Effects of prior exercise on muscle metabolism during sprint exercise in horses

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Effects of prior exercise on muscle metabolism during sprint exercise in horses. J. Appl. Physiol. 87(5): 1914–1922, 1999.—The effect of warm-up exercise on energy metabolism and muscle glycogenolysis during sprint exercise (Spr) was examined in six fit Standardbred horses exercised at 115% of maximal O2 consumption (VO2max) until fatigued, 5 min after each of three protocols: 1) no warm-up (NWU); 2) 10 min at 50% of VO2max (low-intensity warm-up (LWU)); and 3) 7 min at 50% VO2max followed by 45-s intervals at 80, 90, and 100% VO2max (high-intensity warm-up (HWU)). Warm-up increased (P < 0.0001) muscle temperature (Tm) at the onset of Spr in LWU (38.3 ± 0.2°C) and HWU (40.0 ± 0.3°C) compared with NWU (36.6 ± 0.2°C), and the rate of rise in Tm during Spr was greater in NWU than in LWU and HWU (P < 0.01). Peak VO2 was higher and O2 deficit lower (P < 0.05) when Spr was preceded by warm-up. Rates of muscle glycogenolysis were lower (P < 0.05) in LWU, and rates of blood and muscle lactate accumulation and anaerobic ATP provision during Spr were lower in LWU and HWU compared with NWU. Mean runtime (s) in LWU (173 ± 10 s) was greater than HWU (142 ± 11 s) and NWU (124 ± 4 s) (P < 0.01). Warm-up was associated with augmentation of aerobic energy contribution to total energy expenditure, decreased glycogenolysis, and longer run time to fatigue during subsequent sprint exercise, with no additional benefit from HWU vs. LWU.

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SEVERAL INVESTIGATORS have reported beneficial effects of preliminary (warm-up) exercise during subsequent high-intensity exercise in human athletes and have attributed these improvements in performance to a variety of mechanisms, including stimulation of blood flow, augmentation of O2 consumption and maximal heart rate, enhancement of free coordinated movement, and increases in muscle temperature and muscle metabolic processes (1, 20, 36). Augmentation of whole body O2 consumption (VO2) has been proposed to result from acceleration of rate-limiting oxidative phosphorylation reactions, enhanced O2 delivery associated with increased muscle blood flow, and a temperature-induced facilitation of oxyhemoglobin dissociation (23). However, the mechanisms by which warm-up exercise alters intramuscular processes and whole body metabolism are still largely undetermined. Furthermore, beneficial effects of preliminary exercise have not been a consistent finding in all studies that have investigated the use of a warm-up regimen (12, 22, 28). The relative contribution of aerobic and anaerobic sources to total energy production during strenuous exercise varies with exercise intensity and duration (1, 31). In human athletes performing supramaximal exercise lasting 1 min, ~50% of total energy expenditure is estimated to reflect aerobic energy production, with the remainder attributable to a combination of anaerobic and phosphagen sources (31). Recently, Tyler et al. (41) reported an aerobic contribution to total energy requirement in trained Standardbred horses of ~72% for sprint exercise [115% of maximal O2 consumption (VO2max)] of a similar duration (~63 s). When horses completed prior warm-up exercise, this aerobic energy contribution increased to almost 80% (41), reflecting the high aerobic energy capacity and rapid kinetics of gas exchange in the horse compared with many other species (37, 39). This enhancement of aerobic metabolism noted by Tyler and coinvestigators (41) after a 5-min warm-up at speeds calculated to elicit 50% VO2max (6° incline) raises a question as to the effects of warm-up on intramuscular metabolic processes and the potential for warm-up to enhance aerobic energy production in subsequent sprint exercise. To date, there have been no studies that have examined muscular metabolic responses of horses to sprint exercise after warm-up exercise. Furthermore, the effect of warm-up protocols of different intensities on these responses during subsequent sprint exercise (<5 min) has not been investigated.

The primary purpose of this study was to examine the effects of prior (warm-up) exercise on muscular metabolic responses to exercise at a speed equivalent to 115% of VO2max. It was hypothesized that warm-up exercise would alter the relative contributions of aerobic and anaerobic energy to total energy production during subsequent supramaximal sprint exercise. Specifically, our hypothesis was that prior warm-up exercise would increase aerobic energy production and decrease the rate of muscle glycogen degradation and the anaerobic contribution to energy production during subsequent sprint exercise. We further hypothesized that warm-up exercise of higher intensity would result in greater augmentation of the aerobic contribution to total energy production during the sprint.

MATERIALS AND METHODS

All animal experiments were conducted after approval by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University and were performed in compliance with their recommendations.
Experimental design. The effects of prior exercise on skeletal muscle metabolism, muscle temperature, accumulated O2 deficit, and changes in the whole blood lactate and specific muscle metabolites during high-intensity exercise were examined in a three-way, balanced, randomized crossover study. Six horses participated in each of three trials: 1) a control or no-warm-up (NWU) trial, in which there was no exercise before the sprint (Spr); 2) a low-intensity-warm-up (LWU) trial, in which horses completed 10 min of trotting at a speed equivalent to 50% VO2max before Spr; and 3) a high-intensity-warm-up (HWU) trial, in which horses trotted for 5 min at a speed equivalent to 50% VO2max, followed by 45 s intervals at running speeds calculated to elicit 80, 90, and 100% VO2max. For an individual horse, the distance covered during LWU and HWU was the same (-2,700 m). Each horse completed each protocol twice, for a total of six experiments for each subject, with >3 days between trials for individual horses.

Subjects. The subjects were six Standardbred horses (1 gelding and 5 mares), 3–7 yr of age, and weighing 398–442 kg [421.8 ± 7.3 (SE) kg]. All horses were housed indoors during the experimental period and were fed a diet consisting of ~8 kg of timothy grass-alfalfa hay (50:50 mix) and 4 kg of a commercial mixed-grain ration (12% protein; Countrymark Cooperative, Indianapolis, IN), divided between two feedings (0700 and 1600) and had access to a salt-mineral block, with the source and batch of both hay and grain ration maintained constant during the 2 wk before and throughout the experiment. All horses were conditioned and undertaking regular treadmill exercise for at least 3 mo before the study.

Measurement of VO2max. For each horse, VO2max and the relationship between VO2 and speed (by using speeds between 4 m/s and peak VO2) were determined during an incremental exercise test 3 days before the first experiment and within 7 days after the final experiment. The incremental exercise test consisted of the horse running on a high-speed treadmill inclined at 4° for 90 s at 4 m/s, after which the treadmill speed was increased by 1 m/s every 90 s until the horse was no longer able to maintain its position on the treadmill. VO2 was measured every 10 s during the exercise test. VO2max was defined as the value at which VO2 reached a plateau, despite further increases in speed. A plateau was defined as a change in VO2 of <4 ml·min⁻¹·kg⁻¹ with an increase in speed. From linear regression analysis, the running speed that elicited 115% VO2max was calculated for each horse.

Experimental protocol. Food was withheld for 12 h before each experiment. After aseptic preparation and local anesthesia of the overlying skin, a catheter for collection of mixed venous blood samples was placed in the right atrium (via the right jugular vein; PE 240, Becton-Dickinson, Parsippany, NJ), and a copper-constantan thermocouple (IT-14, Physitemp Instruments, Clifton, NJ) attached to a thermometer (BAT-10, Physitemp Instruments) was placed in the right atrium through the catheter. A 5-cm² area of shaved skin over each middle gluteal muscle was aseptically prepared, and local anesthesia was administered subcutaneously to allow measurement of muscle temperature (Tm) and collection of muscle biopsy samples. After measurement of body mass (±0.5 kg, Abso Scales, New Albany, OH), horses were positioned on a high-speed treadmill (Sato) for collection of baseline respiratory gas-exchange measurements and blood samples. During the exercise test, a fan mounted 0.5 m in front of the treadmill was used to maintain an air velocity of 3.5–4 m/s over the anterior and dorsal aspects of the horse. A thermohygro meter (model 3309–60, Cole-Palmer Instruments, Chicago, IL) was used to monitor ambient conditions during all trials. Ambient conditions were similar for all trials; mean ± SE values for room temperature and relative humidity during the experiments were 20.8 ± 0.2°C and 32 ± 1%, respectively.

Exercise test. With the treadmill set at a 4° incline, horses stood quietly for 10 min (NWU) or completed one of the two warm-up protocols (LWU, HWU), followed by 5 min at rest. The horses then walked for 2 min, followed by acceleration of the treadmill to a speed calculated to elicit a running speed equivalent to 115% of their predetermined VO2max [running speed 11.7 ± 0.3 (SE) m/s]. The transition from the walk to the high speed required for the exercise test was accomplished in ~5–6 s. Horses ran at this speed until fatigue, as evidenced by an inability to keep pace with the treadmill despite verbal encouragement. VO2 was measured continuously during the warm-up and Spr.

Respiratory gas-exchange measurements. VO2 was measured with an open-circuit calorimeter (Oxymax-XL, Columbus Instruments, Columbus, OH), as previously described (19). Flow through the system was ~1,500 l/min STP with the horse stationary and 9,000 l/min during running. Expired O2 concentrations were measured with a gas analyzer (Electrochemical cell, Columbus Instruments) at a sample rate of 40 Hz. Data were recorded by a computer-based data-acquisition system (Oxymax-XL, Columbus Instruments) and reported at 10-s intervals; each measurement represents the average gas concentration determined during the 10-s interval. The gas-analysis system was calibrated before the start of each exercise test by using gas mixtures with O2 concentrations that spanned the measurement range. The overall accuracy of the system was verified repeatedly by the nitrogen dilution method (10). Discrepancy between simulated VO2 produced by nitrogen dilution and the value measured by the system was ±3% at nitrogen dilution rates equivalent to a VO2 of 54 ml/min (≈140 ml·kg⁻¹·min⁻¹ for a 385-kg horse). Standard equations were used to calculate VO2 (29).

Calculation of O2 deficit. Accumulated O2 deficit during Spr was calculated as the difference between the expected and actual VO2 during Spr, with the use of previously described assumptions (30). Actual VO2 was calculated by using the trapezoidal rule (14); expected VO2 was calculated from the speed-VO2 relationship determined during the incremental exercise test and the speed of the horse during the exercise tests in the three trials. The speed-VO2 relationship was determined by using VO2 rates below VO2max (29).

Temperature measurements and muscle biopsies. Right atrial blood temperature (Tr) and middle gluteal Tm were measured before and 5 min after the warm-up protocol, at the end of Spr, and after 5 min of recovery. Tm was measured by inserting a needle thermocouple (MT-23; Physitemp Instruments) ~4 cm into the muscle through the lumen of an 18-gauge 37-mm needle. All thermocouples had response times of ~1°C/s and were calibrated in a heated water bath with a precision thermometer (Fisher Scientific, Mississauga, ON). Muscle biopsy samples were collected percutaneously from the middle gluteal muscle by using the needle-biopsy technique described by Lindholm and Piehl (25). Muscle biopsies were obtained before and within 10 s of the termination of Spr. The samples were immediately placed in liquid nitrogen and stored at ~80°C until analysis.

Blood collection. Mixed venous blood samples for measurement of hematocrit, plasma total protein, and whole blood lactate concentrations were collected before and at the end of the warm-up, immediately before Spr, at the point of fatigue, and at 5 min of recovery. Blood samples for measurement of hematocrit and plasma total protein were collected into evacuated glass tubes containing EDTA (Vacutainer, Becton-Dickinson).
Analytic techniques. Whole blood lactate concentrations were measured electrochemically in duplicate (Spor 1500, Yellow Springs Instruments). Hematocrit was measured in duplicate by the microhematocrit technique. Plasma total protein was measured by refractometry (Cambridge Instruments, Buffalo, NY). Muscle metabolite concentrations were measured in freeze-dried muscle samples. Tissue samples were dried to a constant weight, and the change in weight was used to determine tissue water content. A portion of each sample was extracted according to the general procedures of Harris et al. (17); duplicates of these extracts were analyzed for lactate, ATP, phosphocreatine (PCr), and glucose 6-phosphate (G-6-P) content by using enzymatic fluorometric methods (26). Muscle glycogen concentration (as glycogen units) was determined on a second freeze-dried aliquot of muscle, which was extracted and analyzed according to the procedures described by Passoneau and Lauderdale (33). Intra- and interassay coefficients of variation for these assays ranged from 2.8 to 4.6%.

Calculations. The anaerobic ATP provided during Spr (mmol/kg dry muscle) was calculated from the values of ATP, PCr, and lactate in muscle samples collected before and immediately after Spr. Anaerobic ATP production during Spr was calculated as 1.5 × \[\Delta [\text{La}] + \Delta [\text{PCr}] + \Delta [\text{ATP}]\], where \[\Delta [\text{La}]\] is the absolute change in muscle lactate concentration, \[\Delta [\text{PCr}]\] is the absolute change in PCr concentration, and \[\Delta [\text{ATP}]\] is the absolute change in ATP concentration. No correction has been made for lactate efflux during Spr (4, 31). Glycogen utilization was calculated as the difference between the post-warm-up and end-exercise muscle glycogen concentrations. Rates of glycogen degradation and blood and muscle lactate accumulation during Spr were calculated by dividing the change in metabolite concentration by the run time.

Statistical analyses. Data from this study were analyzed as a three-way crossover design by use of a two-way repeated measures analysis of variance [repeated measures on treatment (i.e., NWU, LWU, or HWU) and time factors] or as a one-way repeated-measures analysis (repeated measures on the treatment factor) depending on the data being analyzed (15). Significance was defined as \(P < 0.05\) for each of the main effects of treatment or time and as \(P < 0.1\) for the interaction. Within each warm-up protocol, there was no difference between the two trials for each treatment completed by each horse; therefore, the data were combined for the subsequent analysis. When there was a significant main or interaction effect, differences were identified using a Student-Newman-Keuls test (to detect differences between WU treatments) and Dunnett’s test (to detect differences within a treatment group). Results are expressed as means ± SE.

RESULTS

\(\dot{V}_\text{O}_{2\text{max}}\) speed-\(V\dot{O}_2\) relationship, and body weight. Mean \(\dot{V}_\text{O}_{2\text{max}}\) of the six horses was 151.6 ± 5.4 ml·min\(^{-1}\)·kg\(^{-1}\) or 63.8 ± 1.8 l/min at a treadmill speed of 10.7 ± 0.2 m/s; postexercise mean \(\dot{V}_\text{O}_{2\text{max}}\) of the six horses was 150.4 ± 5.4 ml·min\(^{-1}\)·kg\(^{-1}\). The correlation coefficient for the speed-\(V\dot{O}_2\) regression averaged 0.995 ± 0.001 (\(P < 0.01\)). The slope of the regression line was 0.267 ± 0.01 ml O\(_2\)·m\(^{-1}\)·kg\(^{-1}\), and the ordinate intercept was 6.1 ± 0.6 ml O\(_2\)·min\(^{-1}\)·kg\(^{-1}\). Mean work intensity for all trials was 114 ± 2.2% \(\dot{V}_\text{O}_{2\text{max}}\) and work rate (W) was similar for all trials (Table 1). Body weight, measured immediately before each experiment, was not different for the three trials (Table 1) (\(P < 0.05\)).

\(\dot{V}_\text{O}_2\) and \(O\dot{2}\) deficit. Peak values for absolute \(\dot{V}_\text{O}_2\) (litters of \(O\dot{2}\)/horse) achieved during Spr were significantly higher (\(P < 0.01\)) in LWU (98.9 ± 0.7\% \(\dot{V}_\text{O}_{2\text{max}}\)) and HWU (99.4 ± 0.4\% \(\dot{V}_\text{O}_{2\text{max}}\)) than in NWU (94.0 ± 0.8\% \(\dot{V}_\text{O}_{2\text{max}}\)). Whereas accumulated \(O\dot{2}\) deficit was not different (\(P > 0.05\)) for the three trials (ml \(O\dot{2}\) equivalents/kg body wt: NWU 86.50 ± 3.8; LWU 86.21 ± 7.9; HWU 83.27 ± 4.4), the \(O\dot{2}\) deficit expressed per minute of exercise was significantly higher in NWU than in LWU and HWU (Fig. 1).

Run time to fatigue. Mean run time to fatigue in Spr was significantly (\(P < 0.01\)) greater in LWU than in HWU and NWU (Table 1). HWU run time was also significantly longer than NWU (\(P < 0.05\)). The coefficient of variation for run times within each trial was 4.53 ± 1.05, 4.89 ± 1.29, and 5.94 ± 0.96% for NWU, LWU, and HWU, respectively.

Temperature responses. Warm-up exercise was associated with significant (\(P < 0.0001\)) increases in \(T_m\) at the onset of Spr in LWU (38.3 ± 0.2°C) and HWU (40.0 ± 0.3°C) compared with NWU (36.6 ± 0.2°C) (Fig. 2). The mean rate of rise in \(T_m\) during Spr was significantly greater (\(P < 0.05\)) in NWU (1.51 ± 0.03°C/min) than in LWU (1.09 ± 0.04°C/min) and HWU (0.80 ± 0.03°C/min). Similarly, \(T_m\) was also significantly elevated (\(P < 0.05\)) after warm-up compared with NWU, and these

Table 1. Run time to fatigue, preexercise body weight, peak \(\dot{V}_\text{O}_2\), work rate, rate of anaerobic ATP provision, and data before and immediately after sprint exercise at 115% of \(\dot{V}_\text{O}_{2\text{max}}\) for right atrial temperature, hematocrit, and total plasma protein.

<table>
<thead>
<tr>
<th></th>
<th>NWU</th>
<th>LWU</th>
<th>HWU</th>
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<tr>
<td>Run time, s</td>
<td>124 ± 4</td>
<td>173 ± 10*</td>
<td>142 ± 11*</td>
</tr>
<tr>
<td>Preexercise body weight</td>
<td>416.6 ± 7.8</td>
<td>416.7 ± 8.0</td>
<td>417.2 ± 7.7</td>
</tr>
<tr>
<td>Peak (\dot{V}_\text{O}_2) l/min</td>
<td>60.1 ± 1.1</td>
<td>62.2 ± 1.1*</td>
<td>62.8 ± 1.0*</td>
</tr>
<tr>
<td>Watts at 115% (\dot{V}<em>\text{O}</em>{2\text{max}})</td>
<td>3.093 ± 64</td>
<td>3.081 ± 67</td>
<td>3.096 ± 70.2</td>
</tr>
<tr>
<td>Rate of anaerobic ATP provision, mmol·kg(^{-1}) dry wt·min(^{-1})</td>
<td>1.11 ± 0.11</td>
<td>0.71 ± 0.04*</td>
<td>0.69 ± 0.07*</td>
</tr>
<tr>
<td>(T_m), °C</td>
<td>Rest 36.6 ± 0.2</td>
<td>36.6 ± 0.2</td>
<td>36.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Sprpre 37.6 ± 0.2</td>
<td>37.6 ± 0.1*</td>
<td>38.3 ± 0.3*</td>
</tr>
<tr>
<td></td>
<td>Fatigue 38.6 ± 0.4†</td>
<td>40.9 ± 0.2†</td>
<td>41.4 ± 0.5†</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>Rest 41.5 ± 1.8</td>
<td>45.2 ± 0.8*</td>
<td>48.8 ± 1.8*</td>
</tr>
<tr>
<td></td>
<td>Sprpre 45.8 ± 1.1†</td>
<td>54.3 ± 1.3†</td>
<td>53.5 ± 1.3†</td>
</tr>
<tr>
<td></td>
<td>Fatigue 62.2 ± 2.2</td>
<td>63.7 ± 1.1</td>
<td>64.1 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Sprpre 63.6 ± 1.2</td>
<td>63.5 ± 2.4</td>
<td>66.7 ± 2.1*</td>
</tr>
<tr>
<td></td>
<td>Fatigue 72.9 ± 1.1†</td>
<td>73.8 ± 1.8†</td>
<td>71.8 ± 2.2†</td>
</tr>
</tbody>
</table>

Values are means ± SE. NWU, LWU, and HWU: no, low, and high-intensity warm-up, respectively; \(\dot{V}_\text{O}_2\), \(O\dot{2}\) consumption; \(\dot{V}_\text{O}_{2\text{max}}\) maximal \(\dot{V}_\text{O}_2\); \(T_m\), right atrial blood temperature; Spr, sprint exercise; Rest, before warm-up protocol; Sprpre and fatigue: before and immediately after Spr, respectively. *Significantly different from NWU, \(P < 0.05\). †Significantly different from Sprpre, \(P < 0.05\).
differences in temperature were still evident at the end of Spr (Table 1). However, there was no significant difference in the mean rate of rise in T_{fa} among the three trials (NWU: 0.9 ± 0.08; LWU: 1.1 ± 0.09; HWU: 1.2 ± 0.05°C/min exercise).

Whole blood lactate, hematocrit, and total protein. Hematocrit and plasma total protein concentration were significantly \((P < 0.01)\) increased after the warm-up exercise in LWU and HWU, with no significant difference between these treatments (Table 1). At the end of the exercise test, there were significant increases in hematocrit and plasma protein concentration in each trial but no difference \((P < 0.05)\) in these variables among the three trials. After warm-up exercise and before Spr, whole blood lactate was significantly increased in HWU compared with LWU and NWU. Although whole blood lactate concentrations were not different \((P < 0.001)\) for the three warm-up protocols at the end of Spr (see Fig. 5), the net rate of lactate accumulation during Spr was lower \((P < 0.05)\) in LWU and HWU \((5.14 ± 0.47 \text{ and } 3.21 ± 0.36 \text{ mmol/min, respectively})\) compared with NWU \((6.74 ± 0.35 \text{ mmol/min})\).

Muscle metabolic responses. Water content in pre- and post-Spr muscle samples was not significantly different \((-78\%)\) \((P > 0.8)\). Muscle glycogen concentrations were significantly lower \((P < 0.05)\) in HWU and LWU after the warm-up protocol (Fig. 3A). At the end of
Spr, muscle glycogen concentrations in LWU and HWU were also significantly lower than post-Spr values measured in the NWU trial (P < 0.05). When expressed as a function of run time in Spr, the net rate of muscle glycogenolysis was 40% lower in LWU and 20% lower in HWU compared with NWU. However, only the rate of glycogen degradation during LWU was significantly lower than the no-warm-up trial (P < 0.05) (power 5.0.4733) (Fig. 3B). Although the absolute change in muscle lactate was similar in all three trials (Fig. 4A), the net rate of muscle lactate accumulation during Spr was significantly decreased in LWU and HWU (P < 0.0002) compared with NWU (Fig. 4B).

After warm-up exercise, exercise-associated increases in muscle G-6-P concentration were attenuated (41–45%) (P, 0.001) at the end of Spr (Table 2). Spr resulted in a ~15–17% decrease in ATP, with the magnitude of the decrease being significantly greater in LWU and HWU compared with NWU (Table 2). PCr concentrations at the end of Spr were lower (P < 0.05) in HWU compared with NWU; the decrease in PCr during Spr was significantly less (P < 0.05) in HWU and LWU compared with NWU (Table 2). The rate of anaerobic ATP provision, calculated from the changes ATP, lactate, and PCr was significantly higher (P, 0.05) in NWU compared with LWU and HWU, with no difference between trials with warm-up exercise (Table 1).

DISCUSSION

The most significant findings of the present study were the following: 1) a lower rate of glycogen utilization during the Spr in LWU compared with NWU and HWU; 2) a reduction in the rate of muscle lactate accumulation during Spr after trials with preliminary exercise (HWU and LWU); 3) a lower O2 deficit, expressed per minute of run time, in LWU and HWU compared with NWU; 4) a higher peak VO2 in LWU and HWU; 5) a reduction, after LWU and HWU, in the calculated contribution of anaerobically derived ATP (provided from PCr degradation and glycolysis); and 6) a longer run time to fatigue during Spr when horses completed prior warm-up exercise.

The present study demonstrated that in trials preceded by a warm-up, horses had a larger proportion of energy utilized derived from aerobic metabolism. The greater aerobic energy contribution during Spr in the trials with a prior LWU or HWU is evinced by the attainment of a higher peak VO2 (Table 1). In addition, the lower rate of glycogenolysis, lower O2 deficit per minute of exercise, and the blunted increase in muscle and blood lactate (Fig. 5) during Spr support an attenuation of anaerobic energy production during these trials. As might have been anticipated, in the trials (LWU, HWU) in which a greater proportion of energy was derived from aerobic metabolism, fatigue was delayed as manifest by a longer run time in Spr.

The increased aerobic contribution to total energy expenditure in the high-speed Spr after a warm-up is

Table 2. Middle gluteal muscle metabolite concentrations before and immediately after sprint exercise at 115% of VO2max after NWU, LWU, or HWU

<table>
<thead>
<tr>
<th></th>
<th>NWU</th>
<th>LWU</th>
<th>HWU</th>
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<tbody>
<tr>
<td>ATP</td>
<td>Sprpre</td>
<td>20.02±0.50</td>
<td>21.93±0.59</td>
</tr>
<tr>
<td>Fatigue</td>
<td>18.34±0.49*</td>
<td>18.23±0.61*</td>
<td>17.87±0.94*</td>
</tr>
<tr>
<td>G-6-P</td>
<td>Sprpre</td>
<td>6.18±0.85</td>
<td>7.98±0.61</td>
</tr>
<tr>
<td>Fatigue</td>
<td>18.31±0.78*</td>
<td>15.12±1.10†</td>
<td>15.59±0.74†</td>
</tr>
<tr>
<td>PCr</td>
<td>Sprpre</td>
<td>79.88±3.5</td>
<td>67.68±2.7</td>
</tr>
<tr>
<td>Fatigue</td>
<td>47.38±2.9*</td>
<td>44.28±3.1*</td>
<td>41.83±3.0†</td>
</tr>
</tbody>
</table>

*Significantly different (P < 0.05) from Sprpre. †Significantly different (P < 0.05) from NWU.

Values are means ± SE. G-6-P, glucose 6-phosphate; PCr, phosphocreatine. All muscle metabolites are expressed as mmol/kg dry muscle.
consistent with the findings of Tyler et al. (41). The rate of increase in \( \text{VO}_2 \) at the onset of high-intensity exercise has been demonstrated to be greater in horses than in human subjects (38), and this rate of increase is further accelerated by prior warm-up exercise (41). The more rapid gas-exchange kinetics in the horse most likely are a reflection of the swift mobilization of the splenic reserve of erythrocytes combined with the greater rate of increase and higher maximal heart rate at the onset of exercise (9, 38). In the present study, the significantly higher PCV at the onset of Spr in the trials preceded by a warm-up are consistent with the higher peak \( \text{VO}_2 \) measured in the these two trials (Table 1).

Concurrent with the higher \( \text{VO}_2 \) during Spr preceded by a warm-up, there was a decrease in \( \text{O}_2 \) deficit per minute of exercise. A lower \( \text{O}_2 \) deficit implies a smaller rate of anaerobic ATP provision in the Spr, as reduced \( \text{O}_2 \) deficit, expressed per minute of exercise, in trials with a warm-up (Fig. 1).

The present study demonstrated significant and similar decreases in muscle glycogen after Spr (~18% NWU; 20% LWU; 23% HWU). However, as the time to fatigue for the trials was significantly different, the extent of glycogen degradation during Spr represents notably different rates of glycogenolysis (1.13 ± 0.16, 0.91 ± 0.13, and 0.67 ± 0.17 mmol·s\(^{-1} \cdot kg^{-1} \cdot wt^{-1} \)) for NWU, HWU, and LWU, respectively. In human subjects, estimated rates of glycogenolysis during maximal Spr running of 2- to 3-min duration are 0.15–0.30 mmol·kg\(^{-1} \cdot s^{-1} \cdot wt^{-1} \) (1, 40). Rates of glycogenolysis measured in the present study are within the range of estimates reported for 0.5–3.0 min of intense exercise in human subjects (~0.16 ± 0.25 mmol·kg\(^{-1} \cdot s^{-1} \cdot wt^{-1} \) when expressed as wt), with lower rates in the two warm-up trials. When human subjects completed two bouts (~3 min) of exhaustive exercise separated by 10 min of rest, glycogenolysis was reduced by ~50% and lactate production by ~60% during the second bout of exercise (3). Although part of this decline resulted from the subjects’ inability to sustain a similar work output in the second exercise bout, the reductions cannot be explained solely on the basis of a slightly lower work intensity, but they reflect a substantial decrease in anaerobic ATP resynthesis. Thus the lower rates of glycogenolysis in the present study during Spr preceded by a warm-up most likely reflect changes in the balance of aerobic and/or anaerobic energy contributions, with lowered anaerobic demand resulting from the enhancement of \( \text{VO}_2 \) kinetics at the onset of exercise. Alternatively, differences in initial glycogen concentration may have contributed to a decline in the subsequent rates of glycogenolysis during Spr in which glycogen concentration was decreased by a warm-up.

In certain circumstances, muscle glycogen level can determine the rate of glycogen degradation during exercise (1, 18, 34). In particular, higher glycogenolytic rates have been induced when muscle glycogen storage was increased before submaximal exercise (34). In contrast, there is less evidence that moderate depletion alters glycogen degradation rates during intense exercise (16). Rather, glycogenolytic rates during short-term, high-intensity exercise in human subjects appear to be independent of initial muscle glycogen concentrations above 40–50 mmol/kg wet wt (1, 34). In horses, Davie et al. (8) demonstrated that a 22% reduction in muscle glycogen concentration did not have a measurable effect on glycogenolysis rate during high-intensity exercise. However, severe depletion (55% reduction) has been associated with a shorter run time to fatigue and a decrease in maximal accumulated \( \text{O}_2 \) deficit (24). Although glycogenolytic rates were not measured during these sprints, lower blood lactate concentrations were attributable to lowered glycogenolysis in the glycogen-depleted state. In the present study, the change in glycogen concentration associated with HWU and LWU was estimated as ~15% and, as such, was less than the extent of muscle glycogen degradation induced by Davie et al. (8). Therefore, muscle glycogen...
concentration at the start of Spr is unlikely to have been the determinant of the rate of glycogenolysis.

The lower rates of glycogen degradation after the LWU and HWU were associated with lesser increases in blood lactate concentration. Two major possibilities to explain the decreased blood lactate would include 1) a decrease in anaerobic metabolism, resulting in lower lactate production; and 2) an increase in the rate of lactate uptake within muscle or other tissues. It has been speculated that warm-up exercise contributes to enhanced fuel efficiency by increasing the flux of endogenous energy stores to muscle mitochondria, thereby promoting complete oxidation of substrates. The lower rates of glycogenolysis after warm-up exercise support a decreased anaerobic energy contribution and lower lactate production. Lund et al. (27) also reported a decreased rate of lactate accumulation in trained Thoroughbred horses given a warm-up before exercise to fatigue (at 105% of VO2max) compared with NWU. Their findings provide similar evidence for a reduction in anaerobic energy production in horses when intense exercise is preceded by a warm-up. To explain this decrease in anaerobic energy production, it has been speculated that a slightly higher VO2 after preliminary exercise will translate into large quantities of additional ATP if carbohydrate is oxidized rather than being metabolized to lactate (40). In addition, greater free energy release for ATP hydrolysis due to higher Tm and recruitment of additional muscle fibers and fiber types have also been proposed to contribute to reductions in anaerobic energy production (3, 40).

Lower rates of lactate accumulation during Spr in LWU and HWU may result from decreased lactate production but could also reflect changes in the rate of lactate release and/or uptake by muscle tissue. Increased muscle blood flow after warm-up exercise could increase the rate of lactate release from exercising muscle. During intense exercise of 2- to 3-min duration, such lactate efflux has been estimated to represent as much as 25–35% of the total lactate production (3). As a result of this efflux and the potential for lactate uptake by nonworking muscles and other tissues, accurate quantification of reductions in anaerobic energy production as a result of prior warm-up exercise is difficult. However, the lower rate of provision of anaerobic ATP provision calculated for LWU and HWU is consistent with a decrease in anaerobic energy metabolism and with the finding of a lower rate of accumulation of O2 deficit and a higher peak VO2 in the same trials (Fig. 1, Table 1).

The choice of a protocol that included two different warm-up trials was based on the hypothesis that a HWU would further augment the aerobic contribution to total energy expenditure. This hypothesis was chosen because warm-up exercise strategies employed for two breeds of racehorses (Standardbreds and Thoroughbreds), performing in races of similar duration (~2 min), can vary significantly in terms of their intensity. Studies in human subjects have demonstrated that a warm-up of sufficient intensity to cause a blood lactate concentration ≥4 mmol/l results in an acceleration of VO2 kinetics during subsequent intense exercise. In contrast, low-intensity preliminary exercise has minimal effects on pulmonary gas exchange under similar circumstances (11). Slower VO2 kinetics in human subjects during exercise without a warm-up have been attributable to limitations in blood flow and O2 delivery to working muscles (21). An increase in VO2 kinetics during the exercise after a warm-up have been theorized to result from metabolic changes induced by the prior high-intensity exercise (13). Specifically, an intense warm-up will elevate the concentrations of blood and muscle lactate and decrease muscle pH. These events can result in vasodilation, increases in muscle blood flow, and an acidemia- and temperature-induced rightward shift of the oxygen-hemoglobin dissociation curve, all of which will contribute to enhanced O2 delivery to muscle at the start of a subsequent bout of exercise (7, 13). In contrast to findings reported for human athletes, there is no evidence that prior exercise in horses need be of high intensity. Tyler et al. (41) reported that even a LWU would augment the aerobic contribution to total energy production in horses. In the present study, it was also demonstrated that a LWU would enhance the contribution of aerobic energy to total energy production and decrease the rate of glycogen degradation during intense exercise. Furthermore, it was determined that a higher intensity warm-up did not provide an additional advantage during subsequent Spr.

In this study, run time to fatigue was 30 and 47% longer, respectively, when a HWU and LWU preceded Spr. In each trial, however, maximal O2 deficit and post-sprint concentrations of muscle and blood lactate were similar, with the rates of lactate accumulation being lower in HWU and LWU. If the duration of high-intensity exercise is limited by anaerobic capacity, then the longer run times after the warm-up exercise may represent the effect of a lower rate of anaerobic energy contribution during Spr. Some investigators have argued that an elevated Tm will contribute to enhanced aerobic energy production via increases in muscle VO2 resulting from acceleration of metabolic rate-limiting muscular reactions (Q10 effect) of oxidative phosphorylation (5, 40). Such alterations would contribute to a higher peak VO2 and a lower rate of anaerobic energy production. Elevations in Tm are also purported to augment VO2 after warm-up by enhancing oxyhemoglobin dissociation at any given PO2, thereby increasing O2 availability to the muscle (23). It could therefore be hypothesized that increases in Tm after a warm-up enhanced the aerobic energy contribution during Spr, resulting in a prolongation in run time compared with NWU. In contrast to the potential benefits of an intense warm-up, there is some evidence of long-term (>1 h) effects of prior high-intensity exercise on the kinetics of calcium uptake and release by the sarcolemmal reticulum and/or the rate of decline in neural drive during a second exercise bout (3, 6). Such factors may have contributed to the shorter Spr run time after the more strenuous exercise undertaken during HWU.
In conclusion, warm-up exercise resulted in a higher peak VO_2, reduced O_2 deficit, and a decreased rate of muscle glycogenolysis during a subsequent bout of sprint exercise. This reduction in glycogenolysis was associated with declines in the rate of blood and muscle lactate accumulation during the intense exercise and a decrease in the calculated rate of anaerobic ATP provision during the subsequent sprint. Warm-up exercise in Standardbred horses can increase the rate of oxidative energy metabolism and run time to fatigue during short-term, high-intensity exercise. Furthermore, there appeared to be no additional benefit derived from a high-intensity warm-up compared with a warm-up at 50% VO_2max.

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