Fluid and electrolyte supplementation after prolonged moderate-intensity exercise enhances muscle glycogen resynthesis in Standardbred horses

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Waller AP, Heigenhauser GJ, Geor RJ, Spriet LL, Lindinger MI. Fluid and electrolyte supplementation after prolonged moderate-intensity exercise enhances muscle glycogen resynthesis in Standardbred horses. J Appl Physiol 106: 91–100, 2009. First published October 23, 2008; doi:10.1152/japplphysiol.90783.2008.—We hypothesized that postexercise rehydration using a hypotonic electrolyte solution will increase the rate of recovery of whole body hydration, and that this is associated with increased muscle glycogen and electrolyte recovery in horses. Gluteus medius biopsies and jugular venous blood were sampled from six exercise-conditioned Standardbreds on two separate occasions, at rest and for 24 h following a competitive exercise test (CET) designed to simulate the speed and endurance test of a 3-day event. After the CETs, horses were given water ad libitum, and either a hypotonic commercial electrolyte solution (electrolyte) via nasogastric tube, followed by a typical hay/grain meal, or a hay/grain meal alone (control). The CET resulted in decreased total body water and muscle glycogen concentration of 8.4 ± 0.3 liters and 22.6%, respectively, in the control treatment, and 8.2 ± 0.4 liters and 21.9% in the electrolyte treatment. Electrolyte resulted in an enhanced rate of muscle glycogen resynthesis and faster restoration of hydration (as evidenced by faster recovery of plasma protein concentration, maintenance of plasma osmolality, and greater muscle intracellular fluid volume) during the recovery period compared with control. There were no differences in muscle Na, K, Cl, or Mg contents between the two treatments. It is concluded that oral administration of a hypotonic electrolyte solution after prolonged moderate-intensity exercise enhanced the rate of muscle glycogen resynthesis during the recovery period compared with control. It is speculated that postexercise dehydration may be one key contributor to the slow muscle glycogen replenishment in horses.

skeletal muscle; rehydration; exercise; instrumental neutron activation analysis

IN THE HORSE, BOTH short-term, high-intensity, and prolonged submaximal exercise result in dehydration, loss of water and electrolytes, and depletion of skeletal muscle glycogen (23, 30, 31, 52, 53, 56). Resynthesis of muscle glycogen stores requires 48–72 h in horses, compared with 2.5 h in rats (16) and <24 h in humans (12). Previous studies in horses have altered postexercise nutrition strategies in attempts to enhance muscle glycogen resynthesis. Despite demonstrations that intravenous infusion of large amounts of glucose (3–6 g/kg) modestly accelerated muscle glycogen replenishment (14, 17), conventional feeding strategies have not been successful (23, 53–55). Initial glycogen resynthesis rates in horses after ingestion of meals with varying soluble carbohydrate (CHO) contents ranged from ~8 to ~12 mmol·kg dry wt−1·h−1 for mixed and high-soluble CHO diets, respectively. In contrast, in humans, initial glycogen storage rates are ~40 mmol·kg dry wt−1·h−1, when at least 5 g/kg of soluble CHO are provided during the first 4 h after alternating high- and low-intensity interval exercise (26).

There is considerable anecdotal and practical information that dehydrated horses will not eat, while most forms of glycogen-depleting exercise in horses result in dehydration (37, 56). Water alone is not effective in restoring hydration, as there is also a requirement for electrolytes to osmotically retain water in the appropriate extracellular and intracellular fluid compartments (25, 43, 57). Interestingly, intravenous infusion of large amounts of saline alone (~20 liters in 12 h for a total of 107 g NaCl) resulted in glycogen replenishment rates of ~7.6 mmol·kg dry wt−1·h−1 during the first 12 h of exercise recovery in horses (31), compared with typical rates of ~8 to ~12 mmol·kg dry wt−1·h−1 for mixed and high-soluble CHO diets (33), suggesting a possible role for rehydration in glycogen resynthesis.

The resynthesis of glycogen after exercise requires adequate intracellular water and K+. Within muscle and liver, glycogen is stored in a hydrated form (up to 3 g water/g glycogen) (13), tightly associated with 0.5 mmol K+/g of glycogen (2). Therefore, as muscle contraction occurs and glycogen is utilized, there occurs concurrent release of water and K+ from the glycogen store. In humans and rodents, prolonged exercise results in net losses of water (11) and K+ (36) from contracting muscle; however, this may not be the case in horses. It has previously been reported that intracellular water (37) and K+ (58) may be preserved during exercise in horses, despite ongoing glycogen degradation and extracellular dehydration. However, the concurrent changes in intracellular water, K+, and glycogen content during exercise in horses has not been studied. Furthermore, cell shrinkage (as occurs with dehydration) is associated with decreased glycogen synthesis in skeletal muscle (39) and hepatocytes (21) and increased CHO oxidation and glycogenolysis (29). Therefore, it is reasonable to postulate that postexercise dehydration may be one reason why muscle glycogen replenishment is so slow in horses.

The purpose of the present study was to determine the effect of administering a hypotonic electrolyte solution immediately after prolonged exercise, and before feed is provided, on...
rehydration and muscle glycogen and electrolyte recovery in horses. It was hypothesized that provision of a balanced electrolyte solution, immediately followed by a typical hay and grain meal, after glycogen-depleting exercise, will result in a faster rate of muscle glycogen resynthesis than a grain/hay meal with voluntary access to water alone.

**METHODS**

**Animals.** Six Standardbred geldings (body mass 464 ± 10 kg; age 5–12 yr) from the University of Guelph research herd were used. The study took place in June and July, and horses underwent a 4- to 6-wk diet and exercise acclimation period, during which they were housed in individual box stalls with 7 h of paddock turnout during the day. Horses were exercise conditioned 5 days/wk on a high-speed treadmill (SATO) and outdoor exerciser (Odyssey Performance Trainer, Campbellville, ON, Canada), until able to comfortably perform an ~60-min competitive exercise test (CET) (41, 42) on a high-speed treadmill designed to significantly utilize muscle glycogen stores (47) and result in a total body water loss of 8–10 liters. The CET is designed to simulate the 2nd day (speed and endurance test: classic format) of a one-star Concours Complet International 3-day event and includes the following phases: 10-min walk (1.7 m/s), 10-min trot (3.7 m/s), 2-min gallop (10.0 m/s), 20-min trot (3.7 m/s), 20-min walk (1.7 m/s), 8-min canter (8.0 m/s), and 10-min walk (1.7 m/s).

The horses were maintained on a diet consisting of oats twice daily and mixed grass hay three times daily (Table 1), with free access to water and a salt block. The amount of feed given was increased over this acclimation period such that, during the final 2 wk, the horses were receiving 4 kg of oats and 6 kg of hay daily, and there were no significant changes in the body masses of the horses during this time. The animal care and use procedures were approved by the University of Guelph Animal Care Committee and performed in accordance with the guidelines of the Canadian Council on Animal Care.

**Experimental protocol.** The study consisted of an electrolyte treatment and control; thus each horse performed the CET twice in randomized order, separated by an 8- to 10-day interval, during which time exercise conditioning was maintained (42, 22). A time line of the experimental protocol is shown in Fig. 1. On both sampling days beginning at 7 AM, catheters (14-gauge, 5.25 in.; Angiocath, Becton-Dickinson, Mississauga, ON, Canada) were inserted anterograde into the left and right jugular veins, and four-way stopcocks with 50-cm extensions were attached to the catheters for ease of blood sampling. Patency of the catheters was maintained with sterile, heparinized, 0.9% NaCl.

Preexercise (Pre-Ex) blood and muscle samples were taken at 8 AM, and then the CET was performed. Immediately on completion of the final canter, an “end of exercise” blood sample was taken, following which the horse walked for 10 min. Ten minutes after cessation of exercise, the horse either 1) received by nasogastric tube a commercially available electrolyte solution (Perform’N Win, Buckeye Nutrition, Dalton, OH) designed to replace an 8-liter sweat loss (12 g Na, 24 g Cl, 9 g K, 1 g Ca and Mg), in 8 liters of water (osmolality = 212 mosmol/kgH$_2$O), or 2) stood in stocks for an equivalent amount of time (~10 min). Muscle samples were collected from the gluteus medius by use of the needle biopsy technique (38). Samples were collected by use of aseptic technique after desensitization of the area with 2% meipivacaine (Upjohn, Orangeville, ON, Canada). For each trial, muscle samples were collected at a uniform depth from four standardized sites in the left or right middle gluteal muscles. Biopsies were collected before exercise (baseline), 15 min after cessation of exercise (0 h), and at 4 and 24 h posttreatment. The samples were immediately frozen in liquid nitrogen and stored at ~80°C until analysis. In both treatments, within 20 min of cessation of exercise, the horses were given 2 kg oats and 2 kg hay (0 min of recovery), with access to water ad libitum. Horses were given 2 kg oats and 2 kg hay at 6 h of recovery, and 2 kg hay at 12 h of recovery. Blood samples were taken at 20- to 60-min intervals up to 8 h of recovery, and again at 24 h of recovery; the horses remained in their stalls for the duration of sampling.

**Sample analyses.** Each blood sample was collected into a 7-ml lithium heparinized vacutainer and immediately analyzed for the plasma concentrations of Na$^+$, Cl$^-$, K$^+$, Ca$^{2+}$, and lactate$^-$ using a Nova Stat Profile 9+ (NOVA Biomedical, Waltham, MA). Blood was then transferred into two 1.5-ml Eppendorf centrifuge tubes and centrifuged for 5 min at 15,000 g to separate the plasma. Plasma protein concentration (IPP) was determined by refractometry (coefficient of variation 0.83%) (Atogu clinical refractometer model SPR-T2; Atago, Tokyo, Japan).

Total body water loss during the CET was determined as the change in body mass after accounting for fecal losses. Plasma osmolality was calculated according to the formula of Brownlow and Hutchins (7) for equine plasma such that:

\[
\text{Osmolality (mosmol/kgH}_2\text{O}) = 1.86([\text{Na}^+] + [\text{K}^+]) + [\text{glucose}] + [\text{lactate}] + 9
\]

Muscle biopsies were freeze-dried, dissected of all visible blood, connective tissue, and fat, and powdered for subsequent analysis. Muscle H$_2$O content (l/kg dry wt) was determined as the difference in sample weight before and after freeze-drying. Skeletal muscle creatine, phosphocreatine, ATP, lactate, and glyceral-3-phosphate were determined by enzymatic spectrophotometric assays (1, 20). Glycogen content (as glucosyl units) was determined in duplicate after acid hydrolysis, as described by Passonneau and Lauderdale (46). All muscle measurements were corrected for the highest total creatine measured from each horse.

Muscle ion contents were determined by instrumental neutron activation analysis (INAA) (35). Muscle total tissue water (l/kg wet wt) was determined from muscle H$_2$O contents after correcting for total creatine and muscle wet weight-to-dry weight ratio. Muscle extracellular fluid volume (ECFV) was calculated using two different versions of the Cl$^-$ space method. One version was calculated as per Lindinger and Heigenhauser (35), using an intracellular [Cl$^-$] (where brackets denote concentration) derived from the relationship between membrane potential and plasma [Cl$^-$]. The other version was calculated as per Manery and Bale (40), using the tissue and plasma Cl$^-$ contents and Donnan factors for each ion. The intracellular ion contents (ion$_i$) were calculated from total muscle ion contents (ion$_{ECF}$) measured by INAA and from the quantity of ion in the muscle extracellular space (ion$_{ECF}$) such that:

\[
\text{ion}_i (\text{meq/kg wet wt}) = \text{ion}_{ECF} - \text{ion}_{ECF}
\]

where ion$_{ECF}$ for each individual muscle was determined as:


\[ \text{ion}_{\text{ICF}} = \text{ion}_{\text{plasma}} \times (\text{ECFV} / \text{kg wet wt}) \]

where \( \text{ion}_{\text{plasma}} \) is plasma ion concentration. The intracellular fluid volume (ICFV) for each muscle was calculated as the difference between the total tissue water and the ECFV. The ion, calculated using the method of Manery and Bale (40) were converted to units of concentration by:

\[ [\text{ion}]_{\text{ICF}} = [\text{ion}]_{\text{plasma}} \times (\text{ICFV} / \text{kg wet wt}) \]

**Statistics.** Data are presented as means \( \pm \) SE. Ion contents and concentrations are expressed in equivalents, a form that takes the ionic valency into consideration. For monovalent ions, 1 mmol = 1 meq, and for divalent ions, 1 mmol = 2 meq. Statistical analysis was performed with SigmaStat (Systat Software, San Jose, CA). Changes over time within treatments were assessed by one-way repeated-measures analysis of variance. Differences between treatments and time points during the recovery period were assessed by two-way repeated-measures analysis of variance. When a significant F-ratio was obtained, means were compared using the all-pairwise multiple-comparison procedure of Holm-Sidak. Statistical significance was accepted when \( P \leq 0.05 \) at a power of 0.8.

**RESULTS**

Ambient temperature, humidity, and total body water loss during the control CET were 23.9 \( \pm \) 0.4°C, 68.4 \( \pm \) 2.1%, and 8.4 \( \pm \) 0.3 liters, respectively. Ambient temperature, humidity, and total body water loss during the electrolyte CET were 23.6 \( \pm \) 0.3°C, 70.2 \( \pm \) 2.0%, and 8.2 \( \pm \) 0.4 liters, respectively.

All horses consumed all of the feed offered by the 24-h sample. Every horse finished all of the oats given by 20 min postfeeding (\( \sim 40 \) min postexercise) and had consumed all of the hay given by 3–4 h postfeeding.

**Muscle metabolites.** Exercise significantly decreased middle gluteus muscle glycogen content by 21.9 and 22.6% from the initial (Pre-Ex) values of the electrolyte and control treatments, respectively (Fig. 2, Table 2). Electrolyte enhanced muscle glycogen replenishment such that, at 4 h of recovery, muscle glycogen content was not different from Pre-Ex, with a further significant increase at 24 h. In contrast, in the control treatment, glycogen was still significantly decreased from Pre-Ex at 4 h. By 24 h of recovery, muscle glycogen content was not different from Pre-Ex values in either treatment. Muscle glycogen content at 24 h of recovery was 108.9 and 93.4% of its initial Pre-Ex value in the electrolyte and control treatments, respectively. The rate of glycogen resynthesis from 0 to 4 h of recovery was almost triple in electrolyte (15.0 \( \pm \) 9.0 mmol·kg\(^{-1}\)·h dry wt\(^{-1}\)) compared with control (5.7 \( \pm \) 9.8 mmol·kg\(^{-1}\)·h dry wt\(^{-1}\)) (\( P = 0.440 \)) (Fig. 2). From 4 to 24 h of recovery, the rates of glycogen resynthesis were 5.1 \( \pm \) 1.6 and 3.4 \( \pm \) 2.8 mmol·kg\(^{-1}\)·h dry wt\(^{-1}\) for the electrolyte and control trials, respectively (\( P = 0.723 \)). The overall (0–24 h) mean rates of glycogen synthesis were 6.8 \( \pm \) 2.0 and 3.8 \( \pm \) 1.3 mmol·kg\(^{-1}\)·h dry wt\(^{-1}\) for the electrolyte and control treatments, respectively (\( P = 0.343 \)).

There was no effect of time or treatment on muscle ATP, creatine, phosphocreatine, lactate, or glycerol-3-phosphate in either the electrolyte or control treatment (Table 2).

**Muscle electrolytes and water content.** Measured muscle H₂O contents are shown in Table 2. Calculating muscle ECFV and ion contents, as per Manery and Bale (40), appeared to give more accurate values compared with the method of Lindinger and Heigenhauser (35), which tended to overestimate muscle ECFV and, therefore, resulted in Cl⁻ and Na⁺ contents that appeared to be too low. Therefore, from this point onward, only the muscle ion and fluid contents and concentrations, as calculated per Manery and Bale (40), will be discussed; however, the values calculated as per Lindinger and Heigenhauser (35) are shown in Table 3 for comparison.

Muscle ECFV showed no significant changes over time in either treatment (Fig. 3, top, Table 3). In the electrolyte treatment, ICFV was increased 18% (\( P = 0.038 \)) from Pre-Ex.
Table 2: Glucone metabolic concentrations and water content at rest and during recovery, from a state duration/intensity competition exercise test, after horses were either given 1) a hypotonic electrolyte solution followed by water ad libitum and a typical feeding protocol (electrolyte trial), or 2) water ad libitum and a typical feeding protocol (control).

<table>
<thead>
<tr>
<th>Time, h</th>
<th>G-3P, mmol/kg</th>
<th>G-3P</th>
<th>Lactic, mmol/kg</th>
<th>Lactic</th>
<th>PCr, mmol/kg</th>
<th>PCr</th>
<th>Mg, mmol/kg</th>
<th>Mg</th>
<th>Ca, mmol/kg</th>
<th>Ca</th>
<th>ICF, mmol/kg</th>
<th>ICF</th>
<th>OSMOL, mOsm/L</th>
<th>OSMOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Ex</td>
<td>5.21±0.40</td>
<td>421</td>
<td>0.25±0.10</td>
<td>13.6</td>
<td>2.6±0.1</td>
<td>1.8</td>
<td>0.35±0.10</td>
<td>0.50</td>
<td>0.6±0.1</td>
<td>0.6</td>
<td>0.4±0.1</td>
<td>0.38</td>
<td>0.6±0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>0</td>
<td>4.74±0.23</td>
<td>465</td>
<td>0.12±0.01</td>
<td>12.3</td>
<td>2.2±0.1</td>
<td>1.5</td>
<td>0.4±0.1</td>
<td>0.60</td>
<td>0.5±0.1</td>
<td>0.5</td>
<td>0.3±0.1</td>
<td>0.30</td>
<td>0.5±0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>5.78±0.28</td>
<td>533</td>
<td>0.15±0.01</td>
<td>12.1</td>
<td>2.1±0.1</td>
<td>1.4</td>
<td>0.5±0.1</td>
<td>0.65</td>
<td>0.6±0.1</td>
<td>0.6</td>
<td>0.4±0.1</td>
<td>0.38</td>
<td>0.6±0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>4</td>
<td>5.34±0.26</td>
<td>435</td>
<td>0.13±0.01</td>
<td>12.0</td>
<td>2.2±0.1</td>
<td>1.4</td>
<td>0.5±0.1</td>
<td>0.65</td>
<td>0.6±0.1</td>
<td>0.6</td>
<td>0.4±0.1</td>
<td>0.38</td>
<td>0.6±0.1</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 horses. G-3-P: glucose-3-phosphate; Pre-Ex: preexercise. *Significantly different from baseline (Pre-Ex) time point, P < 0.05. **Significantly different from 0 h time point, P < 0.05.

MUSCLE GLYCOCEN RESYNTHESIS AFTER ELECTROLYTE SUPPLEMENTATION at 4 h of recovery, compared with only 4% (nonsignificant) in the control treatment.

Muscle electrolyte concentrations are shown in Fig. 4. There were no changes in intracellular fluid [Cl] (Cl ICF) in the electrolyte treatment, while, in control, [Cl ICF] was decreased from Pre-Ex at 0 and 4 h of recovery. There were no changes in [Na] (Na ICF) in the electrolyte treatment, while, in control, [Na ICF] at 24 h of recovery was significantly decreased from 0 h. [K] (K ICF) (Fig. 4, bottom left) and [Mg2+] (Mg2+ ICF) (Fig. 4, bottom right) did not change throughout sampling in either treatment.

Gluteus medius muscle electrolyte contents are shown in Table 3. Muscle Ca2+ (not shown) was > 1,000 ppm (6.5 meq/kg wet wt) for all samples and, therefore, below the limits of detection with INAA.

Plasma glucose, protein and osmolality, and water consumption. A detailed analysis of the time course of changes in plasma electrolytes, blood gases, and acid-base parameters can be found in Waller et al. (57). Plasma [glucose] was increased from the end of exercise up to 3 h of recovery in the electrolyte treatment, reaching a maximum of 8.18 ± 0.6 mmol/l at 1 h of recovery (Fig. 5, top). Plasma [glucose] in the control treatment was increased from the end of exercise until 4 h of recovery, reaching a maximum of 7.90 ± 0.4 mmol/l at 2 h of recovery (Fig. 5). There was no difference between treatments (P = 0.377). [PP] was increased at the end of exercise until 1 h of recovery in the electrolyte treatment, and until the end of sampling in the control, with no difference between treatments (P = 0.169) (Fig. 5, middle). Calculated plasma osmolality (Fig. 5, bottom) was increased at the end of exercise, and from 20 min to 1 h and 20 min to 4 h of recovery in the control and electrolyte treatments, respectively. Plasma osmolality decreased throughout the recovery period in the control treatment, such that it was significantly lower than Pre-Ex from 6–7 h of recovery; however, there was no difference between treatments (P = 0.104).

Total water intake (including the 8 liters given via nasogastric intubation) was greater in the electrolyte treatment, such that, at 24 h of recovery, there were 49.5 ± 4.2 and 34.8 ± 2.2 liters in the electrolyte and control treatments, respectively (P = 0.018) (Fig. 6). Total water intake over the entire 24 h of recovery also showed a treatment effect, with total intake in the electrolyte treatment significantly greater than control (P = 0.014).

DISCUSSION

The present study appears to be the first to have determined the effects of administering a hypotonic electrolyte solution immediately after prolonged exercise, and before feed is provided, on skeletal muscle glycogen and electrolyte contents in horses. The reason for early administration of the electrolyte solution was to enhance the rate of recovery of fluid and electrolyte balance and ensure that there was adequate water and K available for glycogen resynthesis. The volume of water and amount of electrolytes administered approximated the sweat losses during exercise. Both the control and electrolyte treatments were designed to mimic recovery protocols typical of the industry and thus included meal feeding with water ad libitum. The nasogastric administration of a hypotonic commercial electrolyte solution in 8 liters of water, followed...
Table 3. Gluteus medius electrolyte and water contents at rest and during recovery from a competition exercise test, after horses were either given 1) a hypotonic electrolyte solution followed by water ad libitum and a typical feeding protocol (electrolyte trial), or 2) water ad libitum and a typical feeding protocol alone (control trial).

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Method of Calculation</th>
<th>Cl⁻, meq/kg dry wt</th>
<th>Na⁺, meq/kg dry wt</th>
<th>K⁺, meq/kg dry wt</th>
<th>Mg²⁺, meq/kg dry wt</th>
<th>ECFV, L/kg wet wt</th>
<th>ICFV, L/kg wet wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Ex</td>
<td>Manery and Bale (40)</td>
<td>4.10 ± 0.3</td>
<td>3.13 ± 0.3</td>
<td>1.59 ± 0.4</td>
<td>93.8 ± 5.6</td>
<td>26.7 ± 1.9</td>
<td>0.157 ± 0.02</td>
</tr>
<tr>
<td>0</td>
<td>Lindinger and Heigerhauser (35)</td>
<td>2.14 ± 0.1</td>
<td>2.38 ± 0.1</td>
<td>1.36 ± 0.6</td>
<td>93.8 ± 5.6</td>
<td>26.6 ± 1.9</td>
<td>0.156 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>Manery and Bale (40)</td>
<td>2.94 ± 0.2</td>
<td>2.42 ± 0.2</td>
<td>1.96 ± 0.6</td>
<td>101.8 ± 11.9</td>
<td>26.8 ± 2.3</td>
<td>0.122 ± 0.01</td>
</tr>
<tr>
<td>24</td>
<td>Lindinger and Heigerhauser (35)</td>
<td>3.90 ± 0.8</td>
<td>2.79 ± 0.1</td>
<td>5.42 ± 2.7</td>
<td>83.4 ± 5.7</td>
<td>25.1 ± 1.2</td>
<td>0.109 ± 0.01</td>
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</table>

Values are means ± SE. ECFV, muscle extracellular fluid volume, ICFV, muscle intracellular fluid volume. *Significantly different (P < 0.05) from Pre-Ex time point.
interpreted with caution. The exercise protocol in the present study (prolonged, moderate intensity) was chosen for its ability to result in significant muscle glycogen depletion after only a single exercise bout, but also for inducing significant water and electrolyte losses, while decreasing the likelihood of musculoskeletal injuries.

The mean rate of glycogen resynthesis from 0 to 4 h of recovery was almost triple in the electrolyte treatment compared with control (15.0 ± 9.0 vs. 5.7 ± 9.8 mmol·kg⁻¹·h⁻¹ dry wt⁻¹, *P = 0.440*), such that, at 4 h of recovery, muscle glycogen content was not different from Pre-Ex in the electrolyte treatment, but was still significantly decreased from Pre-Ex in the control treatment. Glycogen replenishment was also greater in the electrolyte treatment from 4 to 24 h, as evidenced by the significant increase in glycogen content from 4 to 24 h of recovery. The rate of muscle glycogen resynthesis during the first 4 h postexercise in the electrolyte treatment of the present study was not as high as the maximum reported rates in horses following intravenous infusion of glucose. Intravenous infusion of glucose at 6 g/kg body wt after exercise resulted in initial (0–6 h) glycogen storage rates of 19.9 ± 3.8 (14) and 20.9 ± 7.3 mmol·kg dry wt⁻¹·h⁻¹ (17) after ~50% glycogen depletion, while rates up to ~29 mmol·kg dry wt⁻¹·h⁻¹ have been reported with ~80% muscle glycogen depletion (31). Initial glycogen resynthesis rates in horses after ingestion of meals with varying soluble CHO contents range from ~8 to ~12 mmol·kg dry wt⁻¹·h⁻¹ for mixed and high-soluble CHO diets, respectively (33). In contrast, rates of glycogen repletion in humans are two to three times higher than the rates seen in horses, for both CHO ingestion (3, 26) and intravenous glucose infusion (4, 50).

Glycogen synthesis rate is determined by substrate availability and glycogen synthase activity. Recent research suggests that the low rate of glycogen synthesis with oral CHO provision in horses may be due to reduced rate of delivery from the gastrointestinal tract (17), combined with a lack of increase in postexercise insulin sensitivity and membrane GLUT-4 translocation (48). However, intravenous infusion of large amounts of saline alone (~20 liters in 12 h for a total of 107 g NaCl) resulted in rates of replenishment of ~7.6 mmol·kg dry wt⁻¹·h⁻¹ during the first 12 h of exercise recovery in horses (31), suggesting a possible role for rehydration in glycogen resynthesis. Keller et al. (29) demonstrated in vivo in humans that cell swelling induced by hypoosmolality resulted in decreased CHO oxidation and glycogenolysis and stimulated lipolysis, while cell shrinking resulted in increased glycogenolysis. These responses to both increased and decreased cell volume appear to involve signal transduction mechanisms similar to those associated with insulin and growth factor signaling, and it has been suggested that the stimulation of
glycogen synthesis by cell swelling is likely due to dephosphorylation of glycogen synthase via effects on glycogen synthase kinase-3 (39). In support of this cell volume hypothesis, in the electrolyte treatment of the present study, calculated ICFV was increased by 18% from Pre-Ex at 4 h recovery (paired t-test \( P < 0.038 \)), compared with only 4% in the control treatment. Electrolyte administration also resulted in a greater total water intake and faster restoration of hydration status compared with control, as evidenced by faster recovery of [PP]. However, when the horses were provided their normal meals and water ad libitum (control), recovery of hydration status was still incomplete 24 h after exercise. Therefore, it is reasonable to postulate that postexercise dehydration affected muscle glycogen replenishment in the present study. In contrast to humans, equine sweat is hypertonic to plasma (44), resulting in an exercise-induced plasma dehydration that is isotonic to hypertonic. Thus, when electrolytes lost in sweat are not replaced, there is no increase in plasma osmolality to stimulate a thirst response. Indeed, in the present study, plasma osmolality was increased up to 4 h after electrolyte administration, while, in the control treatment, plasma osmolality decreased throughout the recovery period such that it was significantly lower than preexercise by 6 h of recovery. This suggests that a typical feed ration alone is not sufficient, and electrolyte supplementation is required for full recovery of hydration status following exercise.

Interestingly, exercise did not result in decreased ICFV or net loss of K\(^+\) from the gluteus medius of the horses in the present study. In contrast, in humans and rodents, prolonged exercise results in net losses of water (11) and K\(^+\) (36) from contracting muscle, in seeming contrast to the case in horses. It has previously been reported that intracellular water (37) and K\(^+\) (58) may be preserved during exercise in horses, despite ongoing glycogen degradation and extracellular dehydration. The reasons for the apparent preservation of muscle water and K\(^+\) during exercise in horses are not clear, but may be related to the fact that, during locomotion, most muscles in the horse are actively recruited to either maintain posture and balance, or to generate propulsive forces, leaving relatively little tissue mass for redistribution of ions lost from contracting muscle.

While the results of the present study support a difference in glycogen replenishment rate based on hydration and electrolyte status, it is possible that some differences in glucose availability occurred due to the presence of dextrose in the electrolyte...
supplement. The dextrose concentration (31 mmol/l) of the Perform’N Win supplement serves as a direct source of glucose to provide cellular energy to subserve increased rates of epithelial transport of Na\(^+\) and water across the small intestine (15, 18). Although the amount of dextrose administered with 8 liters of supplement (44 g) was insufficient to result in overall differences in plasma [glucose] compared with control, and significant glycemic responses to feeding occurred in both treatments, plasma [glucose] did increase faster with electrolyte supplementation, peaking at 1 h of recovery in the electrolyte treatment vs. 2 h of recovery in control (see Fig. 5). Similar glycemic responses following meals with various soluble CHO content have been demonstrated in horses following the first postexercise feeding (6, 33). This suggests that exercise attenuates the glycemic response, likely due to an increase in glucose transport into skeletal muscle. Interestingly, although identical hay/grain meals were provided at 0 and 6 h of recovery, and each horse consumed all grain fed by 20 min postfeeding, only the first meal resulted in any significant glycemic response. Feeding hay and grain in the same meal has been shown to significantly reduce glycemic response compared with feeding grain alone (33, 45), and it is speculated that hay consumption slows the rate of passage of grain through the small intestine, attenuating the glycemic response.

**Muscle electrolytes.** This study showed no change in glucose mediatus electrolyte contents or concentrations with exercise or electrolyte administration, although changes in plasma electrolyte concentrations did occur (see Ref. 57). The ~8.3-liter sweat loss corresponded to electrolyte losses of ~1.0% of total body K\(^+\), 3.7% of total body Na\(^+\), and 7.6% of total body Cl\(^−\) (9, 44). As expected, the exercise- and electrolyte administration-induced changes in [Na\(^+\)] and [Cl\(^−\)] were seen in the extracellular (plasma) compartment (57), while the estimated 1% decrease in total body K\(^+\) was not sufficient to alter muscle [K\(^+\)] or content. In contrast, a decreased skeletal muscle K\(^+\) content and an increased Na\(^+\) content occur during moderate- to high-intensity exercise in men (5, 10, 51). A possible explanation for the maintenance of muscle K\(^+\) at 0 h of recovery (~20 min postexercise) in the present study is that any sweat losses were countered by the high rates of Na\(^+\)-K\(^+\)-ATPase activity in previously contracting muscles, helping to restore intracellular [K\(^+\)] during initial recovery (36); the high rate of K\(^+\) uptake and Na\(^+\) removal is facilitated by high concentrations of circulating catecholamines and increases in intramuscular [Na\(^+\)], known activators of Na\(^+\)-K\(^+\)-ATPase activity (8).

Studies on electrolyte contents of equine muscle are rare; however, resting values for K\(^+\) and Mg\(^2+\) contents in the present study are similar to those found previously in horses using flame photometer or atomic absorption spectrometry (AAS) (38) or X-ray microanalysis (19), whereas Na\(^+\) and Cl\(^−\) contents are somewhat lower than in previous studies (Na\(^+\) ~10–20 meq/kg wet wt, Cl\(^−\) ~30 meq/kg wet wt) (19, 38). The differences in equine muscle electrolyte contents between the present and previous studies may result from the different methods of analysis, method of estimation of the ECFV (or Cl space), genetic differences, and differences in trained state. Flame photometry, AAS, and X-ray microanalysis require elaborate tissue preparation. In contrast, INAA can be performed with a minimum of sample handling and permits the entire spectrum of strong ions to be measured simultaneously in a small sample of tissue (35). When these methods of ion analysis were compared, similar electrolyte contents were found with INAA and AAS, while the contents of Na\(^+\) and Cl\(^−\) obtained by these techniques were somewhat higher, and that of K\(^+\) lower, than the values obtained by X-ray microanalysis (59). This can be attributed to the fact that, in AAS and INAA, the entire muscle biopsy contents are measured, while in X-ray microanalysis, only the muscle cell components unaffected by extracellular and nonmuscular tissue are determined. Therefore, to avoid the error from nonmuscular tissue in the present study, each sample was creatine corrected (to account for blood contamination), and then the Cl\(^−\) method was used to determine muscle ECFV and ICFV. Calculating muscle ECFV using the Cl\(^−\) space has been used extensively (2, 5, 11, 35, 40); however, it appears to consistently overestimate ECFV and underestimate ICFV, as measured simultaneously by other techniques (35).

**Conclusion.** The nasogastric administration of a hypotonic commercial electrolyte solution, followed by a typical hay and grain meal, resulted in an enhanced rate of muscle glycogen resynthesis during the recovery period compared with control. Additionally, electrolyte supplementation resulted in faster restoration of plasma hydration status compared with control, as evidenced by faster recovery of plasma [protein] and maintenance of plasma osmolality. There were no differences in muscle electrolyte contents between treatments, however. Because postexercise dehydration appears to be an important reason for why muscle glycogen replenishment is so slow in horses compared with other mammals, it is suggested that postexercise rehydration strategies can be actively used to enhance nutritional status in horses during and after fatiguing exercise.

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