Effects of submaximal exercise on adenine nucleotide concentrations in skeletal muscle fibers of horses with polysaccharide storage myopathy

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Objective—To determine whether disruption of adenine triphosphate (ATP) regeneration and subsequent adenine nucleotide degradation are potential mechanisms for rhabdomyolysis in horses with polysaccharide storage myopathy (PSSM) performing submaximal exercise.

Animals—7 horses with PSSM and 4 control horses.

Procedures—Horses with PSSM performed 2-minute intervals of a walk and trot exercise on a treadmill until muscle cramping developed. Control horses exercised similarly for 20 minutes. Serum creatine kinase (CK) activity was measured 4 hours after exercise. Citrate synthase (CS), 3-OH-acylCoA dehydrogenase, and lactate dehydrogenase activities prior to exercise and glucose-6-phosphate (G-6-P) and lactate concentrations before and after exercise were measured in gluteal muscle specimens. Adenine triphosphate, diphosphate (ADP), monophosphate (AMP), and inosine monophosphate (IMP) concentrations were measured before and after exercise in whole muscle, single muscle fibers, and pooled single muscle fibers.

Results—Serum CK activity ranged from 255 to 22,265 U/L in horses with PSSM and 133 to 278 U/L in control horses. Muscle CS activity was lower in horses with PSSM, compared with control horses. Muscle G-6-P, lactate, ATP, ADP, and AMP concentrations in whole muscle did not change with exercise in any horses. Concentration of IMP increased with exercise in whole muscle, pooled muscle fibers, and single muscle fibers in horses with PSSM. Large variations in ATP and IMP concentrations were observed within single muscle fibers.

Conclusions and Clinical Relevance—Increased IMP concentration without depletion of ATP in individual muscle fibers of horses with PSSM during submaximal exercise indicates an energy imbalance that may contribute to the development of exercise intolerance and rhabdomyolysis. (*Am J Vet Res* 2005;66:839–845)

Exertional rhabdomyolysis (ER) has been a common cause of disability in horses for more than 100 years. In one of the first papers to discuss the etiology of ER, it was suggested that muscle necrosis with exercise was the result of lactate accumulation in skeletal muscle, triggered by increased glycogen storage during a few days of rest.[1] Results of a subsequent study[2] indicate that a particular form of ER, **polysaccharide storage myopathy (PSSM)**, is distinguished by the accumulation of high muscle glycogen concentrations and the storage of an abnormal polysaccharide in skeletal muscle. Horses with PSSM have approximately 1.5- to 2-fold higher concentrations of glycogen in their muscles, compared with healthy control horses.[3] However, lactic acid does not appear to cause ER in horses with PSSM because these horses develop high serum **creatine kinase (CK)** activity during submaximal aerobic exercise with low plasma lactate concentrations.[3] Although skeletal muscle energy metabolism has been studied during near-maximal exercise,[3] little information is available about skeletal muscle energy metabolism during submaximal exercise in horses with PSSM at the time they develop muscle necrosis.[4]

Glycogen storage disorders are important causes of rhabdomyolysis in human patients.[5] Various causes for rhabdomyolysis have been postulated in these metabolic myopathies. In lysosomal disorders, such as type II glycogen storage disease (ie, Pompe's disease), accumulation of glycogen-filled inclusions is believed to interrupt normal myofibril positioning and impede force transmission in contracting muscle.[6] Exertional rhabdomyolysis in horses with PSSM, however, precedes the accumulation of large amounts of polysaccharide, making it unlikely that storage products are the primary cause of rhabdomyolysis.[7] In glycogenoses such as myophosphorylase and phosphofructokinase enzyme deficiencies, the inability of the muscle to use glycogen or glucose greatly impairs oxidative metabolism[8,9]; however, horses with PSSM do not have any deficiencies in glycogenolytic or glycolytic enzymes.[10] Polysaccharide storage myopathy in horses is characterized by increased uptake of glucose within skeletal muscle and variably high muscle **glucose-6-phosphate (G-6-P)** concentrations.[3,11] It has been suggested that an increase in G-6-P concentrations in equine muscle may impair oxidative phosphorylation by decreasing the binding of hexokinase to mitochondrial membranes and thus diminishing the supply of mitochondrial **adenine diphosphate (ADP)** for rephosphorylation to **adenine triphosphate (ATP)**.[7,12]

Muscle energy metabolism is tightly regulated by the cytosolic ATP:ADP ratio. A decrease in ATP and an increase in ADP stimulate oxidative metabolism and

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also, when ATP cannot be adequately restored by oxidative metabolism, the myokinase reaction.[13] The myokinase reaction produces ATP and **adenine monophosphate (AMP)** by combining 2 ADPs. Accumulation of AMP activates AMP deaminase, which has particularly high activity in equine muscle,[14] and leads to an increase in muscle **inosine monophosphate (IMP)** concentrations.[13-15] Activation of the myokinase reaction and AMP deaminase with the resultant increase in IMP concentrations normally occurs only during intense exercise or under conditions of considerable metabolic stress.[13,15] It is challenging to elucidate the metabolic disturbances causing ER because it is difficult for affected patients to sustain painful exercise. Furthermore, metabolites are commonly measured in entire muscle specimens, whereas necrosis occurs only in a small fraction of fibers in the sample.[3] Thus, for many metabolic disorders, the precise mechanism linking fatigue and rhabdomyolysis is still not defined.

The aim of the present study was to investigate whether horses with PSSM have a metabolic disorder that can sporadically disrupt cellular energy metabolism during submaximal exercise. The hypothesis was that energy disruption is triggered in individual muscle fibers, leading to ATP degradation and IMP accumulation as a result of an insufficient energy release. To maximize the ability to detect adenine nucleotide degradation in a small number of fibers within a muscle specimen, ATP, ADP, AMP, and IMP concentrations were determined in whole muscle, small pools of muscle fibers, and portions of single muscle fibers that differed in oxidative capacity.

Materials and Methods

Animals—The study included 5 female and 2 castrated male Quarter Horses with PSSM (mean age, 6 years; range, 3 to 11 years) and 4 female control Quarter Horses or crossbreeds (mean age, 9 years; range, 10 to 16 years) that had normal muscle on histologic evaluation and no previously documented episodes of ER or high serum CK activity. Diagnosis of PSSM was based on previously documented episodes of ER, increases in serum CK activity after exercise, and histologic evidence of amylase-resistance abnormal polysaccharide in periodic acid-Schiff staining of muscle tissue. Muscle glycogen concentrations in horses with PSSM and in control horses were previously determined to be 780 ± 97 mmol/kg and 514 ± 155 mmol/kg of dry weight, respectively. All horses were untrained at the initiation of the study.

Exercise protocol—The exercise protocol was designed to be a light submaximal regime that would not be expected to cause adenine nucleotide depletion in healthy horses. The test consisted of repeating 2-minute intervals at a walk (1.9 m/s) and a trot (3 to 4 m/s). The test lasted for a maximum of 20 minutes in control horses. In horses with PSSM, the test lasted until horses had evidence of a tucked-up abdomen and stiffness or shifting lameness, muscle fasciculations, or both. It was anticipated, on the basis of previous studies with these horses, that exercise would last for 20 minutes or less. Blood samples were acquired by jugular venipuncture before exercise and 4 hours after exercise for serum CK activity determination. The duration of exercise for each horse was recorded.

Muscle biopsy specimens—Gluteal muscle biopsy specimens were obtained at rest and immediately following the exercise test through the same incision by use of a percutaneous needle biopsy technique at a standardized site along the gluteal medius muscle.[16] Biopsy specimens were immediately frozen in liquid nitrogen and stored at –80°C.

Frozen muscle specimens were freeze-dried, dissected free of blood and connective tissue, and separated into portions. One portion of muscle biopsy specimens obtained at rest (mean, 1.40 ± 0.05 mg) was taken for analyses of **citrate synthase (CS)**, **3-OH-acylCoA dehydrogenase (HAD)**, and lactate dehydrogenase activities as described by Essén-Gustavsson et al.[17] Other portions of muscle biopsy specimens obtained at rest and following exercise were weighed (mean, 1.54 ± 0.01 mg), homogenized by crushing with a glass rod in 1.5M perchloric acid, and left on ice for 15 minutes. Samples were then centrifuged at 1,200 × *g*, and the supernatant was neutralized with an equal volume of 2M $KHCO_3$. Concentrations of G-6-P and lactate were then measured fluorometrically.[18]

From the remaining portion of the muscle, approximately 80 to 200 fragments of single fibers were dissected from muscle biopsy specimens obtained before and after exercise. One horse with PSSM had visible lipid deposits within the muscle that prevented single muscle fiber dissection. The tip of each fiber fragment was removed and stained for oxidative capacity by use of **nicotinamide adenine dinucleotide (NADH)** staining.[19] Fibers were then subjectively classified with the darkest staining fibers grouped as dark (high oxidative capacity), intermediate staining fibers as medium (moderate oxidative capacity), and lightest staining fibers as light (low oxidative capacity). Because of the short length of many fibers (1 to 2 mm in length), fibers of similar staining intensity were grouped into pools consisting of 3 to 20 fragments and weighed on a microbalance[a] (range, 9 to 58 µg; mean, 28.2 µg). Individual fiber analysis could be performed on selected longer fibers from muscle specimens obtained after exercise from 2 control horses and 5 horses with PSSM; these fibers were weighed on a quartz fiber balance[18] (range, 1.0 to 8.1 µg; mean, 2.63 µg). Pools of single muscle fibers and individual fibers were extracted with 1.5M perchloric acid and neutralized with an equal volume of 2M $KHCO_3$. As a result of the small muscle fiber size, only ATP, ADP, AMP, and IMP could be analyzed on the fiber fragments.

Concentrations of ATP, ADP, AMP, and IMP in muscle specimens as well as in external standards were analyzed with **high-performance liquid chromatography (HPLC)** by use of a reverse-phase column.[20,b] Separation of nucleotides was achieved with a flow rate of 1.0 mL/min, UV detection at 254 nm, and an oven temperature of 40°C.

Statistical analysis—Activity of CK was log transformed to normalize the data. A Student *t* test was used to determine whether significant differences existed in log CK activity, nucleotide concentrations, lactate concentration, G-6-P concentration, and enzyme activities in muscle specimens obtained before and after exercise between PSSM and control horses. A 1-way ANOVA was used to determine whether significant differences existed in nucleotide concentrations among the dark, medium, and light fiber types. Linear regression analysis was performed on IMP concentrations in single muscle fibers versus whole-muscle lactate concentrations. Values are reported as mean ± SEM. Values of $P < 0.05$ were considered significant.

Results

Submaximal exercise test—The mean duration of exercise was 19 ± 7 minutes for horses with PSSM (range, 11 to 34 minutes), and 4 hours after exercise, serum CK activity ranged from 255 to 22,265 U/L with a mean of 4,847 ± 3,010 U/L (**Figure 1**).

The mean duration of exercise was 19 ± 1 minutes for control horses, with serum CK activities averaging 205 ± 31 U/L (range, 133 to 278 U/L). The exercise test was terminated after 16 minutes for one of the control horses because of an inability to maintain pace at a trot. Log-transformed serum CK activity was significantly higher after exercise in horses with PSSM (mean, 3.2 ± 0.28 U/L), compared with control horses (mean, 2.3 ± 0.1 U/L). Serum CK activity was inversely related to the duration of exercise in horses with PSSM.

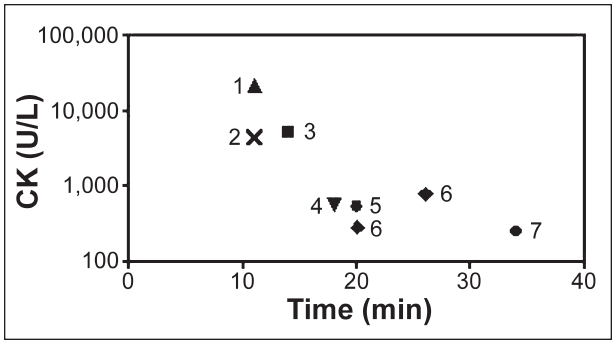

Figure 1—Serum creatine kinase activity (CK) at 4 hours after exercise versus the duration of submaximal exercise in 7 horses with polysaccharide storage myopathy (PSSM). In horse No. 6 (diamonds), serum CK activity was only mildly increased after performing an initial 20-minute exercise session; several months later, this horse performed a second test for 26 minutes and developed moderate stiffness. In horse No. 7 (circle), signs of stiffness did not develop; therefore, the exercise session was terminated at 34 minutes. Mean ± SEM serum CK activity at 4 hours after exercise in 4 control horses was 206 ± 32 U/L (data not graphed).

Table 1—Mean ± SEM activities of citrate synthase (CS), 3-hydroxy-acylCoA dehydrogenase (HAD), and lactate dehydrogenase (LDH) and concentrations of glucose-6-phosphate (G-6-P) and lactate in muscle biopsy specimens obtained before and after exercise in horses with polysaccharide storage myopathy (PSSM) and healthy control horses.

	Horses with PSSM		Control horses	
Variables	**Before**	**After**	**Before**	**After**
CS (mmol/kg/min)	6.5 ± 0.7*	ND	10.5 ± 1.5	ND
HAD (mmol/kg/min)	13.4 ± 0.9	ND	16.3 ± 1.2	ND
LDH (mmol/kg/min)	1865 ± 95	ND	1991 ± 140	ND
G-6-P (mmol/kg)	3.5 ± 0.6	4.8 ± 1.2	4.3 ± 1.1	2.6 ± 0.8
Lactate (mmol/kg)	19.8 ± 4.3	23.5 ± 3.7	14.5 ± 1.8	15.2 ± 1.4

*Significantly ($P < 0.05$) different from control horses.
ND = Not determined.

Whole-muscle analysis—Whole-muscle CS activity was significantly lower in horses with PSSM, compared with control horses. No significant differences were found in whole-muscle HAD and lactate dehydrogenase activities and G-6-P and lactate concentrations before exercise between control horses and horses with PSSM (**Table 1**). No significant differences were found in whole-muscle G-6-P and lactate concentrations after exercise between control horses and horses with PSSM. However, after exercise, a slightly higher whole-muscle lactate concentration was observed in horses with PSSM, compared with control horses, but this difference was not significant ($P = 0.10$).

The concentrations of ATP, ADP, AMP, and IMP in whole muscle were similar in PSSM and control horses at rest (**Table 2**). Exercise resulted in minimal changes in the whole-muscle concentrations of ATP, ADP, and AMP in control horses and horses with PSSM. Compared with before exercise, horses with PSSM had a significant increase in whole-muscle IMP concentration after exercise, which was not apparent in control horses. Whole-muscle IMP concentration was significantly greater in horses with PSSM after exercise, compared with control horses. A modest but significant ($r^2 = 0.40$, $P = 0.05$) correlation was found between whole-muscle lactate and IMP concentrations in all horses after exercise.

Pooled muscle fiber analysis—No detectable difference was found in concentrations of ATP, ADP, AMP, or IMP among pooled muscle fibers with high, medium, and low oxidative capacity, as determined by NADH staining. As a result, data from the different fiber pools were combined for further analysis. No significant differences were found in pooled muscle fiber concentrations of any adenine nucleotides between horses with PSSM and control horses before exercise. Horses with PSSM had a significantly lower pooled muscle fiber concentration of AMP after exercise, compared with control horses (a difference that was not detected by whole-muscle analysis). Pooled muscle fiber concentration of IMP was slightly higher than that of whole-muscle concentrations (Tables 2 and 3). Horses with PSSM had a significant increase in pooled muscle fiber concentration of IMP after exercise, compared with before exercise, and pooled muscle fiber concentration of IMP in horses with PSSM after exercise was significantly higher than in control horses.

Single muscle fiber analysis—No differences were found in concentrations of ATP, ADP, AMP, and IMP

Table 2—Mean ± SEM whole-muscle purine nucleotide concentrations in horses with PSSM and healthy control horses before and after submaximal exercise.

	Whole-muscle purine nucleotide concentrations (mmol/kg of dry weight)							
	ATP		ADP		AMP		IMP	
Muscle specimen	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
PSSM (n = 6)	23.77 ± 0.49	22.13 ± 0.52	5.35 ± 0.17	5.19 ± 0.22	0.35 ± 0.02	0.34 ± 0.02	0.01 ± 0.01	0.36 ± 0.05*†
Control (4)	23.71 ± 0.53	22.08 ± 1.84	5.03 ± 0.09	4.74 ± 0.40	0.31 ± 0.02	0.31 ± 0.02	0.11 ± 0.06	0.09 ± 0.05
P value	0.932	0.976	0.199	0.310	0.199	0.486	0.106	0.004

*Significantly ($P < 0.05$) different from values of control horses after exercise. †Significantly ($P < 0.05$) different from values before exercise. ATP = Adenine triphosphate. ADP = Adenine diphosphate. AMP = Adenine monophosphate. IMP = Inosine monophosphate. n = Number of whole-muscle specimens used in analyses.

Table 3—Mean ± SEM pooled single muscle fiber purine nucleotide concentrations in horses with PSSM and healthy control horses before and after submaximal exercise.

	Pooled muscle fiber purine nucleotide concentrations (mmol/kg of dry weight)							
	ATP		ADP		AMP		IMP	
Muscle fiber pools	Before	After	Before	After	Before	After	Before	After
PSSM	20.32 ± 0.65	21.47 ± 0.85	4.25 ± 0.25	3.81 ± 0.21	0.52 ± 0.09	0.36 ± 0.04*	0.19 ± 0.07	0.45 ± 0.08*†
No. of pools	29	33	29	33	29	33	29	33
Control	20.22 ± 1.03	21.00 ± 0.65	4.33 ± 0.36	4.49 ± 0.31	0.50 ± 0.06	0.74 ± 0.08	0.24 ± 0.13	0.14 ± 0.09
No. of pools	14	23	14	23	14	23	14	23
P value	0.928	0.690	0.865	0.069	0.893	> 0.0001	0.732	0.014

See Table 2 for key.

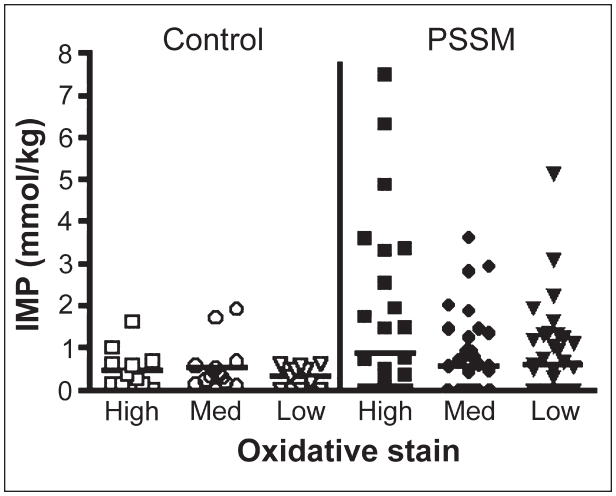

Figure 2—Concentration (bar indicates mean value) of inosine monophosphate (IMP) in single muscle fibers with high, medium (med), and low oxidative capacities of horses with PSSM and control horses after exercise.

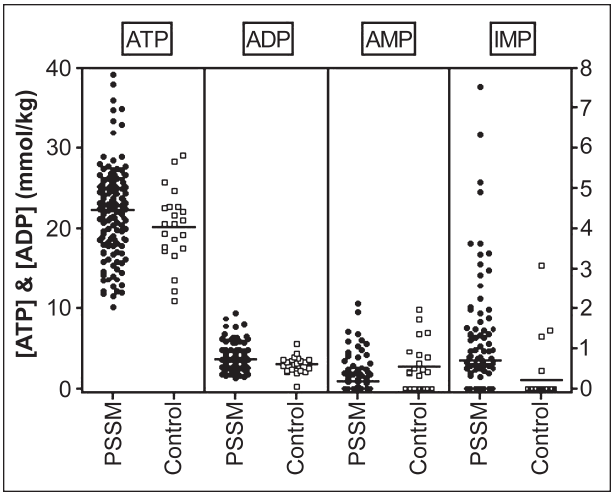

Figure 3—Concentrations (bar indicates mean value) of adenine triphosphate (ATP), adenine diphosphate (ADP), adenine monophosphate (AMP), and IMP in single muscle fibers of horses with PSSM (n = 140 fibers) and control horses (22) after exercise.

among single muscle fibers with high, medium, and low oxidative capacity in horses with PSSM and control horses after exercise (**Figure 2**). A range of ATP concentrations in select muscle fibers was observed in horses with PSSM and control horses after exercise (**Figure 3**). Single muscle fiber concentration of IMP was higher in horses with PSSM (0.70 ± 0.11 mmol/kg) after exercise, compared with control horses (0.15 ± 0.09 mmol/kg). High single muscle fiber concentrations of IMP (> 3 mmol/kg) were found in horses with PSSM after exercise.

Whole-muscle, pooled muscle fiber, and single muscle fiber analyses—Whole-muscle, pooled muscle fiber, and single muscle fiber concentrations of IMP were all consistently higher in horses with PSSM after exercise, compared with control horses. Interestingly, IMP concentrations were highest in single muscle fiber analysis and decreased with increasing fiber populations in samples (ie, single muscle fibers > pooled muscle fibers > whole-muscle homogenates). Detection of adenine nucleotides was possible in single muscle fiber samples that weighed ≥ 1.0 µg, providing results that were within the expected range on the basis of data collected from corresponding whole-muscle and pooled muscle fiber samples. The detection limit for adenine nucleotides at fiber weights of 1 to 2 µg is approximately 0.20 to 0.30 mmol/kg of dry weight. Therefore, if AMP and IMP concentrations were less than this threshold in fibers weighing only 1 to 2 µg, no measurable peak would be found by HPLC; thus, concentrations in these fibers will therefore be detected as 0 mmol/kg of dry weight.

Discussion

In our study, whole-muscle specimens from healthy horses and horses with PSSM did not have significant changes in lactate, G-6-P, ATP, ADP, and AMP concentrations after submaximal exercise. However, increased whole-muscle concentrations of IMP were found after exercise in horses with PSSM and not in healthy horses. The mean concentration of IMP in whole muscle of horses with PSSM (0.36 mmol/kg) was lower than the IMP concentration found in whole muscle of Standardbred trotters performing submaximal exercise at 7 m/s for 48 to 58 minutes until fatigue (1.09 mmol/kg).[21] Thus, based on whole-muscle analysis, only minor changes in muscle metabolism were apparent in horses with PSSM with submaximal exercise, compared with control horses. However, whole-muscle concentrations are mean values of all fibers within the sample and do not reflect the metabolic changes within individual fibers. In addition, whole-muscle specimens contain more connective tissue, blood vessels, and nerve fibers than single muscle

fibers, which could influence the adenine nucleotide concentrations as measured per gram of tissue within whole-muscle samples.

To discern whether small changes in nucleotide concentrations occurred in specific fibers during exercise, additional analyses were done on pooled muscle fibers and small segments of individual muscle fibers of the same oxidative type. Single muscle fiber and pooled muscle fiber analyses revealed more marked accumulation of IMP in some fibers of horses with PSSM that was not apparent in whole-muscle analysis. Concentration of IMP in individual muscle fibers of horses with PSSM after exercise ranged from 0 to 9.8 mmol/kg, compared with 0 to 3.1 mmol/kg in control horses. The IMP concentration in a few single muscle fibers of horses with PSSM was comparable to the IMP concentration reported in single muscle fibers after maximal exercise.[22] Furthermore, the mean IMP concentration for all single muscle fibers after exercise (0.7 mmol/kg) approached whole-muscle IMP concentration (1.09 mmol/kg) in Standardbreds performing fatiguing submaximal exercise.[21] Thus, even with as little as 11 minutes at a walk and a trot, horses with PSSM have adenine nucleotide degradation in some fibers that resembles horses performing at twice the speed for almost 5 times as long.

A diminished nucleotide pool has been proposed to cause fatigue with prolonged submaximal exercise in humans and horses.[21,23,24] Glycogen-depleted fibers were found in these studies, and it was suggested that an impaired ability to resynthesize ATP in the glycogen-depleted fibers results in activation of AMP deaminase.[21,23] Although glycogen depletion patterns were not evaluated in horses with PSSM, little depletion would be anticipated as a result of their high resting glycogen concentrations and because substantial glycogen depletion in select type 1 and type 2 fibers is usually not apparent until after 60 minutes of prolonged slow trotting.[25] Thus, results of our study indicate that the muscle in horses with PSSM undergoes considerable metabolic stress, even when performing short-term aerobic exercise with low lactate concentrations and adequate muscle glycogen concentrations.

Variation in adenine nucleotide concentrations after exercise in individual muscle fibers may be dependent upon the recruitment pattern of muscle fibers and the metabolic properties of different muscle fibers, as well as any fiber type specific metabolic perturbations that may exist in PSSM. Adenine nucleotide degradation was apparent in muscle fibers with high, medium, and low oxidative capacity in horses with PSSM, indicating that they had been recruited during light exercise and that ATP resynthesis was not achieved solely by oxidative or glycolytic metabolism in these fibers. Typing of fibers by NADH staining is subjective and not as accurate as biochemical analysis.[19] Horses with PSSM and control horses used in our study had low oxidative capacities, as measured by CS and HAD activity, compared with other breeds.[26,27] Thus, even fibers subjectively typed as relatively dark in the NADH stain may have had a rather low oxidative capacity. This may explain why no marked differences in adenine nucleotide concentrations between high and low oxidative muscle fibers were found in our study. In addition, the limited number of fibers available to study and the wide variation in ATP and IMP concentrations between fibers decreased the statistical power of analyses and could have impacted our ability to detect differences between oxidative fiber types. It was notable, however, that the low oxidative staining fibers in horses with PSSM, but not control horses, had increased IMP concentrations, possibly indicating a perturbation in muscle metabolism.

The small change in muscle lactate concentrations during submaximal exercise in horses with PSSM further indicated that oxidative metabolism was functioning only to a certain degree in the muscle of horses with PSSM. The lower oxidative capacity of horses with PSSM, compared with control Quarter Horses[11] and other equine breeds,[26,27] may account for the higher muscle lactate concentrations after near-maximal[3] and submaximal exercise in horses with PSSM. The slightly higher whole-muscle lactate measured during submaximal exercise in horses with PSSM may have initiated a decrease in muscle pH and contributed to early activation of AMP deaminase. The extent of lactate accumulation in specific single muscle fibers could potentially be higher than was detected by whole-muscle analysis, resulting in activation of AMP deaminase in these particular fibers and subsequent IMP accumulation. Heightened activity of AMP deaminase in horses with PSSM, compared with control horses, could cause a decrease in AMP concentration and the appearance of IMP in the muscle of horses with PSSM after exercise. Unfortunately, the importance of low AMP concentrations in some single muscle fibers of horses with PSSM was not clear because concentrations were so close to the detection limit of HPLC in some fibers.

Premature adenine nucleotide degradation in horses with PSSM may also reflect a defect in the regulation of the flux of substrates, such as glycogen, glucose, and free fatty acids, through aerobic metabolism. One of the emerging key sensors and regulators of energy metabolism in skeletal muscle is AMP-activated kinase, which monitors astonishingly small shifts in the cellular AMP:ATP ratio.[28,29] The activation of AMP-activated kinase, either by allosteric interactions of AMP, phosphorylation via AMP-activated kinase, or both, triggers a shift from energy-consuming pathways (such as glycogen, fatty acid, and cholesterol synthesis) to ATP generating pathways via the phosphorylation of key regulatory enzymes.[29] Shifts in the concentrations of AMP and IMP in skeletal muscle of horses with PSSM after exercise may well affect the activity of AMP-activated kinase and, thereby, the flux of metabolites directed into ATP generating pathways. An alteration in the ability to switch from 1 metabolic pathway or substrate to another could potentially explain the metabolic stress found in the muscle of horses with PSSM after light and maximal exercise, providing a future direction for research in PSSM. Interestingly, distinct mutations in different isoforms of the gamma subunit of AMP-activated kinase have been implicated in disorders that involve glycogen accumulation in either skeletal muscle of Hampshire pigs or cardiac muscle in humans.[30,31]

The mechanism responsible for rhabdomyolysis in human patients with specific defects in glycogenolysis or glycolysis has also been difficult to discern.[32] The focus of metabolic studies in these patients usually has been to evaluate exercise tolerance and the impact of a block in glycogen or glucose metabolism on muscle metabolism. Methods used include serum biochemical analysis, electromyography, evaluation of muscle biopsy specimens, whole-body maximal oxygen uptake analysis, and measurement of purine nucleotides in muscles of the arm during ischemic exercise within a nuclear magnetic resonance spectroscope.[32-34] Although the mechanisms for early fatigue in myophosphorylase and phosphofructokinase deficiency have not precisely been defined, patients have decreased oxygen uptake, decreased lactate production, depletion of creatine phosphate, and accumulation of ADP and IMP.[35] Human patients with glycogenoses[32,33,35] and horses with PSSM[36] have decreased oxygen uptake, accumulation of IMP, and limited exercise capacity. Depletion of ATP does not appear to cause rhabdomyolysis in human patients with metabolic myopathies[32] or in horses with PSSM, as both control horses and horses with PSSM had similar ranges in ATP concentrations and depletion was not prominent in any muscle fibers regardless of serum CK activity. Thus, more subtle perturbations in energy generation, reflected by increased IMP concentration but not total depletion of ATP, appear to characterize ER in metabolic myopathies. This attenuation of ATP depletion to no more than 50% of resting concentrations in the face of high IMP concentrations is also seen in muscle fibers of athletes performing fatiguing maximal exercise[37] and in some muscle fibers of horses after racing.[23]

In conclusion, horses with PSSM undergoing short-duration submaximal exercise have evidence of purine nucleotide degradation in skeletal muscle. The metabolic stress of 10 to 20 minutes of a walk and trot exercise was best indicated by increased IMP concentration within single muscle fibers of horses with PSSM but not in control horses. Adenosine triphosphate depletion did not occur in conjunction with rhabdomyolysis in horses with PSSM. The increase in muscle IMP concentration in horses with PSSM after light exercise may indicate that a disruption in energy supply occurs in the muscle of horses with PSSM despite adequate glycogen concentrations and normal activity of glycogenolytic and glycolytic enzymes. Exact mechanisms contributing to the muscle cramping and skeletal muscle necrosis in horses with PSSM are still unclear.

a. Cahn 25 Electrobalance, AB Ninolab, Upplands Väsby, Sweden.
b. C:18 (250 × 6 mm) waters, Sverige AB, Sollentuna, Sweden.

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