Effects of exercise and glucose administration on content of insulin-sensitive glucose transporter in equine skeletal muscle

Véronique A. Lacombe, DVM, PhD; Kenneth W. Hinchcliff, BVSc, PhD; Stephen T. Devor, PhD

Objectives—To characterize insulin-sensitive glucose-transporter (GLUT-4) protein in equine tissues and determine effects of exercise and glucose administration on content of GLUT-4 protein in equine skeletal muscle.

Sample Population—Tissue samples from 9 horses.

Procedures—Western blot analyses were performed on crude membrane preparations of equine tissues to characterize GLUT-4. In a crossover, randomized study, horses were strenuously exercised for 3 consecutive days and then administered 13.5% glucose or isotonic saline (0.9% NaCl; control) solution, IV, at similar infusion rates for 12.1 hours. Samples were collected from the middle gluteal muscle before and after exercise and 10.1 hours after completion of an infusion and used for measurements of glycogen concentration and total content of GLUT-4 protein.

Results—Immunoblot analyses detected specifically immunoreactive bands for GLUT-4 in insulin-sensitive tissues. Content of GLUT-4 protein in skeletal muscle increased significantly by 27.3 and 12.3% 22.2 hours after exercise for control and glucose groups, respectively. Intravenous infusion of glucose resulted in a significantly higher rate of glycogenesis, compared with results for the control group (mean ± SD, 3.98 ± 0.61 and 1.47 ± 0.20 mmol/kg/h, respectively). Despite enhanced glycogenesis, we did not detect an increase in content of GLUT-4 protein after glucose infusion, compared with values after exercise.

Conclusions and Clinical Relevance—GLUT-4 protein was expressed in equine skeletal and cardiac muscles. Exercise increased total content of GLUT-4 protein in skeletal muscle, and replenishment of muscle glycogen stores after glucose infusion attenuated the exercise-induced increase in the content of GLUT-4 protein in equine skeletal muscle. *(Am J Vet Res* 2003;64:1500–1506)

During the recovery period after exercise, skeletal muscle is the tissue responsible for most of the uptake and use of glucose. Insulin partially regulates uptake and use of glucose. In rats and humans, movement of glucose across plasma membranes into muscle cells is the rate-limiting step in glucose use. Glucose is used mainly for glycogenesis.[1,2] Movement of glucose from the blood across the muscle sarcolemma is primarily by facilitated diffusion via a family of structurally related proteins, the **glucose transporters** (**GLUTs**; ie, GLUT-1 to GLUT-8).[1] Glucose transporter-4 is the major isoform in muscle cells in rodents and humans.[1] The GLUT-1 and GLUT-5 isoforms are primarily associated with the cell surface and are not insulin-sensitive transporters. However, translocation of GLUT-4 protein from an intracellular (nonactive) pool to the plasma membrane (active site) is largely regulated in nonexercising humans and other animals by an insulin-dependent process.[3] The major factors that induce an increase in glucose transport in muscles are fiber contractions and insulin, although numerous other factors, including catecholamines, hypoxia, growth factors, and corticosteroids, can also alter glucose transport.[1] It has also been suggested[4] that glycogen concentration plays a regulatory role in glucose transport.

In contrast to the extensive investigations conducted in humans and rats, little information is available on glucose transport in other mammals. A GLUT-4-like protein has been characterized by use of immunoblot analysis in insulin-responsive tissues obtained from calves, goats, and sheep.[5,6,a] However, attempts to characterize GLUT-4 in ruminants have led to equivocal results because of the lack of controlled experiments in some studies.[5,6] Furthermore, there is little information regarding factors that influence GLUT-4 content in equine skeletal muscle.

After exercise, replenishment of glycogen stores is a high priority for skeletal muscles. Glucose transport is the rate-limiting step for glucose uptake and glycogen resynthesis in skeletal muscles of rodents and humans[2]; however, the effect of postexercise carbohydrate supplementation on GLUTs has not been investigated in horses. The first objective in the study reported here was to characterize GLUT-4 in nonexercising horses by determining specificity of immunoreactive bands detected during immunoblotting. The second objective was to evaluate the effects of exercise and an infusion of glucose on total GLUT-4 content in equine skeletal muscles. We hypothesized that exercise and glucose infusion would increase total content of GLUT-4 protein in equine skeletal muscles.

Materials and Methods

Animals—Nine horses were used in the 2 parts of the study. In the first portion of the study, tissue samples were

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From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine (Lacombe, Hinchcliff), and the Department of Sport and Exercise Sciences, College of Education (Devor), The Ohio State University, Columbus, OH 43210. Dr. Lacombe's present address is the Department of Science, Division of Pharmacy Practice and Administration, The Ohio State University, Columbus, OH 43210.
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Address correspondence to Dr. Lacombe.

obtained from 3 horses and used for characterization of GLUT-4. In the second part of the study, 6 horses were used to determine the effects of exercise and glucose infusion on total content of GLUT-4 protein in samples of skeletal muscle. All procedures were approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.

Characterization of GLUT-4—Samples of liver and cardiac tissues were collected from 2 horses that were donated to The Ohio State University. Samples were collected within 20 minutes after the horses were euthanatized by IV administration of pentobarbital. Three muscle samples were collected by needle biopsy from the middle gluteal muscle of an adult horse during a 3-week period (1 sample/wk).[7] The horse was a healthy Standardbred gelding that weighed 443 kg. The horse was housed in a box stall (3 × 4 m) and fed timothy grass and alfalfa hay (approx 5 kg/d) and cracked corn (approx 2.5 kg/d), which was sufficient to maintain body weight. All tissue samples were flash-frozen in liquid nitrogen and stored at –80°C until analysis. Several experiments were conducted by use of immunoblot analysis to specifically isolate GLUT-4 protein in these tissues.

Effect of exercise and glucose infusion—Six fit horses were used in a balanced, blinded, randomized, crossover study. The horses were 5 Standardbreds and 1 Thoroughbred that ranged from 2 to 7 years of age (mean ± SE body weight, 433 ± 7 kg). Mean maximum aerobic capacity of the horses was 133.2 ± 6.6 mL/kg/min. All horses were housed in box stalls and fed timothy grass and alfalfa hay (approx 8.5 kg/horse/d) and mixed pelleted grain (approx 2 kg/horse/d) until the beginning of the study.

Each horse was used in 2 experiments separated by a 16-day interval. Each experiment required the horses to complete 3 consecutive days of exercise (days 1 through 3) that depleted muscle glycogen by at least 60% of the initial values, as reported elsewhere.[8] Feed was withheld for 12 hours before the onset of strenuous exercise, and horses were fed 8.5 kg of timothy grass and alfalfa hay/d during the 3 days of exercise. Trace-mineralized salt blocks and water were available to the horses at all times.

The exercise protocol was customized for each horse on the basis of the assessment of each horse's maximum aerobic capacity. Exercise consisted of each horse running on an inclined treadmill (slope, 4°). Horses ran for 5 minutes at a rate of 4 m/s, 15 minutes at a rate calculated to achieve 70% of **maximum oxygen consumption ($\dot{V}O_2$max)**, and 5 minutes at 90% of $\dot{V}O_2$max. Horses were allowed to rest for 30 minutes; they then completed 6 sprints of 1 minute at 100% $\dot{V}O_2$max with 5 minutes of walking between each sprint.

After the completion of the exercise period on the third day of each experiment, horses were infused IV with a 13.5% solution of glucose (in 0.9% NaCl solution) at a rate of 6 g of glucose/kg (glucose group) or with an equivalent volume of isotonic saline (0.9% NaCl) solution (control group), as reported elsewhere.[8,9] Infusions of glucose and isotonic saline solution were initiated within 30 minutes after the end of the strenuous exercise. Infusions were administered for (mean ± SE) 12.1 ± 0.4 hours at a mean rate of 1.68 ± 0.06 L/h via a catheter placed in a jugular vein. Feed was withheld during the glucose or saline infusion. All experiments and infusions were performed at the same time of day for each horse. This experimental design allowed us to investigate the effects of exercise on GLUT-4 (control group) and the combined effects of exercise and glucose infusion on GLUT-4 (glucose group).

Collection of muscle biopsy specimens—Muscle samples were collected at a depth of 6 cm from the middle gluteal muscle by use of needle biopsy.[7] Aseptic conditions were maintained, and the area was desensitized prior to biopsy by injection of a 2% solution of mepivacaine.[b] Muscle samples were flash-frozen in liquid nitrogen and stored at –80°C until subsequent analysis. Muscle samples were collected from the middle gluteal muscle 5 days before the onset of a bout of exercise, within 15 minutes after completion of the strenuous exercise (day 3), and the morning after infusion of saline or glucose solution (10.1 ± 0.4 hours after completion of the infusion; day 4). Samples were used for measurement of muscle glycogen concentration and total content of GLUT-4 protein.

Collection of blood samples—Blood samples were collected via a 14-gauge, 5.25-in catheter[c] placed in a jugular vein. Skin overlying the vein was desensitized by injection of mepivacaine prior to insertion of the catheter. Venous blood samples were collected before the onset of exercise (day 1), before initiation of an infusion (day 3), at 4-hour intervals throughout the infusion, and 10.1 hours after completion of an infusion (day 4). Venous blood samples were collected into chilled 5-mL evacuated tubes containing potassium oxalate and sodium fluoride and used for determination of glucose concentration.

Biochemical analysis—Muscle glycogen content was determined fluorometrically,[d] as described elsewhere.[10] Plasma glucose concentrations were measured in duplicate by use of an automatic analyzer.[e]

Western blot analysis—Total crude extracts of muscle membranes were obtained by a method reported elsewhere.[11] Frozen tissue samples (30 to 60 mg) were homogenized in 4 mL of homogenizing buffer (210mM sucrose, 40mM NaCl, 2mM ethylene glycol-bis [2-aminoethylether] N,N,N',N'-tetraacetic acid, 30mM HEPES [pH, 7.4], and phenylmethylsulfonyl fluoride [0.35 mg/mL; pH, 7.4]) by use of a polytron fitted with a generator.[f] The homogenate was mixed with 3 mL of 58.3mM sodium pyrophosphate and 1.17mM KCl and stored on ice for 15 minutes. Total muscle membranes were then recovered by centrifugation at 100,000 × *g* for 90 minutes at 4°C.[g] Pellets were resuspended in 400 µL of 10mM Tris HCl and 1mM EDTA (pH, 7.4). One hundred microliters of 20% SDS was then added, and samples were centrifuged at 1,100 × *g* for 25 minutes.

Protein concentration of the supernatant was determined spectrophotometrically[h] by use of a detergent-compatible method that included bovine serum albumin as a standard.[i] Total crude muscle membranes were analyzed for GLUT-4 content by use of electrophoresis and subsequent western blotting.[12] Samples were diluted (1:2) in Laemmli sample buffer (2% SDS, 25% glycerol, 0.01% bromophenol blue, 350mM dithiothreitol, and 65.2mM Tris HCl [pH, 6.8]) and separated on a 12% resolving gel by use of SDS-PAGE. Proteins were transferred electrophoretically to a polyvinylidene fluoride membrane[j] by use of semidry blotting for 90 minutes. Membranes were then blocked overnight in a 10% solution of nonfat dry milk at 4°C. After blocking, each membrane was incubated for 1 hour with a polyclonal antibody[k] directed against the last 12 amino acids of the carboxyl terminal of the GLUT-4 protein. Membranes were then washed 3 times in 0.1% Tris-buffered Tween solution (150mM NaCl, 25mM Tris, and 0.1% Tween-20 [pH, 7.4]), and each membrane was incubated for 1 hour with horseradish peroxidase-conjugated anti-rabbit antibody.[l] Quantitative determination of GLUT-4 protein was performed by autoradiography after developing the antibody-bound transporter protein by use of an enhanced chemiluminescence reaction,[m] which was performed in accordance with the manufacturer's recommendations.

Density of the bands on scanned autoradiographs was quantified by use of a computerized densitometry program. Molecular weight markers[n] were used for each gel to determine the relative molecular weight of the labeled bands. Crude membrane preparations of equine cardiac tissues were used as a positive-control standard, and density was expressed as relative units (ie, percentage).

Statistical analysis—Statistical analyses were performed by use of a 1-way or 2-way repeated-measures ANOVA (repeated measures on time and treatment). The null hypothesis (no effect of exercise or carbohydrate administration on measured variables) was rejected at a value of $P < 0.05$. Significant differences between means ($P < 0.05$) were identified by use of the Student-Newman-Keul test. All results were expressed as mean ± SE.

Results

Characterization of GLUT-4—Polyclonal antibodies directed against rabbit GLUT-4 recognized a protein of similar size in crude membranes prepared from rat cardiac tissues (positive-control sample), equine skeletal muscles, and equine cardiac muscles **(Fig 1)**. A positive linear correlation (R^2, 0.99; $P < 0.001$) was obtained between the amount of protein loaded on the gel and density of the bands at approximately 50 **kilodaltons (kd; Fig 2 and 3)**. When compared with the molecular weight markers, calculation of the molecular mass of the band representing GLUT-4 yielded a mean theoretical value of 53.3 ± 0.5 kd (50 to 55 kd). The bands at 50 kd were evident in cardiac and skeletal muscles of horses, whereas we did not detect similar bands in the negative-control sample (ie, equine liver). However, at least 1 additional labeled band was observed at approximately 150 kd in rat and equine muscles. Furthermore, in many analyses, a band was detected at approximately 80 kd. These bands were evident on immunoblots despite the use of various blocking solutions or after additional washes with 0.1% Tris-buffered Tween solution. When nonimmune rabbit serum was used in place of the primary antibody, the 50-kd bands were not evident, whereas weak bands were still observed at 150 and 80 kd (results not shown). An immunoreactive band was not observed on autoradiographs when the membranes were only incubated with horseradish peroxidase antibody prior to the enhanced chemiluminescence, suggesting the lack of nonspecific binding with horseradish peroxidase (results not shown). The 150-kd band, but not the 50-kd band, was evident on the immunoblot when the primary antibody was incubated for 1 hour with GLUT-4 peptide to which the antibody was raised before incubation with the membrane **(Fig 4)**. These results indicated that the band at 50 kd, but not at 150 kd, was specific for GLUT-4 protein in skeletal and cardiac muscles in horses.

Effects of exercise and glucose administration on muscle glycogen concentrations—Muscle glycogen concentrations after exercise on day 3 were significantly ($P < 0.001$) lower than those before exercise

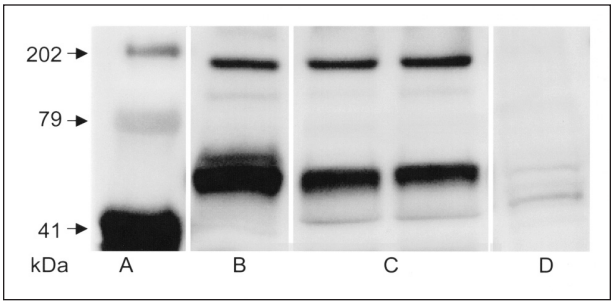

Figure 1—Western blot of crude membrane preparations of tissues obtained from horses. Notice the band for the insulin-sensitive glucose-transporter (GLUT)-4 protein at approximately 50 kilodaltons (kd). Lanes were as follows: A, molecular weight markers; B, cardiac muscle; C, skeletal muscle; and D, liver. Numbers on left side are number of kd.

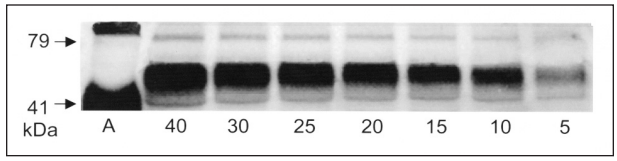

Figure 2—Western blot analysis of GLUT-4 protein in crude membrane preparations of equine skeletal muscle with various amounts of protein. Lane A is the molecular weight markers, and the other lanes are the number of micrograms of protein used for the analysis. Numbers on the left side are number of kd.

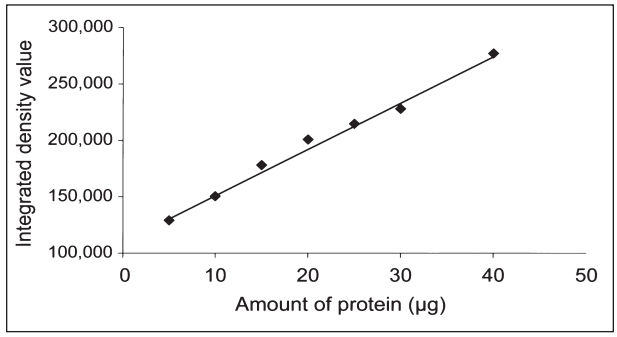

Figure 3—Graph of the relationship between integrated optical density value (arbitrary units) and total content of GLUT-4 protein. There was a high correlation (R^2, 0.991).

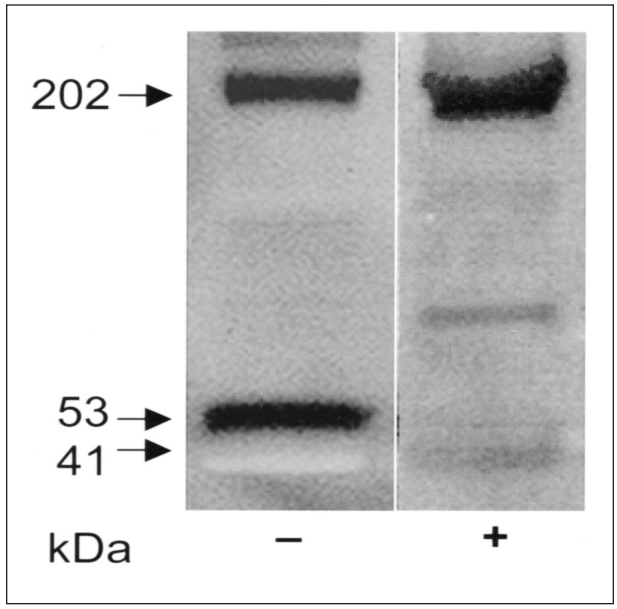

Figure 4—Western blot of GLUT-4 protein in crude membrane preparations of equine gluteal muscle. Western blots were performed with (+) or without (–) the peptide to which the antibody was raised. Numbers on the left side are number of kd.

(**Table 1**). Muscle glycogen concentrations were not significantly different between treatments before and after exercise. Intravenous infusion of glucose after exercise resulted in a significant ($P < 0.001$) increase in muscle glycogen concentrations (91% higher than the pre-exercise value), whereas there was a smaller increase in muscle glycogen stores after infusion of saline solution (39% higher than the pre-exercise value). Intravenous infusion of glucose resulted in a significantly ($P < 0.001$) higher rate of glycogenesis after exercise (3.98 ± 0.61 mmol/kg of wet weight/h), compared with the value for the control group (1.47 ± 0.20 mmol/kg of wet weight/h). Muscle glycogen concentrations on day 4 (22.2 ± 0.3 hours after the end of exercise) were similar to baseline values for the glucose group ($P = 0.34$) but differed significantly from baseline values for the control group.

Effects of exercise and glucose administration on blood glucose concentrations—We detected a significant ($P < 0.001$) time-by-treatment interaction for venous glucose concentrations (**Fig 5**). Glucose concentration was similar between treatments before and after exercise. Intravenous infusion of glucose resulted in a significant ($P < 0.001$) peak plasma glucose concentration (7.6 ± 0.9mM) 4 hours after the initiation of the infusion, whereas glucose concentrations were unchanged throughout the saline infusion. Blood glucose concentration was significantly ($P = 0.01$) lower after exercise (day 3), compared with values 10.1 hours after completion of an infusion (day 4) for the control group. Blood glucose concentrations were similar between treatments after infusion. Although insulin concentrations were not measured in this study, we speculated that the insulin response would have closely followed the glucose response, as has been reported in other studies.[9,o]

Effects of exercise and glucose administration on total content of GLUT-4 protein—We detected a significant ($P < 0.001$) effect of time on the amount of GLUT-4 protein (**Fig 6**). Two values for samples obtained from the control group were considered outliers and removed from the data set. Statistical analysis that included these outliers also revealed a significant ($P = 0.02$) effect of time on content of GLUT-4 protein.

Total content of GLUT-4 protein increased significantly ($P < 0.01$) by 8.3% immediately after exercise

Table 1—Mean ± SE muscle glycogen concentration in samples of skeletal muscle obtained from 6 horses 5 days before onset of an exercise period, after the third day of exercise in an exercise period (ie, day 3), and on the day after infusion of saline (0.9% NaCl) solution (control group) or glucose following completion of the exercise period (ie, day 4)

Group	Before exercise	After exercise	After infusion
Control	120.2 ± 7.0[a]	16.7 ± 2.7[b]	46.6 ± 6.2[b,A]
Glucose	125.3 ± 7.9[a]	26.4 ± 4.8[b]	114.3 ± 7.3[a,B]

Values reported are millimoles of glycogen per kilogram of skeletal muscle (wet weight).
[a,b]Within a row, values with different superscript letters differ significantly ($P < 0.05$) among time periods. [A,B]Within a column, values with different superscript letters differ significantly ($P < 0.05$) between treatment groups.

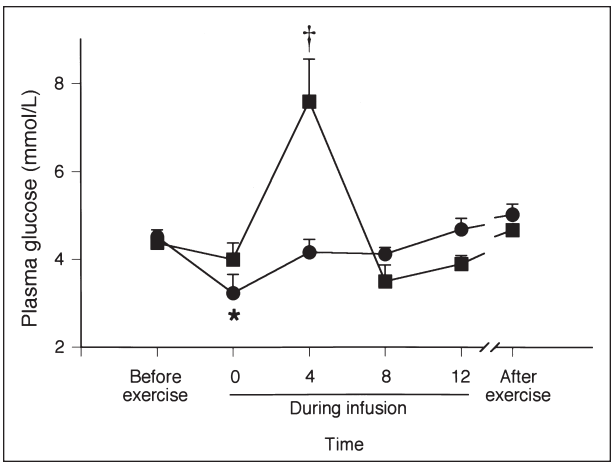

Figure 5—Mean ± SE plasma glucose concentrations in 6 horses before a 3-day period of exercise, after completion of the exercise period and during infusion of glucose (square) or saline (0.9% NaCl) solution (circle), or on the day after the infusion. Time during the infusions represents the number of hours after initiation of the infusion. *Value differs significantly ($P < 0.05$) from value measured after infusion. †Within a time point, value differs significantly ($P < 0.05$) between treatment groups.

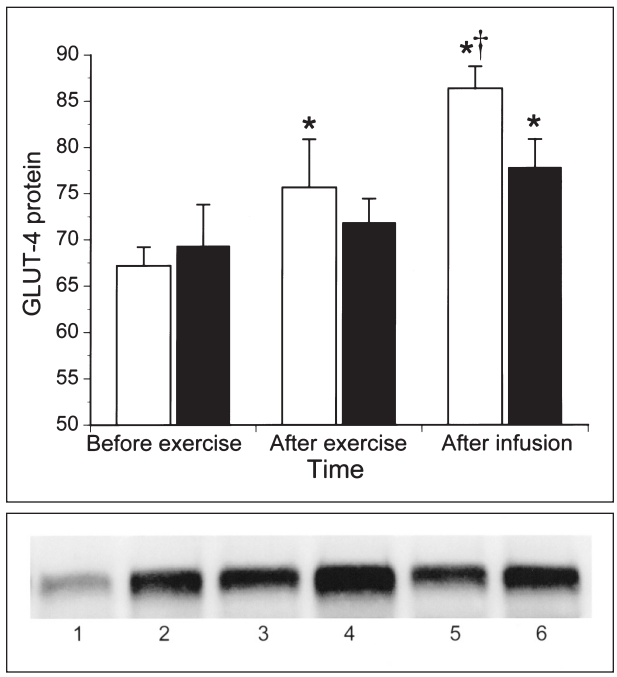

Figure 6—Mean ± SE total content of GLUT-4 protein for 6 horses in samples obtained 5 days before a 3-day period of exercise, immediately after exercise on the third day, and after infusion of glucose (black bar) or saline (white bar) solution on the day after the exercise period (A), and an immunoblot depicting the effects of exercise and carbohydrate administration on content of GLUT-4 protein in crude membrane preparations of equine gluteal muscle (B). Values on the graph represent the content relative to the content in a positive-control sample (ie, equine cardiac tissue).*Within a treatment group, value differs significantly ($P < 0.05$) from the value before exercise. †Within a treatment group, value differs significantly ($P < 0.05$) from value after exercise. Lanes for the immunoblot were as follows: 1, horse in control group before exercise; 2, horse in control group after exercise; 3, horse in glucose group after exercise; 4, horse in control group after saline infusion; 5, horse in glucose group before exercise; and 6, horse in glucose group after glucose infusion.

(day 3), compared with values before exercise. Furthermore, total content of GLUT-4 protein on day 4 was significantly greater (27.3% and 12.3%) after saline and glucose infusion, respectively, compared with values before exercise. Whereas content of GLUT-4 protein increased significantly after saline infusion (12.5% on day 4, compared with values before infusion on day 3), the increase after glucose infusion was smaller but not significantly (P = 0.054) different (8.3%, compared with values before infusion). Content of GLUT-4 protein in the muscle samples collected after infusion on day 4 was higher, but not significantly so, in the control group, compared with values after infusion in the glucose group.

Discussion

The results reported here provide evidence for GLUT-4 protein in equine skeletal and cardiac muscles. In this study, we characterized GLUT-4 by evaluating the specificity of the immunoreactive bands detected by use of immunoblotting, and we determined the molecular weight of the GLUT-4 protein was approximately 50 kd. We also documented that exercise leads to an increase in content of GLUT-4 protein in skeletal muscle, and replenishment of muscle glycogen stores by glucose infusion after exercise attenuated the increase in content of GLUT-4 protein in skeletal muscle.

It is somewhat difficult to perform immunoblotting to specifically identify equine GLUT-4 because of the lack of species-specific antibody. Furthermore, ruling out nonspecific bands is critical when using immunoblotting because nonspecific bands can appear in crude homogenates, especially when investigators use nonradioactive systems of detection.[a] These nonspecific bands could correspond to nonspecific binding of the primary or secondary antibody to the most common proteins found in muscle tissues (ie, myosin heavy chain, actin, troponin, or tropomyosin).[13] Furthermore, dimers of GLUT-4 result in bands of differing molecular weights.[14] Our results clearly documented the specificity of the immunoreactivity of the bands corresponding to GLUT-4 by ruling out evidence of nonspecific bands. Furthermore, GLUT-4 protein was only detected in tissues in which glucose uptake is sensitive to insulin (ie, cardiac and skeletal muscles) and not in noninsulin-sensitive tissues (ie, liver), suggesting an insulin-sensitive protein.[15] Our results also revealed that GLUT-4 proteins in rat and equine muscles have the same molecular weight (approx 50 kd), which is in agreement with results of other experiments conducted in rats and other herbivores.[13] In contrast, a band with a molecular mass of 42 to 45 kd has also been reported in skeletal muscle of rats,[16] calves and goats,[5] and birds.[17] These differences in the theoretic calculation of the molecular mass of GLUT-4 can be explained on the basis of the use of various methods to calculate molecular weight, differing conditions of electrophoresis, or nonspecific binding of the primary antibody to other proteins in muscle tissue when the specificity of the bands was not tested beforehand.[13] Finally, these results also revealed that the use of crude membrane preparations containing sarcolemmal and sarcoplasmic regions is suitable for the sensitive detection of GLUT-4 protein. Therefore, analysis of these results revealed that we used immunoblot analysis to detect insulin-sensitive GLUT-4 in equine skeletal and cardiac muscles.

Two distinct phases are associated with glucose uptake by muscles after exercise: an initial short insulin-independent phase in which GLUT-4 is translocated through a contraction-mediated process, and a second longer phase (up to 48 hours) that is characterized by an increase in insulin sensitivity of the muscles.[18] Similar to the results of studies[19,20] in other species, the study reported here documented that content of GLUT-4 protein in skeletal muscle increased after exercise, especially in the control group, which could suggest an increase in intracellular content of GLUTs or an increase in translocation of GLUT-4 to the plasma membrane for glucose uptake. Similar to results of another study,[15] we determined that content of GLUT-4 protein increased only slightly immediately after exercise. Furthermore, the amplitude of the increase in GLUT-4 content measured 22.2 hours after exercise appeared to be lower than that reported in rodents.[21] The persistent increase in content of GLUT-4 protein 22.2 hours after exercise suggested that increased GLUT-4 content in equine skeletal muscle may also be an insulin-mediated event.[22] Content of GLUT-4 protein after exercise and infusion of glucose or saline solution was higher in horses with persistent depletion of muscle glycogen (control group), compared with values for horses with replenished muscle glycogen stores (glucose group), suggesting that the increase in content of GLUT-4 protein observed after infusion in the control group contributed to the synthesis of muscle glycogen stores. Therefore, analysis of the results for the control group suggested that, similar to other species, exercise induced an increase in content of GLUT-4 protein in a biphasic manner, and this increase was associated with postexercise glycogenesis.

Analysis of the results of our study also revealed that IV infusion of glucose hastened postexercise synthesis of muscle glycogen in horses, which is similar to results reported in humans.[23,24] However, despite a substantial increase in glucose availability and hastened muscle glycogenesis, content of GLUT-4 protein was not significantly different before and after infusion of glucose, whereas there was a significant increase after infusion of saline solution. These results in horses are in contrast with results reported[21,25] in humans and rats in which ingestion of a carbohydrate supplement after exercise enhanced the exercise-induced increase in concentrations of GLUT-4 protein. Because the content of GLUT-4 protein is positively correlated (r, 0.81) with muscle glycogen stores in other species,[21] we expected an increase in content of GLUT-4 protein after glucose infusion in these horses. However, because the concentration of glycogen in muscle of resting horses is higher than for other species,[26] high concentrations of muscle glycogen (observed after glucose infusion) may reverse the increase in insulin sensitivity normally observed after exercise and carbohydrate supplementation[27] and prevent further increases

in GLUT-4 translocation to the plasma membrane via negative feedback.[4] This phenomenon has been described[27] in humans and rodents with supercompensation of muscle glycogen after glycogen-depleting exercise and carbohydrate supplementation. These results provide evidence that replenishment of muscle glycogen stores in the horses reported here was associated with a decrease in total content of GLUT-4 protein, suggesting that muscle concentrations of glycogen may play a regulatory role for GLUT-4.[4] Furthermore, on the basis of analysis of our results, we speculate that GLUT-4 is the rate-limiting factor for glucose uptake and secondary glycogenesis, although we did not measure glucose uptake in this study. Indeed, we speculate that as soon as normal concentrations of glycogen are restored in the muscles after carbohydrate administration, muscle glycogen inhibits further GLUT-4 translocation to the plasma membrane,[4] which will then limit further replenishment of muscle glycogen. In support of this theory is the observation that supercompensation of muscle glycogen is not observed after glycogen-depleting exercise and carbohydrate administration in horses.[28,o]

The lower content of GLUT-4 protein in horses after glucose infusion may also be related to the peculiarities of glucose metabolism observed in herbivores. In roughage-fed animals, a limited amount of glucose is absorbed in the small intestine because most of the glucose supply is from volatile fatty acids produced in the large colon.[29] Therefore, similar to ruminants, peripheral tissues of horses may be less sensitive to insulin, with secondary decreased glucose uptake and GLUT-4 translocation to the plasma membrane.[5] However, it should be mentioned that 1 limitation of the study reported here is that content of GLUT-4 protein was isolated from crude membrane preparations and not from plasma membrane isolations, allowing quantification of total content of GLUT-4 protein but not quantification of GLUTs available at the plasma membrane for glucose uptake. However, studies[21,30] in rodents and humans that used total crude membrane preparations revealed that exercise and feeding increase content of GLUT-4 protein, implying that this technique is adequate to quantify GLUTs. Therefore, the differences in GLUTs observed in our study may reflect differences in glucose metabolism in horses, rather than inadequate muscle preparations.

The study reported here provided biochemical and functional evidence for the insulin-sensitive GLUT-4 protein in equine skeletal muscles. However, additional studies that involve the use of plasma membrane isolations are required to confirm the physiologic role of GLUTs in horses.

[a]Hocquette JF, Castiglia C, Ferré P, et al. Variations in GLUT-4 protein content among bovine adipose tissues (abstr), in *Proceedings*. Nutr Soc 1996;55:21A.
[b]Carbocaine, Abbott Laboratories, North Chicago, Ill.
[c]Angiocath, Deseret, Sandy, Utah.
[d]Sequoia-Turner model 112, Turner Design, Sunnyvale, Calif.
[e]YSI model 1500, Yellow Springs Instruments, Yellow Springs, Ohio.
[f]Polytron homogenizer, Kinematica AG, Littau-Lucerne, Switzerland.
[g]70 Ti rotor ultracentrifuge, Beckman Instruments Inc, Palo Alto, Calif.
[h]Versamax, Molecular Devices Corp, Sunnyvale, Calif.
[i]BCA protein assay, Pierce Biotechnology, Rockford, Ill.
[j]PVDF, Millipore Corp, Bedford, Mass.
[k]Glucose transporter 4, Biogenesis Inc, Sandown, NH.
[l]Anti-rabbit IgG peroxidase conjugate, Calbiochem, San Diego, Calif.
[m]LumiGlo, Kirkegaard & Perry Laboratories, Gaithersburg, Md.
[n]Prestained standards, Bio-Rad Laboratories, Hercules, Calif.
[o]Lacombe VA, Hinchcliff KW, Kohn CW, et al. Post-exercise feeding of meals of varying glycemic index affects muscle glycogen resynthesis in horses (abstr). *J Vet Intern Med* 2002;16:336.

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