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Developmental Onset of Polysaccharide Storage Myopathy in 4 Quarter Horse Foals

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Polysaccharide storage myopathy (PSSM) is characterized by the accumulation of glycogen and an abnormal polysaccharide in the skeletal muscle fibers of Quarter Horse–related breeds. Glycogen storage disorders have been identified in human beings and other animal species that are due to single gene mutations causing deficiencies in the enzyme activities of the glycogenolytic or glycolytic pathways.[1] Biochemical studies have demonstrated that horses with PSSM have functional glycogenolytic and glycolytic pathways.[2] More recently, muscle glycogen and abnormal polysaccharide accumulation in PSSM horses have been suggested to be due to a novel defect in glucose transport in skeletal muscle. Results of IV and oral glucose tolerance tests in adult PSSM horses indicated that affected individuals have enhanced glucose clearance from the bloodstream, which occurs at lower insulin concentrations than in controls.[3]

Clinical signs of PSSM include muscle stiffness, pain, and reluctance to exercise and are usually 1st noticed in adult horses at the commencement of training or when exercise resumes after a lay-up period.[4] Serum creatine kinase (CK) activity may be high at rest in PSSM horses and can increase up to 80,000 IU/L after a 15-minute submaximal exercise test.[5] Although pedigree analysis suggests that PSSM is probably transmitted as an autosomal recessive trait, there is little information about the expression of the disease from birth to the beginning of training at 2–3 years of age.[6] Recently, 2 foals with pneumonia developed severe rhabdomyolysis and were diagnosed with PSSM, suggesting that, under certain circumstances, this disorder may be expressed in young animals.[7] The purpose of this longitudinal study was to follow the offspring from PSSM mares bred to a closely related stallion to determine the age of onset of skeletal muscle glycogen and abnormal polysaccharide accumulation, enhanced glucose clearance, and exertional rhabdomyolysis that are indicative of PSSM. The effects of stall confinement and pasture turnout on serum CK responses to exercise were also evaluated at 2 years of age.

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Materials and Methods

Dams and Sire

Two Quarter Horse mares (Ro and Pa) were donated to the University of Minnesota (St Paul, MN) because of persistent signs of rhabdomyolysis from the beginning of training. A semitendinosus biopsy from Ro at 3 years of age showed abnormal polysaccharide accumulation in 20% of muscle fibers and a muscle glycogen concentration of 1,340 mmol/kg dry weight (normal, 464 ± 47 mmol/kg) (Fig 1). After 3 years on a pasture or a hay diet, muscle glycogen declined to 1,121 mmol/kg. An index of the area under the curve (AUC) for the insulin : glucose ratio after an IV glucose tolerance test (IVGTT) normalized to controls (controls = 100%) was 60% for Ro, indicating enhanced insulin sensitivity.[3] In 13 submaximal treadmill exercise tests of a 2-minute walk and a 2-minute trot, Ro could sustain exercise for only 6–18 minutes before muscle stiffness was evident, with 4-hour postexercise serum CK activity reaching a maximum of 6,020 IU/L (normal, 701 ± 421 IU/L).[8] A muscle biopsy of Pa at 3 years of age showed abnormal polysaccharide accumulation in 1–5% of muscle fibers and a muscle glycogen concentration of 1,384 mmol/kg. After 3 years on a pasture or a hay diet, muscle glycogen concentrations were 604 mmol/kg. The index for the AUC insulin : glucose ratio for Pa was 72%. Submaximal treadmill exercise could be sustained for 30 minutes, with intermittent signs of stiffness in 13 sessions, and the maximum 4-hour postexercise serum CK activity was 1,069 IU/L. A half-brother (Ma) to Pa was also donated to the University of Minnesota (Fig 2). Muscle biopsy showed small linear periodic acid-Schiff (PAS)–positive inclusions between myofibrils and a glycogen concentration of 1,010 mmol/kg (Fig 1). After 3 years on a hay diet, the muscle glycogen concentration was 430 mmol/kg. The stallion's index for the insulin : glucose ratio was 103%. The stallion was lame because of navicular disease; he showed only 1 abnormal increase in CK after submaximal exercise (maximum CK, 1,800 IU/L).

PSSM Foals

Ro and Pa were bred to the stallion MK to produce 2 fillies (identified as L and P) the 1st year of the study. A 3rd related filly (S) developed persistent increases in serum CK activity and muscle atrophy as a weanling (Fig 2). Upon identification of scattered granular PAS-positive inclusions in skeletal muscle biopsies, the foal was donated to the University of Minnesota at 7 months of age and was

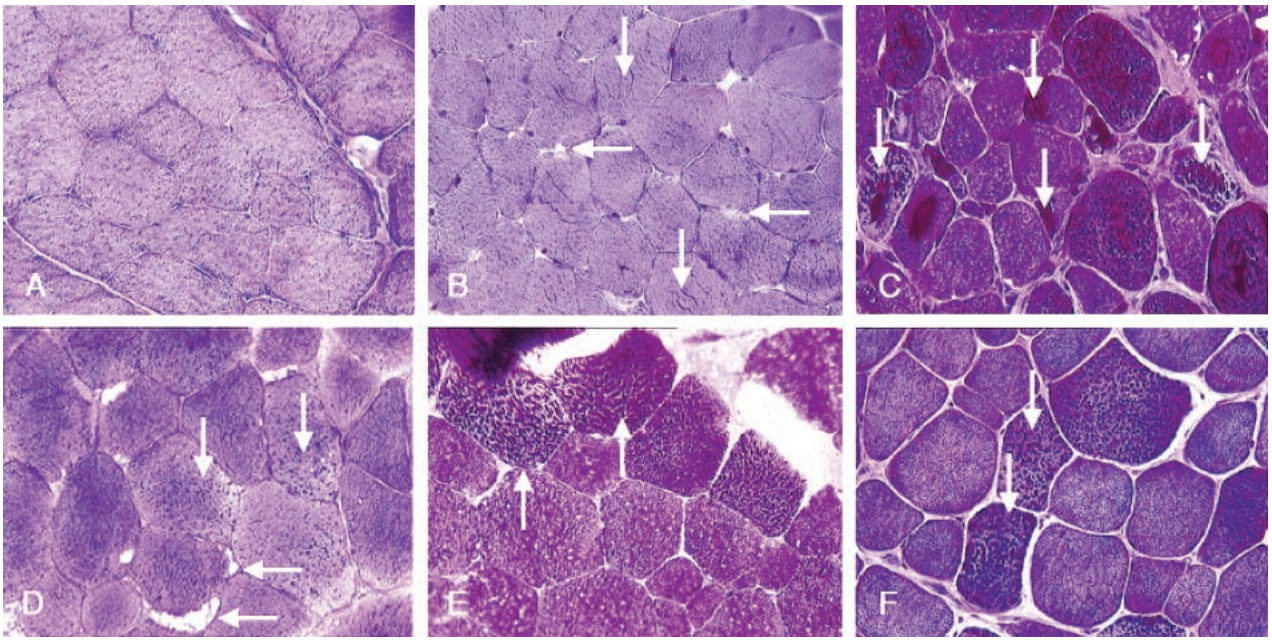

Fig 1. Periodic acid-Schiff (PAS) stains of a cross-section of skeletal muscle from a control animal **(A),** sire MK **(B),** dam Ro **(C),** and foal L at 15 months **(D),** 2 years **(E),** and 4 years **(F)** of age. Vertical arrows indicate aggregates of abnormal polysaccharide. Horizontal arrows indicate subsarcolemmal vacuoles.

included in the study the same year. Afterward, Ro was bred again to MK to produce a 4th colt (M). Foals were housed in box stalls with their dams and turned out for up to 6 hours a day on a grass pasture. They were fed grass hay with access to the 0.5 kg/d of rice bran fed to the dams. At 6 months of age, the foals were weaned, kept together in a pen, and fed grass hay. During the 2nd year, foals P, L, and S were kept on a grass pasture from June to September.

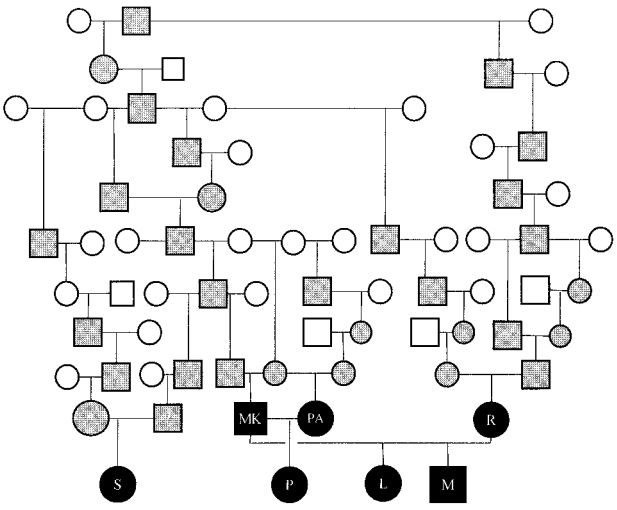

Fig 2. Relationship among the dams and sires of the 4 foals out of the polysaccharide storage myopathy (PSSM) mares used in this study. The dam and sire of foal S had an unknown phenotype and were not available for biopsy. Foal S was included on the basis of abnormal polysaccharide accumulation at 7 months of age and high serum creatine kinase (CK) activity. Note that all foals shared a common bloodline (shaded symbols).

Control Foals

Control foals born and raised at the University of Minnesota consisted of 4 Thoroughbreds (TBs) (3 colts and 1 filly) and 1 TB cross (TB-Paint filly). The dams and sires of these foals had normal muscle glycogen concentrations and no abnormal polysaccharide accumulation in their muscle biopsies. Control mares were fed sweet feed and grass hay, were turned out similarly to the PSSM mares and foals, and were weaned at 6 months of age. Weanlings were housed together in pens and fed grass hay.

Muscle Biopsies

Gluteus medius muscle biopsies were obtained at approximately 6-month intervals from 6 months to 2 years of age (Table 1). Not all foals were precisely the same age at the time of biopsy, and the actual age ranges for these categories were 6 months (range, 4–8 months), 12 months (range, 9–14 months), and 2 years (range, 19–26 months). PSSM foals were biopsied 1–3 times during each time category for the measurement of glycogen concentrations (Table 1). The gluteus medius muscle was sampled with a modified Bergstrom needle at the highest dorsal aspect along a straight line from the point of the tuber coxae to the head of the tail.[9] Initial biopsies of foals were obtained at a depth of 1 cm at 2 months of age, 2.5 cm at 6 months of age, 5 cm at yearling stage, and 7.5 cm at 2 years of age. The biopsy site was alternated from left to right. To avoid the possible effect of a previous biopsy on muscle architecture, muscle histology was reevaluated in the semimembranosus muscle at 4 years of age (foals L, P, and S) and 3 years of age (foal M).

Table 1. Schematic of the tests performed on PSSM (foals L, P, S, and M) and control foals at various ages.[a]

	Age (2 months)	Age (4–8 months)	Age (9–14 months)	Age (15–18 months)	Age (19–26 months)
Muscle glycogen	2 controls; foals, L, P	5 controls; foals L, Px2, Mx2	5 controls; foals Lx2, Px2, Sx2, M	0 controls; foals L, P, S, M	4 controls; foals L, Px2, Sx3, Mx2
IVGTT	ND	All foals	All foals	ND	All foals
Exercise test	ND	Controls	Controls; foal M	Controls; foals L, P, S	Controls; foal M

ND, not done; IVGTT, IV glucose tolerance test; PSSM, polysaccharide storage myopathy.
[a] x2 = twice during that time frame.

Muscle Histochemistry

Gluteal muscle biopsies were oriented in cross-section, frozen in isopentane suspended in liquid nitrogen, and stained with hematoxylin and eosin (H&E) and PAS. Amylase digestion, followed by PAS staining of muscle sections, was performed for 2-year-old horses. The biopsy sections were evaluated for the presence of subsarcolemmal vacuoles (+++, severe; ++, moderate; and +, mild or absent), the presence of abnormal polysaccharide inclusions, and the intensity of PAS staining (subjective assessment by 2 investigators using grades 1–4 to describe staining intensity). A Wilcoxon signed rank test was used to compare the staining intensity between PSSM and control foals. The presence of central nuclei, muscle cell necrosis, and macrophage cells was noted from H&E stains. A total of 24 biopsies from the 4 foals and 14 from the control foals were examined.

Muscle Biochemistry

Samples for glycogen analysis were immersed in liquid nitrogen immediately after collection and stored at −80°C until processing. The number of samples obtained at each age range for glycogen concentrations is shown in Table 1. Samples from 18-month-old control foals were inadvertently thawed and not analyzed. Glycogen was analyzed in samples that were freeze dried and dissected free from blood, fat, and connective tissue; these were then weighed (about 1 mg) and boiled for 2 hours in 1 mL of 1 M HCl. The measurement of total glucose was subsequently performed by fluorometry according to the method of Lowry and Passonneau.[10] Mean muscle glycogen concentrations for each individual were compared to controls by one-way analysis of variance, and changes over time were evaluated by analysis of variance with repeated measures, followed by Bonferroni's multiple comparison tests.

Intravenous Glucose Tolerance Tests

IVGTTs were performed with 0.5 g/kg of glucose administered intravenously over a 10-minute period in foals at 6–8 months of age, at yearling stage, and at 24 months of age (Table 1). Blood samples were collected via jugular catheter before and 2, 4, 8, 16, 30, 60, 90, 120, 150, and 180 minutes after glucose administration. Plasma was separated by centrifugation immediately after blood collection and stored at −80°C. Plasma glucose concentrations were measured by the glucose oxidase method.[11] Plasma insulin concentrations were measured by radioimmunoassay.[12]

Glucose and insulin assays were analyzed by calculating the AUC for plasma insulin concentrations versus time and insulin : glucose ratios versus time for each foal over the 180 minutes of the tolerance tests. Insulin index (%) and insulin : glucose index (%) were determined by dividing the individual foal's AUC by the mean of the age-matched controls' AUC and multiplying by 100. The indices for each foal were compared to those of the control foals by one-way analysis of variance. Analysis of variance with repeated measures was used to compare glucose, insulin, and insulin : glucose ratios over time during the glucose tolerance test performed at 6, 12, and 24 months of age.

CK Activity

Jugular venous blood samples for the measurement of serum CK activity by an automated chemistry analyzer were obtained from foals L and P in the afternoon after turnout in a ½-acre paddock for up to 60 weeks of age. At approximately 18 months of age, PSSM foals L, P, and S were acclimated to run on a high-speed treadmill. Foals were exercised at 2-minute intervals of walk and trot for 5 days, followed by 3 weeks of stall rest. Thereafter, the foals underwent 6 days of exercise, 3 weeks of rest, and 6 days of exercise. In the final trial, foal P missed 1 day because of lameness. Jugular venous blood was obtained 4 hours after exercise from all foals for the measurement of serum CK activity. The amount of time the foals could trot before developing firm, painful muscle cramping was determined from the 1st session, and this time was used as their subsequent exercise time. Three repeated treadmill exercise sessions were also performed for control foals between 6 months and 24 months of age and for foal M at 12 and 24 months of age (Table 1). Foals repeated a 2-minute walk, 2-minute trot for 20 minutes at 6 and 12 months of age, as well as for 30 minutes at 18 and 24 months. Between trials, foals were housed in the same paddock. Control foals showed no evidence of muscle stiffness. Serum CK activities of each foal after exercise were compared to those of the control foals by one-way analysis of variance. A significant difference for all statistic analyses was set at $P < .05$.

Foals L, P, and S were turned out on a 20-acre pasture for 4 months from June through September at 2 years of age. The foals performed a treadmill exercise test when

Table 2. The average intensity of periodic acid-Schiff (PAS) stains, the presence of PAS-positive inclusions, and unstained subsarcolemmal vacuoles in muscle from foals, L, P, S, and M and control foals at different ages.[a]

	PAS Stain Intensity		PAS-positive Inclusions		SS Vacuoles	
	(1–12 months)	(18–24 months)	(1–12 months)	(18–24 months)	(1–12 months)	(18–24 months)
Foal L	3	3	−	+	++	++
Foal P	3.3	3.7	−	+	++	++
Foal S	3	3	+	+	−	−
Foal M	2.7	3.3	−	−	+	+
Controls	2.3[b]	2.3[b]	−	−	−	−

PAS, periodic acid-Schiff; PSSM, polysaccharide storage myopathy; SS, subsarcolemmal.

[a] PAS staining: 4, very intense; 3, intense; 2, moderate; or 1, light staining. PAS inclusions: +, presence; or −, absence of abnormal polysaccharide. SS: +++, severe; ++, moderate; +, mild; or −, absent.

[b] Significantly less than for PSSM foals.

they were brought into the University Teaching Hospital. After 4 months of stall rest, the same exercise test was repeated.

Results

Muscle Histochemistry and Biochemistry

A few subsarcolemmal vacuoles were found in 1 biopsy sample of one of the control foals, but no abnormal polysaccharide was identified in any control foal biopsy sample. The average staining intensity of PAS stains for the control foals was less than that for the PSSM foals (Table 2). Mean muscle glycogen concentrations for control foals were 434 ± 23 mmol/kg, and there was no dramatic change over time.

In foal L, muscle necrosis was evident in 1 biopsy sample at 6 months of age, and a few central nuclei were present at 2 years of age. Subsarcolemmal vacuoles were a consistent feature of the muscle biopsies of foal L by 6 months of age (Table 2; Fig 1). Abnormal PAS-positive inclusions typical for PSSM were 1st noted at 15 months of age and were identified subsequently in all samples, including those in 20% of the fibers of the semimembranosus muscle at 4 years of age (Fig 1). The PAS staining intensity was graded slightly higher than in controls (Table 2). There was no marked difference in glycogen concentration over time, and foal L had a markedly greater mean muscle glycogen concentration of 648 ± 33 mmol/kg than did control foals (Fig 3).

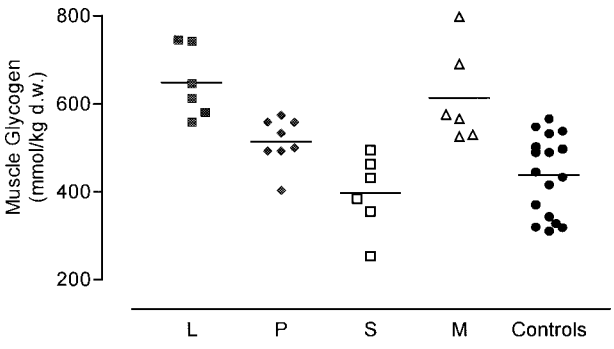

Fig 3. Muscle glycogen concentrations at rest obtained from 2 months to 24 months of age in the 4 polysaccharide storage myopathy (PSSM) foals and control foals.

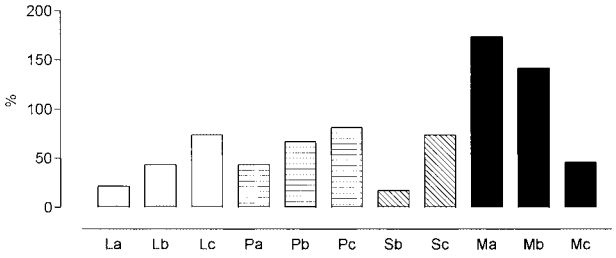

Fig 4. The insulin index is shown for 4 foals at 6 **(a),** 12 **(b),** and 24 **(c)** months of age. Insulin index was calculated as the area under the curve (AUC) from the IV glucose tolerance test (IVGTT) expressed as a percentage of the AUC of age-matched controls. Foal S was included in the study after 7 months of age.

In foal P, muscle necrosis was evident in 1 biopsy at 6 months of age, and a few central nuclei were present at 6 months and 2 years of age. Subsarcolemmal vacuoles were evident in muscle biopsies by 6 months of age. Abnormal PAS-positive inclusions were 1st noted at 18 months of age in gluteal biopsies, as well as in 1–2% of the fibers of the semimembranosus muscle at 4 years of age. The intensity of the PAS stain was graded slightly higher than in controls (Table 2). The mean muscle glycogen concentration of 514 ± 20 mmol/kg was not markedly greater than that of controls (Fig 3).

Necrosis was not evident in biopsy samples from foal S, but at 1 and 2 years of age, numerous centrally located nuclei were present. Subsarcolemmal vacuoles were not identified in any samples. Abnormal PAS-positive inclusions were 1st noted at 7 months of age in a semimembranosus biopsy sample (Table 2). Examination of subsequent biopsy samples of gluteal and semimembranosus muscles revealed no or only a few fibers with PAS-positive inclusions. The PAS stain was graded slightly more intense than in controls, and the mean muscle glycogen concentration (397 ± 35 mmol/kg) was not markedly different from that of controls (Fig 3).

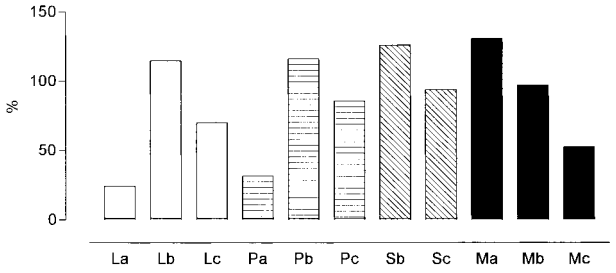

Fig 5. Index for the area under the curve (AUC) for the insulin: glucose ratios in foals at 6 **(a),** 12 **(b),** and 24 **(c)** months of age. Note that, at 12 months, foals L, P, and S showed an increase in the insulin: glucose ratio, indicating the development of an insulin-resistant state. Control foals did not show such a change with age.

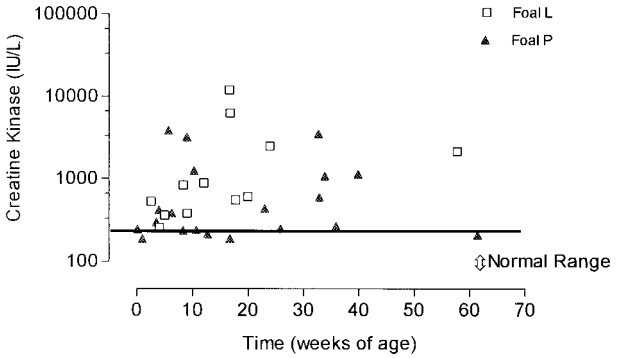

Fig 6. Serum creatine kinase (CK) activity in foals L and P after pasture turnout. CK activity was above the normal range after 1 month of age.

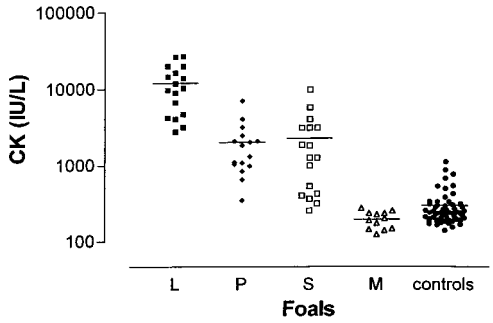

Fig 7. Mean creatine kinase (CK) activity postexercise in polysaccharide storage myopathy (PSSM) and control foals. Foals L, P, and S had markedly higher CK activities than control foals after treadmill exercise. Foal M did not show increases in CK activity at any time.

In foal M, subsarcolemmal vacuoles were present in all biopsy samples after 15 months of age. A few fibers with linear cytoplasmic PAS-positive inclusions were 1st noted at 3 years of age in the semimembranosus biopsy. The PAS stain had a slightly higher staining intensity than in controls (Table 2), and muscle glycogen concentrations were markedly higher (614 ± 44 mmol/kg) than those of control foals (Fig 3).

Intravenous Glucose Tolerance Tests

No dramatic effect of age was seen on the AUC for insulin concentration versus time or for insulin : glucose ratios versus time in the control group glucose tolerance tests.

In foal L, the plasma insulin indices at 6 months and 2 years of age were 21 and 73% of the age-matched controls, respectively (Fig 4). The index for insulin : glucose ratio was also very low for foal L at 6 months (24% of controls) and 2 years of age (85%) (Fig 5). At 1 year of age, the insulin : glucose index increased to 115% of controls, showing a transient insulin resistance.

The insulin index for foal P was 44 and 81% of age-matched controls, respectively, at 6 months and 2 years of age (Fig 4). The index for the insulin : glucose ratio was also low at 6 months (32%) and 2 years of age (70%) (Fig 5). As a yearling, the insulin : glucose index temporarily increased to 116% of controls.

The index for the insulin and insulin : glucose ratio for foal S was 74 and 90%, respectively, of controls at 2 years of age (Figs 4, 5). As a yearling, the insulin : glucose index was 126% of controls.

Foal M showed the opposite developmental pattern for the insulin and insulin : glucose indices (Figs 4, 5). These indices were highest at 6 months of age (174 and 130%, respectively) and declined to less than control values by 2 years of age (46 and 52%, respectively).

Serum CK Activity

Serum CK activity in foal L was increased above 500 U/L 9 of 12 times after turnout from birth to 15 months of age, with values up to 11,900 IU/L (Fig 6). No obvious clinical signs of muscle soreness were observed. Serum CK activity in foal P was increased above 500 IU/L 8 of 16 times after turnout from birth to 15 months of age (Fig 6).

Serum CK activities were in the normal range (57–404 U/L) for control foals at rest and after exercise. The mean duration of exercise was 25 ± 1.0 minutes.

At rest, before the exercise testing, serum CK activity in foal L was 2,420 IU/L. Foal L could exercise for 14 minutes during the 1st exercise session, and when this duration of exercise was repeated, she consistently developed muscle stiffness. Serum CK activity after exercise was markedly higher than in controls, with a maximum CK of 27,140 IU/L (Fig 7).

At rest, before the exercise tests, serum CK activity in foal P was 965 IU/L. Foal P could exercise for 28 minutes for the 1st exercise session, and when this duration of exercise was repeated, she intermittently developed muscle stiffness. Serum CK activity was markedly higher than in controls, with a maximum CK of 7,060 IU/L (Fig 7).

At rest, before exercise testing, serum CK activity in foal S was 280 IU/L. Foal S could exercise for 30 minutes and developed occasional mild muscle stiffness. Mean serum CK activity was markedly higher than in controls, with a maximum of 9,900 IU/L (Fig 7).

Serum CK activity in foal M was not high before or after the 30-minute exercise test (rest, 121 IU/L; maximum postexercise CK, 255 IU/L), and muscle stiffness was not observed (Fig 7).

Pasture Turnout and Serum CK

After 4 months of pasture turnout at 2 years of age, resting CK activity was within normal limits for foals L, P, and S (Table 3). With the 1st exercise test after pasture turnout,

Table 3. CK activity in 3 foals after 4 months of pasture turnout and after 4 months of strict stall rest; the foals performed 20–30 minutes of exercise at a walk and trot on a treadmill.

	Pasture Turnout		Confinement	
PSSM Foals	Before (IU/L)	After (IU/L)	Before (IU/L)	After (IU/L)
Foal L	339	1,282	325	9,450
Foal P	232	332	168	7,543
Foal S	179	167	124	134

CK, creatine kinase; PSSM, polysaccharide storage myopathy.

serum CK activity was normal in foals P and S and was less than 1,300 U/L in foal L, with no clinical signs of stiffness. After a 4-month period of strict stall rest, serum CK activity was higher after exercise in foals L and P, and clinical signs of muscle stiffness were observed in these foals after exercise (Table 3).

Discussion

Determining the pattern of inheritance in horses is confounded by the time and expense of producing large enough families to draw definitive conclusions. Only 4 foals were available to evaluate over a 4-year period in the present study, and these were each treated as individuals to best clinically define the course of PSSM during development. The results of the present study confirm that PSSM is a heritable disease, because breeding mares with PSSM to a closely related stallion produced foals with PSSM. Pedigree analyses in this study and in a previous study demonstrated that the sires and dams of affected horses could be traced back to a common relative (Fig 2). Consanguinity, combined with the fact that all foals in the present study were affected with PSSM, would support an autosomal basis for PSSM and suggests that some individuals are homozygous; however, the trial was too small to draw firm conclusions.

A diagnosis of PSSM in our laboratory is based on the presence of abnormal polysaccharide in skeletal muscle biopsies and has been associated with clinical signs of muscle stiffness after exertion as well as persistent increases in serum CK activity.[13] Fillies L, P, and S could be diagnosed with PSSM by 18 months of age and showed typical clinical signs of PSSM. The biopsy of colt M at 3 years of age resembled that of his sire, with smaller aggregates of polysaccharide in a few muscle fibers and high glycogen concentrations. Colt M did not show persistent serum CK increases or muscle stiffness after exercise. It is possible that the milder phenotype of MK and his son M was an effect of gender, a lack of penetration of the PSSM trait, or a heterozygous state. The inability to identify heterozygotes represents a major difficulty in studying the genetics of PSSM.

Muscle glycogen concentrations in the 2-year-old PSSM offspring in this study (mean, 503 ± 12 mmol/kg) were 2-fold lower than those of their dams and sire at 3 years of age (1,250 ± 120 mmol/kg) and were lower than in other clinical cases of PSSM.[5] PSSM and control foals were not fed grain because there was no means to feed the same amount to each foal within group housing. The lower glycogen concentrations in the foals in this study compared to other reports of PSSM may in part be because of differences in the starch content of their habitual diet. Muscle glycogen concentrations declined in the dams and sire over time after their arrival at the University when their diet was changed by removing grain. Muscle polysaccharide accumulation could well have been higher in PSSM foals if they had been fed a higher starch diet from 6 months to 2 years of age.

Polysaccharide accumulation with PSSM has been associated with enhanced clearance of glucose from the bloodstream, likely into skeletal muscle.[3] One method of comparing glucose tolerance tests across different diets or among a number of individuals is to calculate the AUC for insulin, glucose, or insulin : glucose ratios versus time during the test.[14] The AUC can then be divided by the same value for a control diet or group to provide a comparative index. The indices used in the present study indicated that enhanced insulin sensitivity was evident in PSSM foals at 6 months and 2 years of age. During the IVGTT, the same rate or a faster rate of glucose clearance was achieved, with almost 50% less insulin secretion than in controls. An interesting and transient insulin resistance was noted in the 3 PSSM fillies at 1 year of age. Children experience a transient insulin-resistant state at the beginning of puberty that returns to previous concentrations by the end of puberty.[15] Girls show more insulin resistance than boys.[16,17] Because puberty in fillies is thought to occur between 12 and 24 months of age, this may explain our findings.[18] Previous studies of glucose tolerance in male foals suggested that normal young foals are more insulin resistant than older foals and adults.[19,20] In our study, this was not found in the PSSM fillies or the mixed-gender group of controls, but it was observed in colt M.

Enhanced glucose uptake into skeletal muscle could be a stimulus for enhanced muscle glycogen synthesis and formation of abnormal polysaccharide.[1,21–23] Abnormal polysaccharide accumulation is a rare event in glycogenoses and is usually the result of a less branched glycogen molecule.[1,21,22] We speculated that, over time, the higher insulin sensitivity and glucose transport in PSSM muscle leads to higher muscle glucose-6-phosphate concentrations. With daily exercise and a low-starch diet, muscle glycogen may not accumulate excessively. However, if glucose-6-phosphate is chronically high in skeletal muscle, the synthesis of glycogen with a preponderance of straight chains may be predominant, because glucose-6-phosphate is a potent stimulator of glycogen synthase but not branching enzyme activity.[23] The low-starch diet of PSSM foals may have lowered glycogen concentrations, but it did not completely prevent the accumulation of abnormal polysaccharide. Mild abnormal polysaccharide accumulation has also been reported in 3- and 6-month-old foals with severe rhabdomyolysis.[7]

The earliest evidence of PSSM in the foals of the present study was the development of rhabdomyolysis. By 1 month of age, serum CK activity was above the normal range after pasture turnout (Fig 6). Clinical signs of muscle stiffness, however, were not apparent until the foals were forced to exercise on the treadmill. Thus, one reason why PSSM may not be noted until 2–3 years of age is that foals may regulate the amount of exercise that is comfortable and, unless forced to exercise, can thereby prevent muscle stiffness. Concurrent disease may also induce rhabdomyolysis in sedentary foals with PSSM.[7] Foal S was donated to the University after developing pneumonia as a weanling, with persistent increases in CK activity. In addition, foals L and P developed strangles as yearlings and had high serum CK at that time (Valberg, unpublished data). The cause of rhabdomyolysis with PSSM is unknown. It could be because of a metabolic imbalance created by the enhanced insulin sensitivity and accumulation of glucose-6-phosphate in PSSM skeletal muscle. Accumulation of glucose-6-phosphate in equine muscle decreases binding of hexokinase to mito-

chondrial membranes and slows the generation of adenosine triphosphate via oxidative phosphorylation.[24] This type of metabolic derangement can induce muscle necrosis in animal models.[25]

The extent of rhabdomyolysis and muscle stiffness developed by PSSM horses also seems to be influenced by the amount of daily turnout. After 4 months of free exercise on a 20-acre pasture, foals L, P, and S had normal resting serum CK activity and were able to perform 30 minutes of treadmill exercise without clinical manifestation of muscle stiffness. In contrast, after 4 months of strict stall rest, foals L and P developed muscle stiffness and rhabdomyolysis (Table 3). Free access to a pasture with continuous grazing may alleviate postprandial peaks in glucose and insulin as well as enhance the metabolism of glucose in skeletal muscle through the exercise and increased oxidative metabolism resulting from a training effect.[26]

In conclusion, PSSM is a heritable disorder of skeletal muscle characterized by enhanced insulin sensitivity and rhabdomyolysis as early as 6 months of age. The eventual accumulation of abnormal polysaccharide in skeletal muscle appears to be a later developing secondary characteristic of PSSM. Rhabdomyolysis and polysaccharide accumulation appear to be influenced by diet, amount of daily exercise, other disease states, and possibly gender. Although pathognomonic for PSSM, abnormal polysaccharide accumulation may take up to 3 years to become obvious in muscle biopsy samples. An accurate diagnosis of PSSM in young foals with rhabdomyolysis may require repetitions of the muscle biopsy at a later age or the identification of abnormal polysaccharide in a muscle biopsy of the dam.

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