Insulin sensitivity in Belgian horses with polysaccharide storage myopathy

Anna M. Firshman, BVSc, PhD; Stephanie J. Valberg, DVM, PhD; John D. Baird, BVSc, PhD; Luanne Hunt, BVMS, MS; Salvatore DiMauro, MD

**Objective**—To determine insulin sensitivity, proportions of muscle fiber types, and activities of glycogenolytic and glycolytic enzymes in Belgians with and without polysaccharide storage myopathy (PSSM).

**Animals**—10 Quarter Horses (QHs) and 103 Belgians in which PSSM status had been determined.

**Procedures**—To determine insulin sensitivity, a hyperinsulinemic euglycemic clamp (HEC) technique was used in 5 Belgians with PSSM and 5 Belgians without PSSM. Insulin was infused IV at 3 mU/min/kg for 3 hours, and concentrations of blood glucose and plasma insulin were determined throughout. An IV infusion of glucose was administered to maintain blood glucose concentration at 100 mg/dL. Activities of glycogenolytic and glycolytic enzymes were assessed in snap-frozen biopsy specimens of gluteus medius muscle obtained from 4 Belgians with PSSM and 5 Belgians without PSSM. Percentages of type 1, 2a, and 2b muscle fibers were determined via evaluation of > 250 muscle fibers in biopsy specimens obtained from each Belgian used in the aforementioned studies and from 10 QHs (5 with PSSM and 5 without PSSM).

**Results**—Belgians with and without PSSM were not significantly different with respect to whole-body insulin sensitivity, muscle activities of glycogenolytic and glycolytic enzymes, or proportions of muscle fiber types. However, Belgians had an increased proportion of type 2a and decreased proportion of type 2b muscle fibers, compared with proportions in QHs, regardless of PSSM status.

**Conclusions and Clinical Relevance**—PSSM in Belgians may be attributable to excessive glycogen synthesis rather than decreased glycogen utilization or enhanced glucose uptake into muscle cells. (Am J Vet Res 2008;69:818–823)

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Polysaccharide storage myopathy was first described in 1992 in 9 QH-related breeds that had a history of chronic exertional rhabdomyolysis. Histologic evaluation of frozen sections of skeletal muscle from affected horses revealed intracytoplasmic PAS-positive inclusions that were resistant to amylase digestion. Subsarcolemmal vacuoles; necrosis, atrophy, and regeneration of myofibers; and high concentrations of amylase-sensitive glycogen were also detected. In addition, muscle concentrations of glycogen and glucose-6-phosphate in horses with PSSM were between 1.4 and 2.5 times as high as concentrations in healthy horses. Since that report was published, PSSM has been detected in various other breeds of horses including draft horse and Warmblood breeds. Clinical signs of PSSM in draft horses or Warmbloods vary; affected horses may have no clinical signs or they may develop muscle soreness, exertional rhabdomyolysis, gait abnormalities, and severe weakness and become recumbent. Results of histopathologic evaluation of skeletal muscle biopsy specimens obtained from affected draft horses and Warmblood horses are identical to results reported for QHs with PSSM.

Studies of QHs with PSSM reveal that glycogen accumulation in affected horses may be attributable to excessive synthesis of glycogen rather than a defi-
ciency of glycolytic or glycogenolytic enzymes. During exercise, glycogen utilization is similar or increased in QHs with PSSM, compared with glycogen utilization in horses without PSSM. Increased glycogen synthesis in QHs with PSSM may be attributable to an increase of insulin-stimulated glucose uptake into skeletal muscle. Results of oral and IV glucose tolerance tests indicate that glucose is cleared from the bloodstream of QHs with PSSM faster than it is cleared from the bloodstream of QHs without PSSM. Furthermore, testing by use of the HEC technique, a more rigorous assessment of insulin sensitivity, reveals high insulin sensitivity in QHs with PSSM. However, the mechanism relating increased glucose uptake and glycogen synthesis with muscle necrosis during exercise in QHs with PSSM is unknown.

Studies of the capacity of skeletal muscle of draft horses with PSSM to break down glycogen and glucose are few in number. In a study, glycogen concentration and activities of glycolytic and glycogenolytic enzymes in skeletal muscle specimens from various breeds of horses with PSSM, including draft horses, were measured. Investigators reported that glycogen concentrations were high but detected no specific enzyme deficiencies. Little is known about glucose uptake by muscle cells or insulin sensitivity in draft horse breeds. The purpose of the study reported here was to determine insulin sensitivity, proportions of muscle fiber types, and activities of glycogenolytic and glycolytic enzymes in Belgians with and without PSSM.

Materials and Methods

Animals—Horses used in this study were selected from a sample of 103 Belgians from which biopsy specimens of gluteal muscle had been obtained to determine the prevalence of PSSM in another study. In that study, frozen sections of gluteus medius muscle were stained with H&E, PAS, and amylase-PAS, and muscle glycogen concentrations were measured. A diagnosis of PSSM was made when abnormal amylase-resistant polysaccharide inclusions were detected in at least 2 muscle fibers/biopsy sample.

In the study reported here, in which insulin sensitivity testing was performed, Belgians were selected from a group of 33 horses located at 1 farm. Owner consent was obtained. To choose horses with the widest possible range in muscle glycogen concentrations, Belgians with PSSM (PSSM horses) were defined as those in which analysis of muscle biopsy specimens obtained in the aforementioned study revealed an accumulation of glycogen > 175 mmol/kg and amylase-resistant polysaccharide inclusions within muscle fibers; Belgians without PSSM (control horses) were defined as those in which muscle glycogen accumulation was < 120 mmol/kg. In addition, to increase specificity when diagnosing PSSM, horses with the highest and lowest PSSM scores for muscle biopsy specimens (as described elsewhere) were chosen for PSSM and control groups, respectively. Horses selected for the PSSM group consisted of 3 geldings and 2 mares; mean ± SD age and muscle glycogen concentration were 6.7 ± 3.1 years (range, 3 to 8 years) and 209.3 ± 45.1 mmol/kg of muscle (wet weight), respectively. Control horses consisted of 5 geldings; mean ± SD age and muscle glycogen concentration were 8.0 ± 1.0 years (range, 7 to 9 years) and 99.3 ± 14.1 mmol/kg of muscle (wet weight), respectively. Values for mean serum creatine kinase and aspartate aminotransferase activities in all 10 horses were obtained in the aforementioned study in which the horses were involved. At the time of that study, values for the PSSM horses were within reference ranges (293.8 ± 88.8 U/L and 410.3 ± 169.0 U/L, respectively) and were not significantly different (P = 0.18) different from values for control horses (224.0 ± 21.8 U/L and 272.5 ± 30.9 U/L, respectively). No history of exertional rhabdomyolysis was reported for any horse.

For evaluation of glycogenolytic and glycolytic enzyme activities, horses were randomly selected from the sample of 103 Belgians. Horses with PSSM consisted of 3 mares and 1 stallion; mean ± SD age was 11.0 ± 2.2 years. Horses without PSSM (control horses) consisted of 4 mares and 1 gelding; mean ± SD age was 3.3 ± 1.0 years. One of the control horses used for evaluation of enzyme activities was also used for evaluation of insulin sensitivity.

For evaluation of fiber types in gluteus medius muscle, all horses included in insulin sensitivity testing and enzyme activity assays were used. For comparison, 10 QHs were also used. Five of the QHs had PSSM on the basis of detection of amylase-resistant polysaccharides in skeletal muscle biopsy specimens and muscle glycogen concentrations > 175 mmol/kg of muscle (wet weight). The rest did not have PSSM on the basis of results from histologic examination of muscle biopsy specimens. Samples of gluteal muscle used for determining the type of muscle fibers for QHs were collected during another study. The Institutional Animal Care and Use Committee of the University of Minnesota approved all procedures.

Determination of insulin sensitivity via testing by use of the HEC technique—The technique used for the HEC was the same as that described for the evaluation of PSSM in QHs. One affected and 1 control horse were evaluated at the University of Minnesota; the remaining 4 PSSM 4 control horses were evaluated on a farm in New York. Investigators were not aware of the PSSM status of all horses. All horses were clinically normal on physical examination and were fed similar diets of grass hay and grain. Horses were allowed to rest in stalls for 1 day prior to testing, and food was withheld for 12 hours prior to testing. An IV catheter was placed in each jugular vein of each horse prior to testing. Horses were placed in stocks and groomed by an attendant to maintain a calm state during testing.

Before testing by use of the HEC technique began, 3 blood samples were obtained at 10-minute intervals from one of the catheters (reserved for blood collection) and baseline blood glucose concentrations were determined by use of a validated handheld glucose meter. Prior to infusion, insulin was mixed in 500 mL of physiologic saline (0.9% NaCl) solution and 2 mL of homologous blood. Insulin was subsequently initiated (time 0 = start of infusion) through the second jugular catheter (reserved for treatments) via a volumetric infusion pump at a constant rate of 3 mU/kg/min for 3 hours. Blood glucose concentrations
were monitored at 10-minute intervals. A 50% dextrose solution was infused through the second catheter via another volumetric infusion pump at a rate that maintained blood glucose concentration within 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 equation⁴,

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\text{Space correction} = (G1 - G2) \times 0.019
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where G1 is the first blood glucose concentration and G2 is the subsequent glucose concentration. The rate of glucose infusion during testing by use of the HEC technique was calculated for each horse; a rate of glucose infusion corrected for changes in glucose space⁴ was also calculated. In addition, the ratio of the mean corrected glucose infusion rate to mean insulin concentration was calculated.

Plasma samples were obtained at 20-minute intervals by centrifugation of blood samples immediately after collection. Plasma was harvested, shipped overnight on ice packs, and stored at −80°C. Plasma obtained from thawed samples was analyzed for insulin concentration by use of a radioimmunoassay.

Hyperinsulinemia was defined as plasma insulin concentration ≥ 200 µU/mL. Insulin clearance during 60 to 180 minutes of testing by use of the HEC technique was also calculated on the basis of plasma insulin concentration and insulin dose.³⁻⁵

Determination of activities of muscle enzymes by use of biochemical analysis—Activities of glycogenolytic and glycolytic enzymes were assessed in 10-mg samples of snap-frozen biopsy specimens obtained from the gluteus medius muscle of horses. Activities of enzymes that are known to cause nonfatal disorders of skeletal muscle glycogen storage in other animal species were selected for assessment. For analyses of activities of PKG, PGAM, and LDH, muscle specimens were homogenized in a 10% (wt/vol) solution of 30mM KF and 1mM EDTA (pH, 7.5). For analysis of PFK activity, muscle specimens were homogenized in a 10% (wt/vol) solution of 40mM β-glycerophosphate, 40mM NaF, 10mM EDTA, and 20mM mercaptoethanol (pH, 6.8). Enzyme activities were assayed by use of a spectrophotometer.¹⁷

Determination of proportions of muscle fiber types by use of histochemical analysis—To minimize artifacts caused by freezing, muscle biopsy specimens were rolled in talcum powder before snap-freezing in liquid nitrogen. Biopsy specimens were cut into 10-µm-thick sections by use of a cryostat, incubated at pH 4.25 and pH 4.55, and stained for myosin ATPase activity to identify type 1, type 2a, and type 2b muscle fibers.²⁶

For each biopsy specimen, fiber types of ≥ 250 muscle fibers were determined and percentages of type 1, 2a, and 2b fibers were calculated.

Statistical analysis—Values are expressed as mean ± SD. Blood glucose and plasma insulin concentrations obtained during use of the HEC technique were compared via repeated-measures ANOVA. Rate of glucose administration during 30-minute intervals of the HEC procedure (corrected for changes in glucose space) was compared between PSSM and control horses by use of a Student t test. Unpaired 2-tail Student t tests were used to compare PSSM and control horses with respect to resting glucose and insulin concentrations, ratio of mean corrected glucose infusion rate to mean insulin concentration, proportions of muscle fiber types, and muscle enzyme activities.
Hyperinsulinemia was at P, Muscle glycogen concentrations were significantly (P = 0.001) higher in PSSM horses, compared with concentrations in control horses.

Statistical analyses were conducted by use of computer software programs. For all analyses, values of P ≤ 0.05 were considered significant.

Results

Insulin sensitivity—Hyperinsulinemia was attained approximately 60 minutes after the HEC technique was initiated. Steady-state conditions for blood glucose and plasma insulin concentrations were achieved in each horse (Figure 1). Mean ± SD baseline blood glucose concentrations obtained prior to testing were similar (P = 0.19) between PSSM horses (80.9 ± 20.0 mg/dL) and control horses (89.5 ± 14.3 mg/dL). Baseline serum insulin concentrations were also similar (P = 0.67) between PSSM horses (14.7 ± 28.6 µU/mL) and control horses (8.7 ± 10.9 µU/mL). During 60 to 180 minutes of the HEC procedure, blood glucose concentrations were not significantly (P = 0.38) different between PSSM horses (99.2 ± 13.9 mg/dL) and control horses (95.8 ± 13.2 mg/dL). Serum insulin concentrations during the same interval were also not significantly (P = 0.92) different between PSSM horses (278.9 ± 168.2 µU/mL) and control horses (289.1 ± 130.3 µU/mL).

Rate of glucose infusion during 60 to 180 minutes of the HEC procedure was not significantly (P = 0.86) different between PSSM horses (4.6 ± 1.4 mg/kg/min) and control horses (4.7 ± 2.0 mg/kg/min; Figure 2). Mean rate of glucose infusion (corrected for changes in glucose space) during the same time period was 5.6 ± 4.8 mg/kg/min for PSSM horses, which did not differ significantly (P = 0.49) from the rate in control horses (6.5 ± 5.8 mg/kg/min). Ratio of the mean corrected glucose infusion rate to mean insulin concentration was similar (P = 0.47) between PSSM horses (2.0) and control horses (2.2). Clearance of insulin during 60 to 180 minutes of the HEC procedure was not significantly (P = 0.66) different between PSSM horses (0.015 ± 0.0088 mL/kg/min) and control horses (0.013 ± 0.0056 mL/kg/min).

Activities of muscle enzymes—Concentrations of glycogenolytic or glycolytic enzyme activities of horses with PSSM were not significantly different from those of control horses (Table 1). Muscle glycogen concentrations were significantly (P = 0.001) higher in PSSM horses, compared with concentrations in control horses.

Proportions of muscle fiber types—Proportions of muscle fiber types in biopsy specimens of gluteus medius muscle obtained from 18 Belgians and 10 QHs, regardless of PSSM status. When proportions of muscle fiber types were compared between Belgians and QHs, a significant difference in the proportion of type 1 fibers was not detected. In contrast, Belgians had significantly (P = 0.002) more type 2a and significantly (P = 0.008) less type 2b muscle fibers, compared with proportions in QHs (Table 3).

Discussion

Testing by use of the HEC technique is regarded as a more accurate method for assessment of insulin sensitivity, compared with other more commonly used methods such as oral and IV glucose tolerance testing. The HEC technique provides maximal steady-state insulin concentrations, during which the rate of glucose infusion required to maintain euglycemia provides an indication of the insulin sensitivity of muscle and adipose tissues. In contrast with oral and IV glucose tolerance testing, an advantage of testing by use of the HEC technique is that the exogenously induced hyperinsulinemia inhibits endogenous insulin secretion, thereby preventing fluctuations in glucose homeostasis.
technique can also be used to study other disorders of glucose metabolism in horses, such as equine motor neuron disease, hyperlipemia, and hyperadrenocorticism. Results of testing by use of the HEC technique indicate that excessive storage of glycogen and abnormal polysaccharides in QHS with PSSM is associated with a 2-fold increase in the rate of uptake of glucose into muscle cells, compared with the rate of glucose uptake by muscle cells in QHS without PSSM. The study reported here did not detect the same differences in insulin sensitivity between control and PSSM Belgians that has been reported elsewhere for PSSM and control QHS.

We were unable to perform all testing by use of the HEC technique in a hospital setting, and this factor may have contributed to the large SD values and higher insulin concentrations obtained in this study, compared with results obtained by our laboratory group for studies conducted in QHS. Individual variation among Belgians and other draft horses may also be greater than that among QHS. Nevertheless, mean values were remarkably similar between control and PSSM Belgians in the study reported here. Insulin sensitivities were not significantly different between the 2 horses evaluated in a hospital setting and the 8 horses evaluated on the farm; however, low numbers of horses within each group may have limited the power to detect a significant difference.

During testing by use of the HEC technique in the study reported here, the rate of glucose infusion required to maintain normoglycemia in PSSM horses (4.6 ± 1.4 mg/kg/min) and control horses (4.7 ± 2.0 mg/kg/min) was higher than that required in healthy QHS (2.03 ± 0.27 mg/kg/min) and similar to that required in PSSM QHS (3.63 ± 0.47 mg/kg/min) evaluated by use of the same technique in another study. Discrepancies between values may have been attributable to breed differences in insulin sensitivity. Other factors, such as dietary imbalances (eg, vitamin E deficiency) and differences in age, stress, type of diet, and environmental conditions, may have contributed to the difference in insulin sensitivity detected between the Belgians reported here and QHS reported in other studies. The Belgians reported here and QHS reported in other studies were fed grass hay and grain in similar proportions to their body weight. Interestingly, compared with muscle in QHS, gluteal muscle of Belgians had a significantly higher proportion of slower contracting, higher oxidative type 2a fibers relative to the proportion of faster contracting, lower oxidative type 2b muscle fibers. In rats, glucose uptake and insulin sensitivity determined via use of the HEC technique are significantly higher in slow-twitch muscle fibers, compared with values in fast-twitch muscle fibers. Furthermore, in humans and rats, the ratio of oxidative fibers to glycolytic fibers may be important for determination of whole-body insulin sensitivity. Thus, the approximately 2-fold higher insulin sensitivity in Belgians in the study reported here, compared with insulin sensitivity in QHS in another study, may also be related to an increased ratio of type 2a to type 2b fibers in Belgians. Physical fitness and diet of horses were unlikely to have contributed to the differences in insulin sensitivity between breeds of horses evaluated in that study or the study reported here because none of the QHS or Belgians were trained regularly or fed a diet high in fat or grain.

In humans and other animals, high concentrations of glucose-6-phosphate and glycogen in skeletal muscle are associated with various forms of glycogenoses caused by deficiencies in glycolytic or glycogenolytic enzymes. Glycolytic disorders can also develop in other species and include myophosphorylase deficiency in Charolais cattle, acid maltase deficiency in Shorthorn cattle, PFK deficiency in English Springer Spaniels, debranisher deficiency in German Shepherd Dogs, and glycogen branching-enzyme deficiency in QH foals. Phosphofructokinase deficiency (glycogenosis type VII) is similar to PSSM in many respects; both disorders can result in the development of exertional rhabdomyolysis and accumulation of abnormal polysaccharides that are resistant to amylase digestion. Accumulation of abnormal polysaccharides in animals with PFK deficiency is believed to be attributable to a high concentration of glucose-6-phosphate that accumulates as a result of interruption of the glycolytic pathway. Glucose-6-phosphate is a potent activator of glycogen synthase; thus, a high concentration of glucose-6-phosphate results in a high ratio of glycogen synthase to branching-enzyme activity. In the Belgians evaluated in our study, no deficiencies were identified in the activities of PFK, PPL, PGAM, PGK, or LDH, regardless of whether the horses had PSSM. Glycogen branching-enzyme activity was not measured in this study because a deficiency in this enzyme causes a fatal disorder that prevents affected horses from reaching adulthood. Results of the study reported here concur with those of a study of various breeds of horses with PSSM (including draft horses). Analysis of our results suggests that a deficiency of one of these enzymes is not the cause of PSSM in Belgians. Additional studies of enzymes involved in glycogen synthesis, such as glycogen synthase, as well as those involved in the regulation of energy metabolism, such as AMP kinase, are warranted. Such studies may reveal the cause of PSSM in draft horses.

References


