Time course of insulin sensitivity and skeletal muscle glycogen synthase activity after a single bout of exercise in horses

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The time course of insulin sensitivity, skeletal muscle glycogen and GLUT4 content, and glycogen synthase (GS) activity after a single bout of intense exercise was examined in eight horses. On separate days, a euglycemic-hyperinsulinemic clamp (EHC) was undertaken at 0.5, 4, or 24 h after exercise or after 48 h of rest [control (Con)]. There was no increase in glucose infusion rate (GIR) with exercise (0.5-, 4-, and 24-h trials), and GIR was significantly decreased at 0.5 h postexercise (GIR: 8.6 ± 2.7, 6.7 ± 2.0, 9.0 ± 2.0, and 10.6 ± 2.2 mg·kg⁻¹·min⁻¹ for Con and at 0.5, 4, and 24 h, respectively). Before each EHC, muscle glycogen content (mmol glucosyl units/kg dry muscle) was higher (P < 0.05) for Con (565 ± 102) than for other treatments (317 ± 84, 362 ± 79, and 382 ± 74 for 0.5, 4, and 24 h, respectively) and muscle GLUT4 content was unchanged. Pre-EHC active-to-total GS activity ratio was higher (P < 0.05) at 0.5, 4, and 24 h after exercise than in Con. Post-EHC active GS and GS activity ratio were higher (P < 0.05) in Con and at 24 h. There was a significant inverse correlation (r = -0.43, P = 0.02) between glycogen content and GS activity ratio but no relationship between GS activity and GIR. The lack of increase in insulin sensitivity, determined by EHC, after exercise that resulted in a significant reduction in muscle glycogen content is consistent with the slow rate of muscle glycogen resynthesis observed in equine studies.

MUSCLE GLYCOGEN CONTENT IS AN IMPORTANT DETERMINANT OF BOTH HIGH- AND MODERATE-INTENSITY EXERCISE PERFORMANCE IN HORSES (24). After an intense bout of exercise, muscle glycogen content may be reduced to less than half of the initial levels (10, 24). Therefore, for horses required to undertake exercise on successive days, an adequate rate of postexercise muscle glycogen resynthesis becomes a critical issue. Studies in humans and rodents have shown that the rate of skeletal muscle glycogen synthesis after exercise is dependent on several factors, including initial muscle glycogen content, carbohydrate availability, glucose transport into muscle, and the activity of glycogen synthase (GS) (6, 7). In these species, there is an increase in whole body and skeletal muscle insulin sensitivity and a concurrent increase in insulin-stimulated GS activity in skeletal muscle after a single bout of exercise (6, 7, 31). Furthermore, the magnitude of these postexercise increases in insulin sensitivity and GS activity is inversely related to muscle glycogen content, suggesting that muscle glycogen itself regulates the enhanced insulin action on glucose metabolism after exercise (17, 31).

Postexercise increases in insulin sensitivity are thought to facilitate rapid muscle glycogen synthesis after glycogen-depleting exercise (26, 37, 47, 48) and potentially enable the process of glycogen storage to allow supercompensation (i.e., contents higher than before exercise). In this context, it has been suggested that insulin action on glucose metabolism is unchanged after exercise in horses (23, 25), potentially accounting for the slow rate of postexercise muscle glycogen resynthesis (up to 48–72 h) observed in this species (8, 25). However, to date, there have been no measurements of insulin sensitivity following a single bout of exercise in horses. Therefore, the purpose of this study was to investigate the time course of insulin sensitivity, skeletal muscle glycogen and GLUT4 protein contents, and muscle GS activity after a single bout of glycogen-depleting exercise in horses. Given previous observations of slow postexercise muscle glycogen resynthesis (9, 17, 32, 35, 43), we predicted no change in insulin sensitivity during a 24-h period after a single bout of intense exercise.

MATERIALS AND METHODS

Animals. All procedures were approved by the University of Guelph’s Animal Care Committee, and all protocols were in accordance with the guidelines of the Canadian Council on Animal Care. Eight mature Standardbred horses [6 geldings, 2 mares, 428 ± 35 (SD) kg body wt, age 4–5 yr] that had undergone at least 2 mo of physical conditioning by running on a treadmill (3° incline; Sato) before the experimental trials were used in the study. Horses were housed in 3.5-m × 4-m box stalls and fed a daily ration of 5–6 kg of mixed grass hay and 4–5 kg of a pelleted concentrate (dry matter basis: 13% crude protein, 38.2% neutral detergent fiber, 14.2% fat). Salt and water were available ad libitum. Horses were turned out into a small paddock for ∼3 h daily during which time horses were fitted with muzzles to prevent grazing.

Experimental design. A replicated 4 × 4 Latin square design was used to determine the effects of time after a single bout of exercise on dependent variables. A 120-min euglycemic-hyperinsulinemic clamp (EHC) was administered in the resting state [no exercise in the previous 48 h; control (Con)] and at 0.5, 4, or 24 h after an ~1-h bout of exercise. The exercise was undertaken between 0700 and 0800. Therefore, the Con, 0.5-h, and 24-h EHCs were administered between ~0800 and 1100, whereas the 4-h EHC was commenced between 1200 and 1300. In all experiments, feed was withheld starting at 1900 the day before the EHC. In the 0.5- and 4-h treatments, horses were denied access to feed during the period between the end of exercise and the start of the EHC. In the 24-h postexercise treatment, horses...
received ~3 kg of hay at 12 h postexercise. For each horse, the order of trials was randomized, and there was a 10-day interval between trials.

**Incremental exercise test.** Before the experiments, horses completed an incremental exercise test for measurement of the peak rate of oxygen consumption (\(\dot{V}O_{2\text{peak}}\)). With the treadmill set at a 3° incline, horses completed a 5-min warm up at 4 m/s, and then a loose-fitting face mask was applied for collection of respiratory gases. Horses resumed trotting at 4 m/s for 90 s, after which the speed of the treadmill was increased by increments of 1 m/s every 60 s until horses reached fatigue. Fatigue was defined as the point at which the horse could no longer keep pace with the treadmill despite verbal encouragement. Oxygen consumption was measured continuously by use of an open-circuit indirect calorimeter (Oxymax-XL; Columbus Instruments, Columbus, OH), and the highest value recorded was designated as \(\dot{V}O_{2\text{peak}}\). The oxygen and carbon dioxide analyzers were calibrated against gases of known composition before each experiment, and the overall accuracy of the system was verified by the nitrogen dilution technique. For each horse, linear regression analysis was used to determine the slopes corresponding to 50, 75, and 100% of \(\dot{V}O_{2\text{peak}}\), and these slopes were used for the exercise protocol.

**Exercise protocol.** Horses completed an exercise protocol on a treadmill set at a 3° incline. The exercise consisted of a 5-min warm up at 4 m/s, followed by 15 min at a speed that elicited 50% \(\dot{V}O_{2\text{peak}}\), 20 min at 75% \(\dot{V}O_{2\text{peak}}\), and four 1-min sprints at 100% \(\dot{V}O_{2\text{peak}}\), each separated by a 5-min walk (1.6 m/s). This protocol was used because it could be accomplished by all horses in the trial and, without invoking a period of feed deprivation, would consistently deplete glycogen stores by ~45–50% of the initial value, a quantity sufficient to stimulate glycogen repletion.

**EHC.** A 120-min EHC was conducted at the designated times after exercise or at rest. Either before exercise (0.5-h treatment) or 45 min before the EHC (all other treatments), catheters (14 gauge, 5.25 in.) were inserted into both jugular veins after aseptic preparation and desensitization of the overlying skin. One of these catheters was subsequently used for infusion of glucose and insulin and the other for collection of blood samples.

Initial blood samples were collected into tubes (Vacutainer, Fisher Health Care, Chicago, IL) containing either no additive (to harvest serum) or potassium EDTA (for plasma). After this collection, blood samples (20 mL) were homogenized in buffer (50 µg/mL) and centrifuged at 7,000 g at 4°C for 5 min, and aliquots of the supernatant (cytosolic fraction) were used for determination of active (I form) and total GS (I + D forms) activity. The reaction was started with the addition of 8 mM UDP-glucose and stopped by heating at 90°C for 2.5 min. Samples were centrifuged, and the supernatant was removed for fluorometric assay of UDP-GS activity. GS activity was calculated as nanomoles of UDP-glucose incorporated into glycogen per hour per milligram of protein. Muscle protein concentration of GS homogenates was measured with a bicinchoninic acid reagent kit (Pierce, Rockford, IL), and the ratio of the GS activities at 0 and 10 mM glucose-6-phosphate (G6P; I + D forms) was calculated.

**Muscle biopsy.** Five minutes before and at the end of the EHC (before termination of the insulin and glucose infusion), samples of middle gluteal muscle were taken by use of the needle biopsy technique (27). Specimens (~300–500 mg wet wt) were collected under aseptic conditions after desensitization of the area with 2% mepivacaine (Carbocaine, Deseret, Sandy, UT). The samples were collected at a uniform depth and site, alternating between the left and right middle gluteal muscles. Muscle samples were immediately flash frozen in liquid nitrogen and stored at ~−80°C until analysis of glycogen and GLUT4 protein content and GS activity.

**Blood analyses.** Serum immunoreactive insulin and cortisol were measured in duplicate by radioimmunoassay with commercial kits (Coat-a-Count, Diagnostic Products, Los Angeles, CA) validated for use in samples obtained from horses. Intra-assay coefficients of variation for insulin and cortisol were 7.3% and 6.1%, respectively. Plasma glucose and NEFA concentrations were measured by using a spectrophotometrically with a microplate reader and commercially available kits (glucose from Infinity Reagent, Thermo Electron, Waltham, MA; NEFA from WAKO Chemicals USA, Richmond, VA). Plasma lactate concentrations were measured by use of an autoanalyzer (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). Intra-assay coefficients of variations for the glucose, NEFA, and lactate assays were, respectively, 3.9, 4.5, and 2.5%.

**Muscle analyses.** A piece of each muscle sample was lyophilized and dissected free of visible blood, fat, and connective tissue. Aliquots of each sample (~20 mg dry wt) were powdered and subjected to acid hydrolysis before spectrophotometric measurement of glycogen content (as glucosyl units) as described previously (4).

Frozen wet muscle (~10 mg) was removed from each biopsy under liquid nitrogen for the determination of GS activity as previously described (34). Briefly, samples were homogenized in buffer (50 µg/mL) and centrifuged at 7,000 g at 4°C for 5 min, and aliquots of the supernatant (cytosolic fraction) were incubated for 45 min at 37°C for the determination of active (I form) and total GS (I + D forms) activity. The reaction was started with the addition of 8 mM UDP-glucose and stopped by heating at 90°C for 2.5 min. Samples were centrifuged, and the supernatant was removed for fluorometric assay of UDP-GS activity. GS activity was calculated as nanomoles of UDP-glucose incorporated into glycogen per hour per milligram of protein. Muscle protein concentration of GS homogenates was measured with a bicinchoninic acid reagent kit (Pierce, Rockford, IL), and the ratio of the GS activities at 0 and 10 mM glucose-6-phosphate (G6P; I + D forms) was calculated.

**Western immunoblotting for GLUT4.** Immunoblot analysis was performed to determine total GLUT4 content in muscle homogenates as previously described (24, 29). In brief, frozen muscle (30 mg) was homogenized on ice in protein lysis buffer, and equal amounts of protein (40 µg) were resolved on a 10% SDS-polyacrylamide gel before transfer to a nitrocellulose membrane (Hybond ECL, Amer sham Biosciences, Buckinghamshire, UK). Membranes were incubated with a polyclonal antibody directed against rabbit GLUT4 (Biogenesis, Poole, UK) and subsequently an anti-rabbit horseradish peroxidase-linked antibody (Cell Signaling Technology, Beverly, MA). Protein bands were visualized with an enhanced chemiluminescence detection system (Alpha-Innotech FluorChem IS-9900, San Leandro, CA). GLUT4 protein was corrected to α-tubulin (Sigma-Aldrich, St. Louis, MO) protein bands imaged on the same membrane.

**Statistical analyses.** Descriptive statistics of continuous variables are expressed as means ± SD. Normality was tested using the Kolmogorov-Smirnov statistic. A two-way repeated-measures ANOVA was used to determine the effects of treatment and the time of the clamp on EHC, muscle glycogen, total GS, and GS activity ratio, and plasma lactate, NEFA, and cortisol and serum insulin concentrations. Data for GLUT4 protein content were analyzed by one-way repeated-measures ANOVA. Pearson correlation coefficients were calculated to determine the relationship between GS activity and muscle glycogen content and between muscle glycogen content and GIR. The null
hypothesis was rejected at \( P < 0.05 \). Significant differences between means were identified by use of the Bonferroni \( t \)-test. A software program (Sigmastat 3.0, SSPS, Chicago, IL) was used for statistical analyses.

**RESULTS**

The mean \( \dot{V}_{O_{2peak}} \) of the horses was 119.5 ± 17.8 ml·kg\(^{-1} \)·min\(^{-1} \). Mean running speeds corresponding to 50, 75, and 100% \( \dot{V}_{O_{2peak}} \) during the exercise trials were 6.2 ± 0.8, 8.7 ± 0.7, and 11.8 ± 0.9 m/s, respectively. One horse did not complete the 24-h treatment due to lameness. Accordingly, the data for the 24-h treatment represent the means ± SD for seven horses.

Blood glucose concentration before the start of the EHC was higher in the 0.5-h treatment (5.3 ± 0.7 mM) than in the other treatments (\( P < 0.001 \); 3.8 ± 0.3, 3.6 ± 0.4, 4.0 ± 0.1 mM for the Con and 4- and 24-h treatments, respectively). In all trials, a steady-state blood glucose concentration was reached after 40–55 min of the commencement of the EHC (Table 1). Serum insulin concentrations before and during the EHC were similar among the four trials, increasing significantly from resting values (<10 \( \mu \)U/ml) and reaching a plateau of ~260–280 \( \mu \)U/ml after 45 min, which was sustained until the end of the EHC (Table 1). In each EHC, there was no effect of time during the clamp (60–90 min vs. 90–120 min) on mean GIR (Fig. 1). Over the final 60 min of each EHC, mean GIR at 0.5 h (6.7 ± 2.0 mg·kg\(^{-1} \)·min\(^{-1} \)) was significantly \(( P < 0.05 \) lower than that shown for the other treatments (Con: 8.6 ± 2.7; 4 h: 9.0 ± 2.0; 24 h: 10.6 ± 2.2 mg·kg\(^{-1} \)·min\(^{-1} \)). Mean GIR at 4 and 24 h did not differ from GIR of Con.

Plasma lactate concentration at the start of the EHC (0 min) was significantly higher at 0.5 h than at the other times (Table 2). Similarly, plasma cortisol concentration was higher in 0.5 h than in Con, 4 h, and 24 h at the start of the EHC and after 60 min but not different compared with the other treatments after 120 min (Table 2). Prior exercise (0.5, 4, and 24 h) resulted in higher \(( P < 0.001 \) plasma NEFA concentrations at 0 min of the EHC compared with Con (Table 2). Plasma NEFA after 60 min of the EHC remained higher at 0.5 and 4 h than that shown for Con. In all trials, there was a marked decrease in plasma NEFA between 0 and 120 min of the EHC.

In all trials preceded by exercise, mean muscle glycogen content was significantly lower than for Con (44%, 36%, and 33% in 0.5, 4, and 24 h groups, respectively; Fig. 2). In all treatments, pre- and post-EHC muscle glycogen content did not differ. There was no significant relationship between GIR and muscle glycogen content (Fig. 3). Mean muscle GLUT4 protein content, analyzed in samples obtained before each EHC, did not differ among treatments (arbitrary units; Con: 1.1 ± 0.2; 0.5 h: 0.9 ± 0.15; 4 h: 1.2 ± 0.2; 24 h: 1.0 ± 0.1).

There was no effect of treatment or sampling time on total GS activity (10 mM G6P). Total GS activities for Con, 0.5 h, 4 h, and 24 h were 90 ± 15, 102 ± 19, 84 ± 14, and 93 ± 10 nmol·h\(^{-1} \)·mg protein\(^{-1} \), respectively, in pre-EHC samples and 94 ± 12, 99 ± 11, 90 ± 10, and 97 ± 13 nmol·h\(^{-1} \)·mg protein\(^{-1} \), respectively, in post-EHC samples. However, for pre-EHC samples, GS in the active or I form (0 mM G6P) was significantly higher in the trials preceded by exercise (Fig. 4A).

![Fig. 1. Glucose infusion rate (GIR) during the euglycemic-hyperinsulinemic clamp (EHC) in the 4 treatments. For each treatment, mean GIR did not differ between the 2 periods of assessment (60–90 min and 90–120 min). Data are means ± SD for 8 horses under control conditions (Con) and at 0.5 and 4 h after exercise and for 7 horses at 24 h after exercise. *Significant difference (\( P < 0.05 \)) between 0.5 h and all other treatments.](http://jap.physiology.org/)

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**Table 1. Serum insulin and blood glucose concentrations at rest and during the 2-h EHC in each treatment**

<table>
<thead>
<tr>
<th>Glucose, mM</th>
<th>Con</th>
<th>0.5 h</th>
<th>4 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>3.8±0.3a</td>
<td>5.3±0.7b</td>
<td>3.6±0.4a</td>
<td>4.0±0.1a</td>
</tr>
<tr>
<td>30 min</td>
<td>4.8±0.5</td>
<td>5.2±0.3</td>
<td>4.7±0.4</td>
<td>4.8±0.6</td>
</tr>
<tr>
<td>60 min</td>
<td>5.1±0.2</td>
<td>5.1±0.2</td>
<td>5.2±0.3</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td>90 min</td>
<td>5.1±0.1</td>
<td>5.2±0.1</td>
<td>5.1±0.2</td>
<td>4.9±0.2</td>
</tr>
<tr>
<td>120 min</td>
<td>5.0±0.2</td>
<td>5.1±0.2</td>
<td>5.0±0.2</td>
<td>5.1±0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insulin, ( \mu )U/ml</th>
<th>Con</th>
<th>0.5 h</th>
<th>4 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>6.3±1.1</td>
<td>8.8±2.2</td>
<td>8.4±1.6</td>
<td>6.7±1.5</td>
</tr>
<tr>
<td>30 min</td>
<td>208±24.1a</td>
<td>225±27.3a</td>
<td>242±12.9a</td>
<td>239±20.2a</td>
</tr>
<tr>
<td>60 min</td>
<td>346±23.9a</td>
<td>284±24.6a</td>
<td>264±19.1a</td>
<td>250±19.9a</td>
</tr>
<tr>
<td>90 min</td>
<td>263±24.4a</td>
<td>281±32.1a</td>
<td>288±18.9a</td>
<td>279±24.7a</td>
</tr>
<tr>
<td>120 min</td>
<td>273±29.2a</td>
<td>282±29.3a</td>
<td>277±20.1a</td>
<td>281±15.6a</td>
</tr>
</tbody>
</table>

Values are means ± SD for 8 horses under control (Con) conditions and at euglycemic-hyperinsulinemic clamp (EHC) 0.5 and 4 h after exercise and for 7 horses at EHC 24 h after exercise. Means within rows with different superscript letters differ significantly \(( P < 0.05 \). *Significant \(( P < 0.05 \) difference from 0 min within treatment.

**Table 2. Serum cortisol, plasma NEFA, and plasma lactate concentrations during the 2-h EHC**

<table>
<thead>
<tr>
<th>Cortisol, ( \mu )g/ml</th>
<th>Control</th>
<th>0.5 h</th>
<th>4 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>8.3±1.6a</td>
<td>15.5±3.3b</td>
<td>6.9±2.5a</td>
<td>9.1±2.3a</td>
</tr>
<tr>
<td>60 min</td>
<td>6.8±2.1a</td>
<td>10.4±2.3a</td>
<td>5.7±1.8a</td>
<td>7.2±2.7a</td>
</tr>
<tr>
<td>120 min</td>
<td>7.7±1.6a</td>
<td>9.6±2.6a**</td>
<td>7.5±3.1a</td>
<td>7.7±1.4a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NEFA, mM</th>
<th>0 min</th>
<th>0.15±0.10a</th>
<th>0.58±0.30a</th>
<th>0.52±0.20b</th>
<th>0.34±0.20b</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 min</td>
<td>0.03±0.02**</td>
<td>0.10±0.06**</td>
<td>0.11±0.06b*</td>
<td>0.07±0.03**</td>
<td></td>
</tr>
<tr>
<td>120 min</td>
<td>0.03±0.02**</td>
<td>0.05±0.03**</td>
<td>0.03±0.03**</td>
<td>0.03±0.01**</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lactate, mM</th>
<th>0 min</th>
<th>0.8±0.1a</th>
<th>5.8±1.9b</th>
<th>0.9±0.2a</th>
<th>0.9±0.3a</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 min</td>
<td>0.9±0.1a</td>
<td>1.8±0.4b*</td>
<td>1.0±0.2b</td>
<td>1.1±0.3a</td>
<td></td>
</tr>
<tr>
<td>120 min</td>
<td>1.0±0.1a</td>
<td>1.2±0.2**</td>
<td>1.0±0.3a</td>
<td>1.1±0.4a</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD for 8 horses in Con and 0.5- and 4-h groups and for 7 horses in the 24-h group. NEFA, nonesterified fatty acid. Means within rows with different superscript letters differ significantly \(( P < 0.05 \). *Significantly different \(( P < 0.05 \) from 0 min.
In the Con and 24-h trials, both active GS and the GS activity ratio were significantly higher in post- vs. pre-EHC samples (Fig. 4, A and B). Similarly, the ratio of active to total GS activity (I/I + D) in pre-EHC samples was higher (P < 0.01) in the 0.5-, 4-, and 24-h treatments than in Con (Fig. 4B; 307%, 272%, and 233% of Con for 0.5-, 4-, and 24-h treatments, respectively). However, in the 0.5- and 4-h treatments, there was no change in active GS or the GS activity ratio when pre- and post-EHC samples were compared.

There was a significant inverse linear relationship (r = −0.43, P = 0.02) between muscle glycogen content and the GS activity ratio in pre-EHC samples (Fig. 5A). There was also an inverse relationship (r = −0.36) between muscle glycogen content and active GS (0 mM G6P) in pre-EHC samples, but this did not achieve statistical significance (P = 0.06) (Fig. 5B). The relationships between GIR and active GS (r = 0.13, P = 0.59) and between GIR and the GS activity ratio (r = 0.07, P = 0.65) also were not significant.

**DISCUSSION**

The present study examined the time course of changes in insulin sensitivity and skeletal muscle GS activity in trained horses after a single bout of exercise. The main findings were 1) a decrease in whole-body insulin sensitivity (assessed by mean GIR during the final 60 min of the EHC) measured 0.5 h after exercise and no change in insulin sensitivity in the 4- and 24-h postexercise treatments compared with the control condition, 2) an increase in the GS activity ratio after hyperinsulinemia in nonexercised horses (Con), 3) a pre-EHC increase in the GS activity ratio in all exercise treatments with a further (insulin-induced) increase in this ratio at 24 h, but not at 0.5 and 4 h postexercise, and 4) no change in crude muscle membrane GLUT4 in any treatment following exercise. The lack of increase in insulin sensitivity observed after exercise in this study may help to explain the slow rate of postexercise glycogen replenishment observed following glycogen-depleting exercise in horses (8, 9, 15, 32, 35, 43).

This study utilized a 2-h EHC, a procedure that has been used in previous equine studies (42, 44). One limitation was the lack of measurement of EGP, with verification that the insulin infusion protocol suppressed EGP in each treatment condition. Nonetheless, in preliminary studies, the identical infusion protocol completely suppressed EGP, and it is therefore reasonable to assume that values for GIR during the final 60 min of the clamp were reflective of whole-body glucose disposal. We also had no measure of skeletal muscle glucose uptake, although, at least in rodents and humans, skeletal muscle (~50% of body mass in horses) is the major site of glucose disposal under conditions of insulin stimulation such as an EHC (11).
Postexercise, a time frame in which any contraction-mediated enhancement of insulin sensitivity would be expected to occur (12, 36).

Exercise-induced alterations in counterregulatory hormones such as cortisol, glucagon, and epinephrine and increases in NEFA could mask enhancement of insulin sensitivity shortly after exercise (2, 12, 13, 30, 33). In the present study, serum cortisol concentrations were higher than Con in the 0.5-h trial but were not different from Con in the 4- and 24-h trials. This elevation in serum cortisol evident 30 min after exercise may have contributed to the lower GIR noted in the 0.5-h postexercise treatment. Although exercise-induced changes in NEFA (45) and lactate concentrations (5) have been implicated in modulating changes in insulin sensitivity, no relationship between NEFA or lactate concentrations and insulin sensitivity was evident in this study.

Insulin and exercise/contractile activity are the most important physiological stimuli of glucose uptake and each can contribute to increases in GS activity in skeletal muscle. Both acute and chronic exercise have been demonstrated to enhance insulin-stimulated GS activity (1, 6, 20, 47, 48) although exercise-associated increases in the GS activity ratio noted in horses are less marked than observed in humans (3, 19, 47). Lacombe et al. (25) reported a GS activity ratio in resting horses similar to that noted in the nonexercise treatment in the present study, with a doubling of the ratio after strenuous exercise but no further increase in this ratio when a high-carbohydrate meal was consumed immediately after exercise. The absence of an insulin-induced increase in basal GS and the GS activity ratio at 0.5 and 4 h is consistent with the observations of these investigators. In our study, there was a prolonged time course (at least 24 h) of increased GS activity in the horses, and this sustained increase in the GS activity ratio at 24 h may reflect the lack of change in muscle glycogen content compared with that measured in the early postexercise treatments.

Insulin alone can stimulate GS by promoting its dephosphorylation via inhibition of glycogen synthase kinase-3 (GSK3) (α and β) after phosphorylation of this enzyme by Akt/protein kinase B at NH2-terminal serine residues (Ser21 in GSK3α and Ser9 in GSK3β). In humans, insulin stimulation has been shown to increase Akt phosphorylation and GS activity, following enhanced GSK3α deactivation/GS activation (1, 19, 20, 47). In the present study, GS activity increased after the EHC in the resting state (Con) or 24 h after exercise, a finding consistent with an insulin-induced increase in GS activity.

In human studies, increased GS activity has also been noted following exercise, with an associated increase in Akt phosphorylation and enhanced phosphorylation of GSK3α on inhibitory site Ser21 (39). An increase in the GS activity ratio was evident after all exercise treatments in the present study, but this activity ratio was not further enhanced by insulin stimulation in EHCs performed in the early (0.5 and 4 h) postexercise period. In a companion study using a similar protocol in horses (29), the authors of the present study observed that exercise resulted in increased Akt phosphorylation (at Ser727) and GSK3α phosphorylation (at Ser21) (but not GSK3β) at Ser21 detectable at 30 min but not at subsequent time points following exercise, possibly reflecting a short timeframe for Akt phosphorylation/activation after muscle contraction (40, 41). Whereas hyperinsulinemia resulted in a further increment in Akt phosphorylation in each treatment, additional insulin-
induced increases in GSK3α phosphorylation were only observed in the nonexercised and 24-h postexercise treatments (29), a finding consistent with unchanged GS activity ratio noted following the EHC at 0.5 and 4 h postexercise in the present study.

In the skeletal muscle samples obtained before each EHC, there was no detectable change in crude muscle membrane GLUT4 protein content. This observation is similar to that of Nout et al. (32) in which no changes in muscle GLUT4 protein or mRNA content were observed in horses after 60 min of moderate-intensity treadmill exercise, which lowered muscle glycogen content to ~50% of its initial value. On the other hand, increases in GLUT4 protein (23) and mRNA content (18) were observed in middle gluteal skeletal muscle of horses after 3 consecutive days of intense exercise, which lowered muscle glycogen content by >70%. In this instance, the changes in muscle GLUT4 measured after the final day of exercise were likely the result of exercise training adaptations (14, 21). The absence of a postexercise increase in insulin sensitivity in the horses in this study would be consistent with little increase in the relative permeability of the cell membrane for glucose transport. The finding that equine skeletal muscle GLUT4 content is not enhanced after a single bout of exercise observed in this study and others (8, 15, 32, 35) would support whether the rate and extent of GLUT4 translocation to the cell membrane can explain the unchanged insulin sensitivity and slow rate of postexercise glycogen resynthesis in this species. In summary, the results of this study demonstrate that insulin sensitivity in horses is not increased by an acute bout of moderately intense exercise that results in a 50% reduction in muscle glycogen content. Exercise increased the GS activity ratio, and this ratio was not further increased by induced insulinemia at 0.5 and 4 h after exercise. Little change in muscle glycogen content up to 24 h following exercise was consistent with the lack of increase in insulin sensitivity during this period. These findings contribute to the understanding of the mechanism associated with the slow postexercise replenishment of muscle glycogen observed in horses compared with other species.

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REFERENCES


