Am J Vet Res* 1984;45:1750–1756.

13. Schneiter P, Tappy L. Kinetics of dexamethasone-induced alterations of glucose metabolism in healthy humans. *Am J Physiol* 1998;275:E806–E813.

14. Robeiro WP, Valberg SJ, Pagan JD, et al. The effect of varying dietary starch and fat content on serum creatine kinase activity and substrate availability in equine polysaccharide storage myopathy. *J Vet Intern Med* 2004;18:887–894.

15. Cartmill JA, Thompson DL, Gentry LR, et al. Effects of dexamethasone, glucose infusion, adrenocorticotropin, and propylthiouracil on plasma leptin concentrations in horses. *Domest Anim Endocrinol* 2003;24:1–14.

16. Reimers TJ, Cowan RG, McCann JP, et al. Validation of a rapid solid-phase radioimmunoassay for canine, bovine, and equine insulin. *Am J Vet Res* 1982;43:1274–1278.

17. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979;237:E214–E223.

18. Lowry OH, Passonneau JV. Typical fluorometric procedures for metabolite assays. In: Lowry OH, Passonneau JV, eds. *A flexible system for enzyme analysis*. New York: Academic Press Inc, 1972;68–92.

19. McMahon M, Gerich J, Rizza R. Effects of glucocorticoids on carbohydrate metabolism. *Diabetes Metab Rev* 1988;4:17–30.

20. Tappy L, Randin D, Vollenweider P, et al. Mechanisms of dexamethasone-induced insulin resistance in healthy humans. *J Clin Endocrinol Metab* 1994;79:1063–1069.

21. Coderre L, Srivastava AK, Chiasson JL. Effect of hypercorticism on regulation of skeletal muscle glycogen metabolism by insulin. *Am J Physiol* 1992;262:E427–E433.

22. Block NE, Buse MG. Effects of hypercortisolemia and diabetes on skeletal muscle insulin receptor function in vitro and in vivo. *Am J Physiol* 1989;256:E39–E48.

23. Frolow J, Milligan CL. Hormonal regulation of glycogen metabolism in the white muscle slices from rainbow trout (*Oncorhynchus mykiss* Walbaum). *Am J Physiol Regul Integr Comp Physiol* 2004;287:R1344–R1353.

24. Firshman AM, Valberg SJ, Bender JB, et al. Epidemiologic characteristics and management of polysaccharide storage myopathy in Quarter Horses. *Am J Vet Res* 2003;64:1319–1327.

25. Valentine BA, Van Saun RJ, Thompson KN, et al. Role of dietary carbohydrate and fat in horses with equine polysaccharide storage myopathy. *J Am Vet Med Assoc* 2001;219:1537–1544.

26. Hardie DG. Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. *Endocrinology* 2003;144:5179–5183.