Muscle glycogen depletion and subsequent replenishment affect anaerobic capacity of horses

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Lacombe, Veronique A., Kenneth W. Hinchcliff, Ray J. Geor, and Carole R. Baskin. Muscle glycogen depletion and subsequent replenishment affect anaerobic capacity of horses. J Appl Physiol 91: 1782–1790, 2001.—The purpose of this study was to determine the effect of muscle glycogen depletion and subsequent replenishment on anaerobic capacity of horses. In a blinded crossover study, seven fit horses performed glycogen-depleting exercise on two occasions. Horses were infused after glycogen-depleting exercise with either 6 g/kg body wt of glucose as a 13.5% solution in 0.9% NaCl (Glu) or with 0.9% NaCl (Sal) of equivalent volume. Subsequently, horses performed a high-speed exercise test (120% of maximal rate of oxygen consumption) to estimate maximum accumulated oxygen deficit. Replenishment of muscle glycogen was greater (P < 0.05) in Glu [from 24.7 ± 7.2 (SE) to 116.5 ± 7 mmol/kg wet wt before and after infusion, respectively] than in Sal (from 23.4 ± 7.2 to 47.8 ± 5.7 mmol/kg wet wt before and after infusion, respectively). Run time to fatigue during the high-speed exercise test (97.3 ± 8.2 and 70.8 ± 8.3 s, P < 0.05), maximal accumulated oxygen deficit (105.7 ± 9.3 and 82.4 ± 10.3 ml O2 equivalent/kg, P < 0.05), and blood lactate concentration at the end of the high-speed exercise test (11.1 ± 1.4 and 9.2 ± 3.7 mmol/L, P < 0.05) were greater for Glu than for Sal, respectively. We concluded that decreased availability of skeletal muscle glycogen stores diminishes anaerobic power generation and capacity for high-intensity exercise in horses.

maximal accumulated oxygen deficit; oxygen consumption; lactate; glucose

CARBOHYDRATE IN THE FORM OF muscle glycogen is the energy source for anaerobic glycolysis during vigorous exercise in humans and horses. However, repeated bouts of maximal intensity exertion or a combination of prolonged running and repeated sprints results in declines of up to 50% in muscle glycogen concentration of horses (8, 17, 19, 26, 37, 38). Furthermore, many horses undertake several events in a single day, and the interval between exercise bouts may be inadequate for complete restoration of the muscle glycogen pool (37). It is possible that a reduction in muscle glycogen stores contributes to a decline in subsequent high-intensity exercise performance in horses, although this issue has received scarce attention. Whereas a reduction in muscle glycogen concentration by 22% did not have a measurable effect on duration of high-intensity exercise (8), exercise that depleted muscle glycogen by at least 55% of its initial value was associated with a marked reduction in anaerobic power generation during subsequent high-speed exercise (26). These latter results suggest that lower glycogen availability may contribute to a decline in anaerobic power generation during high-intensity exercise. However, this latter study did not conclusively demonstrate such an effect of muscle glycogen depletion because of the confounding effects of other exercise-induced changes, such as dehydration and musculoskeletal pain, on athletic capacity. Therefore, the effect of preexercise muscle glycogen depletion on athletic capacity of horses remains uncertain.

It is well recognized that provision of supplemental carbohydrate to humans increases the time to fatigue during moderate to mild exercise (6). Ingestion of foods with a high-glycemic index by humans before exertion increases preexercise muscle glycogen concentration and the time to fatigue and delays the decrease in muscle glycogen concentration with a subsequent increase in duration of exercise (23, 31, 35). Consumption of a high-carbohydrate diet for 3–4 days before exercise improves exercise capacity during high-intensity exercise, although this effect is less consistent than that with endurance exercise (28, 29). Whereas the observation that provision of supplemental glucose to horses performing endurance exercise on the treadmill increases the duration of exercise provides evidence that energy supplies limit performance in endurance exercise of horses (10), most athletic events involving horses are briefer or require repeated bouts of exercise in a shorter period of time. Furthermore, as previously noted, intramuscular glycogen stores may be a limiting factor during this type of activity in horses (26). Our hypothesis was that an exercise-induced reduction in muscle glycogen concentration of horses would reduce anaerobic capacity and that subsequent replenishment of muscle glycogen stores by glucose infusion would...
increase anaerobic capacity over values for horses with continued muscle glycogen depletion.

MATERIALS AND METHODS

Experimental design. The effect of muscle glycogen depletion on exercise performance was examined in a longitudinal and blinded study, using a partially counterbalanced randomized crossover design. Seven fit adult horses performed two trials separated by an 8-day interval (trials 1 and 2). Both trials involved the horses completing 3 consecutive days of strenuous exercise that depleted muscle glycogen by at least 55% of its initial values. After the last bout of glycogen-depleting exercise in each trial, horses were infused intravenously with either 6 g/kg body wt of glucose as a 13.5% solution in 0.9% NaCl (Glu) or with equivalent volume of isotonic (0.9%) NaCl (Sal). The maximal rate of oxygen consumption (Vo2 max), measured during an incremental exercise test, and maximal accumulated oxygen deficit (MAOD), measured during a single high-speed exercise test at 120% Vo2 max, were used to estimate, respectively, aerobic and anaerobic capacities of the horses. MAOD and Vo2 max were measured 3 and 4 days before the horses undertook the glycogen-depleting exercise and 12 and 36 h after the end of glucose or saline administration, respectively. The order of trials for each horse was randomized, but the overall design was balanced; the horses performed aerobic and anaerobic tests both in the depleted state and after repletion of the muscle glycogen stores (Fig. 1). This protocol was approved by the Institutional Laboratory Animal Care and Use Committee of the Ohio State University.

Horses. The subjects were seven clinically normal horses (6 Standardbreds and 1 Thoroughbred; 2 geldings and 5 females) with ages ranging from 2 to 7 yr and had body weight of 436 ± 7 kg (mean ± SE).

The animals had been accustomed during a 1-mo period to the treadmill barn, to walk and run on the treadmill, and to wearing the mask of the open-circuit indirect calorimeter. Horses were conditioned by running on the treadmill (at a 2° incline) 5 days/wk for 2 mo. The conditioning consisted of the horses trotting at 4 m/s for 5 min, walking at 2 m/s for 2 min, trotting at 4 m/s for 5 min, walking at 2 m/s for 2 min, and galloping at 8 m/s for 5 min. To maintain a basic level of fitness during the experimental period, the horses continued to run on the treadmill at least 3 days/wk. During the conditioning and experimental period, the horses were housed in box stalls (3 × 4 m) and fed timothy grass and alfalfa hay (~8.5 kg·horse⁻¹·day⁻¹) and mixed grain (~2 kg·horse⁻¹·day⁻¹) with an estimated digestible energy intake of 19 Mcal·horse⁻¹·day⁻¹, which was sufficient to maintain body weight. Extending from 48 h before the glycogen-depleting exercise until the end of the trial, the horses were fed 8.5 kg of mixed hay per day, with estimated digestible energy of 13 Mcal·horse⁻¹·day⁻¹. Feed was withheld for 12 h before the first day of the glycogen-depleting exercise. Trace mineralized salt blocks and water were available at all times.

Indirect calorimetry. The horses were positioned on the treadmill, and a loose-fitting mask was applied on their face for measurement of respiratory gas exchange. Oxygen consumption (Vo2) and carbon dioxide production were measured with an open-circuit indirect calorimeter (Oxymax-XL, Columbus Instruments, Columbus, OH) during MAOD and Vo2 tests. Flow through the system was ~1,500 l/min with the horse stationary and 10,000 l/min during running (see below for details). The oxygen and carbon dioxide sensors of the open-circuit calorimeter (electrochemical cell and single-beam nondispersive infrared sensor, respectively) were calibrated against gases of known composition within 10 min of the start of each exercise test, during which the horse stood quietly on the treadmill. The overall accuracy of the system was verified daily by nitrogen dilution. Discrepancy between stimulated Vo2 produced by nitrogen dilution and the values measured by the system was ±3% at nitrogen flow rates equivalent to a Vo2 of 54 l/min (~140 ml·min⁻¹·kg⁻¹ for a 385-kg horse).

Determination of Vo2 max. The Vo2 max of each horse was measured by indirect calorimetry during an incremental exercise test 4 days before the glycogen-depleting exercise and 2 days after the glycogen-depleting exercise, after Glu or Sal infusion (Fig. 1). The incremental exercise test consisted of running horses on a treadmill inclined at 4° for 90 s at 4 m/s, with subsequent increases of 1 m/s every 90 s until the horses were unable to maintain their position on the treadmill. Vo2 was measured every 10 s. Vo2 max was determined as the value of Vo2 when it reached a plateau (defined as a change in Vo2 <4 ml·min⁻¹·kg⁻¹ for an increase in speed). The speed-Vo2 relationship for each horse was determined by linear regression of Vo2 and speed during the incremental exercise test.

Determination of MAOD. Anaerobic capacity was estimated by the calculation of the MAOD during a single high-speed exercise test 3 days before and 1 day after the glycogen-depleting exercise. The single high-speed exercise test consisted of running the horses on a treadmill (4° incline) for 5 min at 3 m/s (warm-up), at the speed calculated to require a Vo2 of 120% of the Vo2 max until fatigue (sprint), and for 5 min at 3 m/s (cool down). Fatigue was assessed by the same observer, who was blinded to treatment, and was based on the horse’s inability to maintain its position on the treadmill.

Fig. 1. Time line of the experimental protocol. MB, muscle biopsy; IET, incremental exercise test; SHET, single high-speed exercise test; Glu, glucose treatment; Sal, saline treatment. Nos. in parentheses, no. horses receiving treatment.
Despite vigorous, humane (verbal) encouragement. Time to fatigue was recorded for each horse. Oxygen deficit was calculated by subtracting the actual VO₂ measured during the single high-speed exercise test, from the estimated oxygen demand (9). Oxygen demand was calculated from the speed-VO₂ relationship and was determined by measuring the rate of VO₂ at each speed during the incremental exercise test. The regression equation describing the speed-VO₂ relationship for each horse was developed from these values and used to estimate oxygen demand at higher speeds.

Glycogen-depleting exercise protocol. The horses performed 3 days of strenuous exercise intended to deplete muscle glycogen stores. Exercise for each horse was individualized based on the assessment of aerobic capacity. This strenuous, repeated exercise consisted of running horses on a treadmill (with a 4° slope) for 5 min at 4 m/s, 15 min at 70% of VO₂max, 5 min at 90% of VO₂max, and, after 30-min rest, for six sprints of 1 min at 100% VO₂max with 5 min of walking between each sprint. We have demonstrated that this exercise protocol depletes muscle glycogen by at least 55% of its initial value (26). The single high-speed exercise test and the incremental exercise test were repeated 24 and 48 h, respectively, after the last bout of the glycogen-depleting exercise for each trial.

Treatment infusions. For each trial, 30 min after the last bout of the glycogen-depleting exercise, the horses received randomly and in a blinded fashion either Glu or Sal of equivalent volume and delivered at an equivalent rate to that of the Glu infusion. Fluid administration began within 30 min of the end of the strenuous exercise. Fluid was administered at a mean rate of 225.5 g glucose/h for 11.9 min of the end of the strenuous exercise. Fluid administration began within 30 min of the end of the strenuous exercise. Fluid was administered at a mean rate of 225.5 g glucose/h for 11.9 min (mean ± SE) through a catheter (14 gauge, 5.25 in., Angiocath, Deseret, Sandy, UT) placed in a jugular vein. Similar glucose infusion has previously been shown to replenish at least 80% of muscle glycogen concentration in horses (7). The infusion was repeated 30 min after the end of the single high-speed exercise test, following the protocol described above, to ensure partial or complete restoration of muscle glycogen concentration before the incremental exercise test. The horses were confined in their stalls during the infusion and until the subsequent exercise test.

Weight. The horses were weighed on entry to the laboratory before each exercise test.

Biochemical analysis. Muscle samples were collected by needle biopsy of the middle gluteal muscle at a depth of 6 cm, under aseptic conditions, after desensitization of the area with 2% mepivacaine (Carbocaine, Abbott, North Chicago, IL). Samples were collected before each incremental exercise and single high-speed exercise test and within 10 min of the end of the last bout of glycogen-depleting exercise. The muscle samples were flash frozen in liquid nitrogen and then stored at −70°C until analysis. Muscle glycogen concentration was determined in duplicate with a fluorometer (Sequoia-Turner model 112, Turner Design, Sunnyvale, CA) placed in a jugular vein. Similar glycogen depletion was determined by measuring the variable speed oxygen demand (9). Oxygen demand was calculated from the microhematocrit technique and refractometry (Cambridge Instruments, Buffalo, NY), respectively. Samples for glucose assays were collected into chilled 5-ml evacuated tubes containing potassium oxalate and sodium fluoride. Plasma glucose concentrations were measured by using an automatic analyzer (model 1500, Yellow Springs Instruments, Columbus, OH). The area under the curve for blood glucose concentration was calculated by using the trapezoidal rule. Venous samples for measurement of blood lactate concentration were collected into total blood lactate tubes (YSI total blood lactate tube with 2315 YSI blood lactate preservative kit, Yellow Springs Instruments). Blood lactate was determined in duplicate by an electrochemical method (YSI 1500 Sport lactate analyzer, Yellow Springs Instruments). The onset of blood lactate accumulation was estimated by measuring the variable speed at which a blood lactate concentration of 4 mmol/l was reached during the incremental exercise test (VL4). The apparent rate of lactate production (expressed in mmol·kg⁻¹·s⁻¹) was calculated by using the following equation:

\[
\frac{[\text{Lac}]_{\text{end}} - [\text{Lac}]_{\text{beg}}}{t_{\text{sprint}}}
\]

where \([\text{Lac}]_{\text{end}}\) is the blood lactate concentration at the end of the sprint (expressed in mmol/l), \([\text{Lac}]_{\text{beg}}\) is the blood lactate concentration at the end of the warm-up (expressed in mmol/l), and \(t_{\text{sprint}}\) is the time of sprint (measured in seconds).

Statistical analysis. Before the study, we calculated that the power to detect a 10% difference in MAOD and an 8% difference in VO₂max using seven horses was 90%. Statistical analyses were performed by using either a two-way repeated-measures analysis of variance (repeated measures on time and treatment) or a three-way repeated-measures analysis of variance (time, treatment, and trial), as appropriate for the dependent variable. The null hypothesis (no effect of Glu infusion on muscle glycogen concentration and exercise performance) was rejected at \(P < 0.05\). Significant differences between means (\(P < 0.05\)) were identified by using Student-Newman-Keuls test. All results are expressed as means ± SE.

RESULTS

Muscle glycogen concentration. Three days of strenuous exercise resulted in substantial reductions (\(P < 0.001\)) in muscle glycogen concentration for Glu and Sal (78 and 79% reduction from values before depletion, respectively, Fig. 2). Intravenous infusion of Glu resulted in replenishment of muscle glycogen concentration by 78.8% from the values before infusion after the glycogen-depleting exercise (from 24.7 ± 7.2 to 116.5 ± 7.0 mmol/kg wet wt before and after infusion, respectively; \(P < 0.05\)), whereas Sal infusion resulted in a significantly smaller increase in muscle glycogen concentration (from 23.4 ± 7.2 to 47.8 ± 5.7 mmol/kg wet wt; \(P < 0.05\)) (Fig. 2).

\(\text{VO}_2\text{max and speed-VO}_2\text{ relationship.} \text{ VO}_2\text{max of the seven horses before the glycogen-depleting exercise of trial 1 was 142 ± 15 m O}_2\text{-min}^{-1}\cdot\text{kg}^{-1}\text{ at a treadmill speed of 9.9 ± 0.4 m/s. The average correlation coefficient of the speed-VO}_2\text{ relationship was 0.992 ± 0.002 (}\text{P < 0.01,}\text{ the slope of the regression line was 14.7 ± 0.6 m O}_2\text{-min}^{-1}\cdot\text{kg}^{-1}\text{, and the ordinate intercept was...}

\[\text{J Appl Physiol • VOL 91 • OCTOBER 2001 • www.jap.org}\]
3.7 ± 0.9 ml O\textsubscript{2}·min\textsuperscript{-1}·kg\textsuperscript{-1}. There was no significant difference in the rate of total VO\textsubscript{2max} (l/min) and in the rate of VO\textsubscript{2max} per unit body weight (ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) between treatments before and after the glycogen-depleting exercise (Table 1). VO\textsubscript{2max} per unit body weight (ml·kg\textsuperscript{-1}·min\textsuperscript{-1}), but not total VO\textsubscript{2} (l/min), was increased in both groups after the glycogen-depleting exercise.

**MAOD and run time to fatigue.** Replenishment of muscle glycogen concentration by Glu infusion was associated with a 30% greater (P < 0.05) MAOD compared with values after Sal infusion. A significant reduction in MAOD was observed for the Sal treatment after the glycogen-depleting exercise (from 111.3 ± 9.3 ml O\textsubscript{2} equivalent (O\textsubscript{2eq})/kg for values before depletion to 82.4 ± 10.3 ml O\textsubscript{2eq}/kg for values after depletion, P < 0.05), but not in the Glu-supplemented horses (from 100.5 ± 9.3 to 105.7 ± 9.3 ml O\textsubscript{2eq}/kg). A significant (P < 0.05) reduction in run time to fatigue during the single high-speed exercise test was observed for the Sal treatment after depletion of the muscle glycogen stores (from 129.9 ± 8.2 to 70.8 ± 8.6 s, respectively, before and after depletion), whereas there was no significant reduction in run time to fatigue for the Glu treatment (from 112.1 ± 8.2 to 97.3 ± 8.2 s, respectively, before and after infusion). Run time during the single high-speed exercise test was 28% longer in horses with glycogen replenishment compared with horses with persistent glycogen depletion.

**Biochemical analysis.** There was a significant treatment effect (P < 0.05) on blood glucose concentration at each speed, starting at 6 m/s during the incremental exercise test (Fig. 3). The area under the curve was significantly greater (P = 0.03) after Glu than Sal treatment. Maximum concentration of venous blood glucose during the incremental exercise test was significantly higher for horses that received the Glu infusion after the glycogen-depleting exercise (from 4.04 mmol/l before the glycogen-depleting exercise to 5.83 mmol/l after the glycogen depleting exercise; P = 0.02). No significant difference in blood glucose concentration was observed during the single high-speed exercise test after Glu or Sal treatment (Table 2). VL\textsubscript{4} during the incremental exercise test was significantly higher after Sal treatment (7.6 ± 0.3 and 8.5 ± 0.3 m/s before and after depletion, respectively; P = 0.025), whereas VL\textsubscript{4} was not significantly affected by Glu administration (from 7.2 ± 0.3 to 7.3 ± 0.3 m/s). Venous blood lactate concentration at the end of the sprint and during re-

### Table 1. VO\textsubscript{2max}, hematocrit, and total protein concentration during the incremental exercise test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>VO\textsubscript{2max} ml·kg\textsuperscript{-1}·min\textsuperscript{-1}</th>
<th>VO\textsubscript{2max} l/min</th>
<th>Highest PCV, %</th>
<th>Highest TP, g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Glucose</td>
<td>Saline</td>
<td>Glucose</td>
</tr>
<tr>
<td>Before depletion</td>
<td>143.3 ± 6\textsuperscript{a}</td>
<td>139 ± 4</td>
<td>61.7 ± 2.1</td>
<td>59.9 ± 2.1</td>
</tr>
<tr>
<td>After depletion</td>
<td>147.3 ± 6\textsuperscript{a}</td>
<td>147.7 ± 7.7\textsuperscript{a}</td>
<td>60.8 ± 2.1</td>
<td>62.0 ± 2.1</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 7 horses. VO\textsubscript{2max}, maximal rate of oxygen consumption; PCV, packed cell volume (hematocrit); TP, total protein. \textsuperscript{a}P < 0.05 before depletion vs. after glucose or saline infusion.
covery (5 and 10 min) during the single high-speed exercise test was significantly \( (P < 0.05) \) lower after Sal treatment than after Glu treatment (Fig. 4). There was no treatment effect on the apparent rate of lactate production during the single high-speed exercise test after the glycogen-depleting exercise \( (0.10 \pm 0.01 \text{ mmol} \cdot l^{-1} \cdot s^{-1}) \), respectively, for Sal and Glu; \( P = 0.6 \)). Moreover, the apparent rate of lactate production during the sprint period was not statistically different before and after the glycogen-depleting exercise \( (0.09 \pm 0.01 \text{ mmol} \cdot l^{-1} \cdot s^{-1}) \), respectively; \( P = 0.06 \)). The highest hematocrit and plasma total protein concentrations, measured during the incremental exercise test, were similar for both treatments after the glycogen-depleting exercise (Table 1). Hematocrit and total protein concentration were similar between treatment groups before, during, and after each single high-speed exercise test. Hematocrit values were significantly \( (P < 0.05) \) lower after the glycogen-depleting exercise for each speed level during the single high-speed exercise test.

**Body weight.** Body weight before the incremental exercise test decreased after the glycogen-depleting exercise for both treatments (from 432.3 ± 7.7 to 430.5 ± 7.7, 414.3 ± 7.7 to 421.4 ± 7.7 kg, for Sal and Glu, respectively; \( P < 0.001 \)). In a similar fashion, body weights measured before the single high-speed exercise test decreased after the glycogen-depleting exercise for both treatments (from 430.1 ± 7.1 to 427.6 ± 7.1 to 406.1 ± 7.1 and 412.9 ± 7.1 kg for the Sal and Glu treatments, respectively; \( P < 0.001 \)). However, body weight was not affected by treatment after the glycogen-depleting exercise (414.3 ± 7.7 and 421.4 ± 7.7 kg before the incremental exercise test, and 406.1 ± 7.1 and 412.9 ± 7.1 kg before single-high speed exercise test for Sal and Glu, respectively).

**DISCUSSION**

We demonstrated that, in horses, muscle glycogen depletion was associated with significant decreases in run time to fatigue, MAOD, and blood lactate concentration during a single high-speed exercise test. Furthermore, replenishment of muscle glycogen stores after substantial depletion was associated with restoration of the MAOD, run time to fatigue, and blood lactate concentration to values similar to those recorded before glycogen depletion. In contrast, depletion and subsequent replenishment of muscle glycogen stores were not associated with a change in \( \overline{V}O_2 \text{max} \). Demonstration that replenishment of muscle glycogen concentration by glucose infusion restores anaerobic capacity confirms a role for muscle glycogen concentration in limiting anaerobic capacity of horses. To our knowledge, this is the first evidence that provision of supplemental energy as glucose to horses, a herbivore that relies on short-chain (volatile) fatty acid production for a significant proportion of its resting energy

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**Table 2. Hematocrit, total protein, and glucose concentrations during the single high-speed exercise test**

<table>
<thead>
<tr>
<th></th>
<th>Before Depletion</th>
<th></th>
<th>After Depletion and Glu or Sal Infusion</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>W</td>
<td>S</td>
<td>R5</td>
</tr>
<tr>
<td>TP, g/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sal</td>
<td>6.4 ± 0.2</td>
<td>6.8 ± 0.2</td>
<td>7.4 ± 0.2</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>Glu</td>
<td>6.44 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>7.4 ± 0.1</td>
<td>6.9 ± 0.1</td>
</tr>
<tr>
<td>PCV, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sal</td>
<td>41.1 ± 1.4</td>
<td>49.4 ± 0.8</td>
<td>54.5 ± 1.0</td>
<td>51.8 ± 1.3</td>
</tr>
<tr>
<td>Glu</td>
<td>41.3 ± 1.6</td>
<td>48.7 ± 1.4</td>
<td>53.6 ± 1.6</td>
<td>50.9 ± 1.2</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sal</td>
<td>4.4 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>6.7 ± 0.3</td>
</tr>
<tr>
<td>Glu</td>
<td>4.7 ± 0.2</td>
<td>4.7 ± 0.3</td>
<td>4.4 ± 0.2</td>
<td>6.3 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE for \( n = 7 \) horses. Sal, saline group; Glu, glucose group; R, rest; W, warm-up; S, sprint; R5, 5 min postrecovery; R10, 10 min postrecovery.

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**Fig. 4. Blood lactate concentration at rest, at the end of the warm-up, at the end of the sprint, and at the end of the recovery during each SHET and before (a) and after (b) GDE. R-5, 5 min postrecovery; R-10, 10 min postrecovery. Values are means ± SE. *P < 0.05 vs. corresponding value before glycogen depletion.**

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needs, restored muscle glycogen pool and preserved anaerobic capacity during high-intensity exercise.

Muscle glycogen and exercise performance. Prolonged, submaximal running, repeated bouts of maximal-intensity exertion, or a combination of prolonged running and repeated sprints results in significant declines in muscle glycogen concentration in horses (8, 17, 19, 26, 37, 38). Moreover, repletion of muscle glycogen stores does not occur within 24 h in Thoroughbred racehorses after a high-speed training run (37). Therefore, exercise causes muscle glycogen depletion that may persist at the time of subsequent exercise. While preexisting muscle glycogen depletion in humans decreases endurance time and increases the time required to cover a given distance during submaximal exercise (1, 24), the effect of preexisting muscle glycogen depletion on high-intensity exercise is less well established.

During high-intensity exercise, the major pathways for ATP resynthesis are the breakdown of creatine phosphate (CP) and the degradation of muscle glycogen to lactic acid. It has been demonstrated that the contribution of glycogen to anaerobic ATP provision is fourfold greater than that of CP during 30 s of maximal isokinetic cycling exercise (20). The decline in muscle glycogen that occurs during repeated, high-intensity exercise could theoretically contribute to impaired exercise performance via a reduction in substrate for phosphorylase and subsequent glycolytic flux (15). Several investigators reported conflicting results regarding the role of low intramuscular glycogen concentration on high-intensity exercise performance in humans. High-intensity exercise performance was not impaired by low intramuscular glycogen concentration (15, 40), and the decline in exercise performance during high-intensity, intermittent exercise was not related to a reduction in muscle glycogen but was more likely induced by reduced CP availability, impairment in sarcoplasmic reticulum (SR) function, or some other fatigue-inducing agents (16). Similarly, Symons and Jacobs (40) found no effect of lowering muscle glycogen concentration on electrically evoked muscle force, maximal voluntary isometric force, or repeated maximal isokinetic leg extensions. However, in these studies, muscle glycogen pool was not severely depleted, and it can be argued that a greater degree of glycogen depletion is required before changes in performance during high-intensity exercise can be detected. Theoretically, given that in vitro $K_m$ of glycogen phosphorylase is reported to be low, a muscle glycogen concentration of 150 mmol/kg dry wt will provide sufficient fuel to perform high-intensity exercise for 115–405 s (4). In the present study, the decrease in exercise duration in Sal was likely related to the greater reduction in preexercise muscle glycogen concentration with that treatment. Conversely, some authors maintain that variations in muscle glycogen concentration should have no effect on the duration of short-term maximal exercise because intramuscular concentration remains high at the point at which fatigue develops (40). However, at an exertion intensity of 150% $VO_2max$, which was maintained for ~8 min, quadriceps femoris muscle glycogen concentration declined from 62 to 13 mmol/kg (11). Therefore, short-term, high-intensity exercise can induce substantial depletion of muscle glycogen stores.

Because the major pathways for anaerobic ATP resynthesis are the breakdown of CP and the degradation of muscle glycogen to lactic acid, reduced glycogen availability could contribute to a decline in anaerobic energy production and exercise performance (25), leading several investigators to speculate that substantial reduction of muscle glycogen availability may limit performance during high-intensity exercise (18, 21). In support of this theory is the observation that a decrease in maximal isokinetic force generation by the leg extensors was reported 1 h after exercise designed to deplete intramuscular glycogen (22). Similarly, intense knee-extensor performance, during two exercise bouts separated by 1 h, is maintained in one leg with elevated muscle glycogen, whereas performance is reduced in the contralateral leg with reduced muscle glycogen (2). In the present study, evidence to support an inhibition of anaerobic metabolism by glycogen depletion included a reduction in run time, MAOD, and blood lactate concentration in the Sal treatment group during the single high-speed exercise test.

The importance of muscle glycogen reserves to prevent fatigue is also highlighted by studies that manipulated muscle glycogen stores with different diets. A decrease in time to fatigue during anaerobic exercise occurs in humans fed a low-carbohydrate diet (27, 30), and performance during supramaximal intermittent exercise was decreased in the low-carbohydrate diet group (23). Compared with a low-carbohydrate diet, a moderate and high consumption of dietary carbohydrate can at least maintain supramaximal intermittent exercise performance (23).

In horses, it is possible that reduction in preexercise muscle glycogen stores contributes to a decline in high-intensity exercise performance, although this issue has received scarce attention. Davie et al. (8) reported that a 22% decrease in muscle glycogen concentration did not have a measurable effect on high-intensity exercise performance. A concern with the study of Davie et al. is that the muscle glycogen concentration might not have been reduced sufficiently to produce detectable effects on athletic performance, given the small number of animals included in the study. In contrast, a decrease in preexercise muscle glycogen concentration by 41% impaired the capacity for anaerobic work in draught horses dragging a sled (41), leading the authors to suggest a reduction in anaerobic capacity. Recently, it has been demonstrated that exercise that induces substantial depletion of muscle glycogen stores in horses is associated with decreased run time to fatigue and decreased anaerobic capacity during a subsequent high-speed exercise test (26). Furthermore, low muscle glycogen concentration is associated with a decrease in blood lactate concentration at identical work intensities, suggesting reduced glycolysis in these horses. However, in this study, impairment of anaerobic capacity, evident as a decrease in MAOD and run time to...
fatigue, cannot be attributed only to the decrease in muscle glycogen stores because the previous high-intensity exercise may have played another role in the development of fatigue.

In summary, the effect of muscle glycogen depletion on short-term, high-intensity exercise duration has remained controversial in both human and equine studies. One explanation for this controversy could be the diversity of exercise protocols and experimental designs, and might be related to differences in preexercise muscle glycogen concentration. For instance, it appears that glycogen availability impairs performance when muscle glycogen stores are severely depleted (4). Moreover, in some studies, intramuscular glycogen concentrations were assumed to be low but were not measured, raising the issue as to what extent the described protocols actually altered muscle glycogen concentration (4, 23, 30). A further challenge of investigations of the relationship between muscle glycogen and athletic capacity resides in the difficulty to control extraneous factors that may also affect athletic capacity. For instance, the role of muscle glycogen stores as a factor limiting high-intensity exercise performance has been questionable because the previous exercise may be a more potent determinant of fatigue than glycogen availability (14). However, in the present study, the restoration of muscle glycogen depletion by glucose infusion accounted for the effect of such extraneous factors and confirmed the role of muscle glycogen as a factor limiting anaerobic capacity during high-intensity exercise in horses. Another limitation when the role of glycogen substrate availability on anaerobic capacity is investigated is the method used to assess anaerobic capacity. In the present study, the use of the MAOD test as a gold standard for the assessment of anaerobic capacity in horses is problematic because MAOD is an indirect measured of anaerobic capacity. Despite accurate measurement of submaximal $V_O2$, the MAOD technique relies on the extrapolation of the $V_O2$ vs. speed regression to predict the $O_2$ demand for exercise at supramaximal intensities (9). Therefore, the accuracy of estimation of oxygen demand at supramaximal intensity is unknown, and, hence, the accuracy of the MAOD test cannot be assessed because of the absence of other measures of anaerobic capacity (9). However, these assumptions have been widely accepted, and MAOD have been used as a measure of anaerobic capacity in horses (9).

The lack of an effect of glycogen depletion on aerobic capacity noted in the present study is consistent with previous reports in both humans and horses. Similar $V_O2$ values during exercise were reported, despite varying the initial muscle glycogen concentration in humans (3), and a change in $V_O2_{max}$ was not detected in horses with muscle glycogen depletion (26).

Glycogen and glycogenolysis. A marked reduction in blood lactate concentration was apparent in horses with preexisting muscle glycogen depletion at the end of the sprint and during recovery from the single high-speed exercise test. Furthermore, replenishment of muscle glycogen stores after substantial depletion was associated with restoration of blood lactate concentration to similar values to those recorded before glycogen depletion during the single high-speed exercise test. Blood lactate concentration of humans after high-intensity exercise is lower after a low-carbohydrate diet and higher after a high-carbohydrate diet (13). One explanation for the decreased lactate concentration in the glycogen-depleted state would be a lack of substrate for glycogenolysis and glycolysis during anaerobic metabolism and/or by a reduction in work performed (4, 18). However, the existence of a direct relationship between muscle glycogen content and blood lactate accumulation has been questioned in humans, with similar postexercise blood lactate concentrations being observed over a wide range of preexercise muscle glycogen concentrations (13, 21). Moreover, it has been reported that the rate of muscle glycogen degradation and of lactate production during short, intense contraction is not affected by the initial glycogen concentration (2, 33).

The decrease in high-intensity exercise performance, observed when glycogen levels are low, may be attributable to the alteration of the blood acid-base status induced by exercise and dietary intervention. Furthermore, glycogen depletion-induced reductions in lactic acid production reduce cellular acid production, which plays a prominent role in the regulation of sympathetic vasoconstriction, through the activation of the skeletal muscle metaboreflex system. Therefore, glycogen depletion-induced reduction in lactate production may attenuate sympathetic vasoconstriction by reduction of the metaboreceptor stimulation (36) and may contribute to the decrease in high-intensity exercise performance.

Glycogen and fatigue. Decreased availability of muscle glycogen stores likely reduces the rate of anaerobic glycogenolysis and lactate production and, therefore, limits anaerobic ATP synthesis. We speculate that failure to maintain ADP homeostasis at the contractile site due to a relative impairment of ATP resynthesis secondary to reduced glycogen availability results in decreased anaerobic power generation (12). Potential intracellular sites where ADP could limit the muscular contraction process are actin-myosin interaction, the reuptake of calcium by the SR, the maintenance of the Na$^+$/K$^+$ gradient, the membrane potential over the sarcolemma, and the signal transduction between T tubuli and SR (12, 34). Exercise with low initial glycogen stores results in rapid development of fatigue and in a more pronounced formation of IMP and NH$_3$ than exercise with normal glycogen levels, which may reflect the large increases in free ADP and AMP at the enzymatic site during the contraction (33, 39). Therefore, the decrease in substrate availability may impair glycolysis as evidenced by the decreased phosphofructokinase activity and tricarboxylic acid cycle intermediates in humans with depleted glycogen stores (39). Moreover, because of the possible functional coupling between ATP supplied by glycolysis and ATP utilized within the SR T tubule, reduction in muscular force,
inhibition of contractile proteins, and failure of calcium release observed during fatigue have been linked to reduced muscle glycogen stores (5). Therefore, in the present study, all of these mechanisms of interaction between muscular fatigue and substrate glycogen availability may partly explain the accelerated onset of fatigue observed in horses in a glycogen-depleted state.

In summary, these data demonstrate that repletion of muscle glycogen stores restored anaerobic capacity of horses with preexisting exercise-induced depletion of muscle glycogen. Therefore, we concluded that decreased availability of skeletal muscle glycogen stores causes a decline in anaerobic power generation in horses.

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