Muscle metabolites and performance during high-intensity, intermittent exercise

MARK HARGREAVES,1 MICHAEL J. McKENNA,2 DAVID G. JENKINS,4 STUART A. WARMINGTON,1 JIA L. LI,2 RODNEY J. SNOW,3 AND MARK A. FEBBRAIO1

1Department of Physiology, The University of Melbourne, Parkville 3052; 2Department of Human Movement, Recreation, and Performance, and 3Exercise Metabolism Unit, Department of Chemistry and Biology, Centre for Rehabilitation, Exercise, and Sport Science, Victoria University of Technology, Footscray 3011; and 4Department of Human Movement Studies, The University of Queensland, Brisbane 4072, Australia

Hargreaves, Mark, Michael J. McKenna, David G. Jenkins, Stuart A. Warmington, Jia L. Li, Rodney J. Snow, and Mark A. Febbraio. Muscle metabolites and performance during high-intensity, intermittent exercise. J. Appl. Physiol. 84(5): 1687–1691, 1998.—Six men were studied during four 30-s “all-out” exercise bouts on an air-braked cycle ergometer. The first three exercise bouts were separated by 4 min of passive recovery; after the third bout, subjects rested for 4 min, exercised for 30 min at 30–35% peak O2 consumption, and rested for a further 60 min before completing the fourth exercise bout. Peak power and total work were reduced (P < 0.05) during bout 3 [765 ± 60 (SE) W; 15.8 ± 1.0 kJ] compared with bout 1 (1,168 ± 55 W, 23.8 ± 1.2 kJ), but no difference in exercise performance was observed between bouts 1 and 4 (1,094 ± 64 W, 23.2 ± 1.4 kJ). Before bout 3, muscle ATP, creatine phosphate (CP), glycogen, pH, and sarcoplasmic reticulum (SR) Ca2+ uptake were reduced, while muscle lactate and inosine 5′-monophosphate were increased. Muscle ATP and glycogen before bout 4 remained lower than values before bout 1 (P < 0.05), but there were no differences in muscle inosine 5′-monophosphate, lactate, pH, and SR Ca2+ uptake. Muscle CP levels before bout 4 had increased above resting levels. Consistent with the decline in muscle ATP were increases in hypoxanthine and inosine before bouts 3 and 4. The decline in exercise performance does not appear to be related to a reduction in muscle glycogen. Instead, it may be caused by reduced CP availability, increased H+ concentration, impairment in SR function, or some other fatigue-inducing agent.

Methods

Subjects. Six male subjects [26 ± 4 (SD) yr, 80.6 ± 7.0 kg] agreed to participate in this study after being informed of all procedures, risks, and stresses and providing their written consent. The study was approved by the Human Research Ethics Committee of the University of Melbourne. Peak pulmonary O2 uptake (VO2peak) was measured during incremental cycling to fatigue; VO2peak averaged 4.03 ± 0.29 l/min.
Subjects reported to the laboratory at a time that was at least 6 h postprandial. They had abstained from exercise and from intake of alcohol and caffeine for the previous 24 h. Subjects lay supine on a couch while a catheter was inserted into an antecubital vein and a resting blood sample was obtained. The catheter was kept patent by periodic flushing with saline containing a small amount of heparin (10 IU/ml). A muscle sample was then obtained from the vastus lateralis by using the percutaneous needle-biopsy technique with suction. A portion of this sample (20–30 mg) was used immediately for determination of the peak SR Ca\(^{2+}\) uptake rate. The remaining sample was quickly frozen in liquid N\(_2\) for later analysis of muscle [H\(^+\)] and metabolites. Subjects then moved to an air-braked cycle ergometer (Repcor, Melbourne, Australia). After subjects rested for at least another 5–10 min in the sitting position, a preexercise exercise bout was obtained before subjects completed four 30-s “all-out” cycling bouts. The first three exercise bouts were separated by 4 min of passive recovery. After the third exercise bout, subjects rested on the cycle ergometer for 4 min, cycled for 30 min at a work load requiring 30–35% VO\(_2\)peak, and then rested for a further 60 min in the supine position before completing the fourth all-out exercise bout. This protocol was chosen to facilitate removal of lactate and H\(^+\) from blood and muscle while minimizing resynthesis of muscle glycogen (12). Power output, which was assumed to be proportional to the cube of pedal frequency, and total work were recorded by a work-monitor unit (Repcor) during each exercise bout. Venous blood was sampled immediately before and during the last 5 s of each exercise bout and after 4 min of passive recovery from each bout. Blood was analyzed for hemoglobin concentration, for hematocrit, and for plasma [H\(^+\)] and concentrations of lactate and K\(^+\) ([K\(^+\)]). Additional muscle samples were obtained immediately before the third and fourth exercise bouts for analysis of peak SR Ca\(^{2+}\) uptake rate, H\(^+\), and metabolites.

Analytical methods. Hemoglobin concentration was measured in duplicate spectrophotometrically (OSM-2 hemoximeter, Radiometer, Copenhagen), and hematocrit was measured in duplicate on a blood-gas/metabolite analyzer (Ciba-Corning 865, Ciba-Corning Diagnostics Group, Medfield, MA). Lactate concentration was measured in duplicate on deproteinized plasma extracts by using an enzymatic, fluorometric method (20). For biochemical analyses, muscle samples were freeze dried, dissected free of visible connective tissue, and powdered. One portion was extracted (16) and analyzed for ATP, CP, creatine, and lactate contents by using enzymatic, fluorometric methods (20). Adenine nucleotides, IMP, hypoxanthine, and inosine were measured by HPLC (33). A second portion was extracted in 250 µl of 2 M HCl at 100°C for 2 h, neutralized with 750 µl of 0.67 M NaOH, and assayed for glycogen (as glucosyl units) by using an enzymatic method (20). Muscle metabolites, except glycogen and lactate, were adjusted to the peak total creatine for each subject. A third portion was homogenized in buffer (200 µl/mg) and was analyzed for [H\(^+\)] at 37°C (27) by using an M 410 microelectrode (Microelectrodes, Londonderry, NH). SR Ca\(^{2+}\) uptake was measured on a muscle homogenate as described previously (31). Briefly, muscle samples (20–30 mg) were homogenized in buffer (8 µl/mg) containing 10 mM sodium azide, 5 mM oxalate, 5 µM N,N,N',N'-tetraakis (2-pyridylmethyl)ethylendiamine, 40 mM KCl, 40 mM HEPES, and 250 mM sucrose. A 100-µl sample was added to a quartz cuvette with 2 ml of assay buffer comprising 40 mM HEPES, 40 mM KCl, 5 mM MgATP, 10 µM CaCl\(_2\) (free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_0\)) = 1–1.5 µM), and 7.5 µM fura 2 and maintained at 37°C. The change in cuvette [Ca\(^{2+}\)]\(_0\), as a result of Ca\(^{2+}\) uptake by SR vesicles, was monitored by the ratio of fura-2 fluorescence at 510 nm after excitation at 340 and 380 nm (Cairn, UK). SR Ca\(^{2+}\) uptake was calculated from the peak rate of change of [Ca\(^{2+}\)]\(_0\), which occurred within the first 20 s after homogenate injection. Homogenate total protein was measured by using the Coomassie blue method (8). The data were analyzed by one-way, repeated-measures ANOVA, with significance at the P < 0.05 level. All data are reported as means ± SE.

### RESULTS

Peak power output and total work production during exercise bouts 2 and 3 were significantly lower than the values achieved during bout 1 (Table 1). On average, peak power and total work were reduced by ~15 and 35% during bouts 2 and 3, respectively. In contrast, there were no differences in peak power and total work between bouts 1 and 4 (Table 1).

Plasma [H\(^+\)] and lactate increased progressively after exercise bouts 1, 2, and 3; however, values immediately before bout 4 were not different from those obtained before bout 1 (Table 2). The 4-min postexercise plasma [H\(^+\)] and lactate values for bouts 1 and 4 were not different (Table 2). Plasma [K\(^+\)] increased during

### Table 1. Peak power and work output during four 30-s exercise bouts

<table>
<thead>
<tr>
<th>Exercise Bout</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak power, W</td>
<td>1.168 ± 55</td>
<td>1.007 ± 55</td>
<td>765 ± 60†</td>
<td>1.094 ± 64†</td>
</tr>
<tr>
<td>Work, kJ</td>
<td>23.8 ± 1.2</td>
<td>19.8 ± 1.3*</td>
<td>15.8 ± 1.0*</td>
<td>23.2 ± 1.4†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. *Significantly different from bout 1, P < 0.05; †significantly different from bout 2; P < 0.05; ‡significantly different from bout 3; P < 0.05.

### Table 2. Changes in plasma volume, plasma [H\(^+\)], [lactate], and [K\(^+\)] before, during the last 5 s, and at 4 min after the four 30-s exercise bouts

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Pre-Bout 1</th>
<th>Bout 1, 30-s</th>
<th>Pre-Bout 2</th>
<th>Bout 2, 30-s</th>
<th>Pre-Bout 3</th>
<th>Bout 3, 30-s</th>
<th>4-min Post-Bout 3</th>
<th>Pre-Bout 4</th>
<th>Bout 4, 30-s</th>
<th>4-min Post-Bout 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔPV, %</td>
<td>0</td>
<td>-7.3 ± 0.5</td>
<td>-9.5 ± 2.9*</td>
<td>-15.6 ± 1.9*</td>
<td>-17.9 ± 2.5*</td>
<td>-16.5 ± 2.5*</td>
<td>-18.9 ± 2.6*</td>
<td>-16.3 ± 3.1*</td>
<td>4.3 ± 3.1*</td>
<td>-6.0 ± 4.0</td>
<td>-10.3 ± 3.4</td>
</tr>
<tr>
<td>[H(^+)], mM</td>
<td>44.2 ± 1.1</td>
<td>45.2 ± 0.1</td>
<td>48.2 ± 0.8</td>
<td>77.6 ± 3.7*</td>
<td>73.4 ± 1.9*</td>
<td>90.0 ± 6.4*</td>
<td>84.1 ± 2.6*</td>
<td>91.6 ± 5.3*</td>
<td>45.8 ± 0.9</td>
<td>51.7 ± 2.8</td>
<td>72.3 ± 2.1</td>
</tr>
<tr>
<td>[Lactate], mM</td>
<td>1.2 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>3.2 ± 0.4</td>
<td>11.7 ± 1.3*</td>
<td>12.6 ± 0.4*</td>
<td>14.7 ± 1.1*</td>
<td>16.9 ± 2.2*</td>
<td>19.0 ± 2.1*</td>
<td>22.2 ± 0.3</td>
<td>52.2 ± 2.1*</td>
<td>11.7 ± 1.1</td>
</tr>
<tr>
<td>[K(^+)], mM</td>
<td>4.3 ± 0.0</td>
<td>4.4 ± 0.2</td>
<td>6.2 ± 0.2</td>
<td>4.0 ± 0.1</td>
<td>5.7 ± 0.3*</td>
<td>4.2 ± 0.2</td>
<td>5.1 ± 0.3*</td>
<td>4.3 ± 0.1</td>
<td>4.5 ± 0.1</td>
<td>6.1 ± 0.3</td>
<td>3.9 ± 0.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. ΔPV, change in plasma volume; [H\(^+\)], H\(^+\) ion concentration; [Lactate], lactate concentration; [K\(^+\)], potassium ion concentration. *Significantly different from corresponding value for exercise bout 1, P < 0.05.
DISCUSSION

The results of the present study suggest that the decline in exercise performance with repeated bouts of exercise is not related to a reduction in muscle glycogen. Rather, it may be caused by reduced CP availability, increased [H\(^+\)], impairment in SR function or some other fatigue-inducing agent, although our experimental protocol could not separate their relative importance. This conclusion is based on the observation that exercise performance in bout 4 was no different from that in bout 1 (Table 1), despite lower muscle ATP and glycogen levels (Table 3). Of note, muscle [H\(^+\)] and SR Ca\(^{2+}\) uptake were similar before bouts 1 and 4, whereas muscle CP levels were actually higher before bout 4 (Table 3).

Although the importance of muscle glycogen for endurance-exercise performance is well accepted, its role in determining intermittent, high-intensity-exercise performance is less clear. 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. Recently, it has been observed that reduced dietary carbohydrate intake, and, by inference, low availability of muscle glycogen, resulted in reduced work output during the initial three 30-s bouts, but not a fourth 30-s bout of maximal cycling exercise (10). Furthermore, intense knee-extensor exercise performance during two exercise bouts separated by 1 h was maintained in a leg with elevated muscle glycogen, whereas it was reduced in the contralateral leg with reduced muscle glycogen (3).

However, lactate production and muscle glycogen utilization were not influenced by preexercise availability of muscle glycogen, and the relationship between glycogen content and exercise performance could not be resolved (3). In the present study, exercise performance in bouts 1 and 4 was similar, despite large differences in muscle CP and peak performance (5, 6). Thus, the reduced muscle CP before bout 3 in the present study was likely to have contributed to the lower peak power, and possibly to lower total work, in this bout. After 4 min of recovery from bout 2, muscle CP remained

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Table 3. Muscle metabolites, [H\(^+\)], and peak SR Ca\(^{2+}\) uptake immediately before exercise bouts 1, 3, and 4

<table>
<thead>
<tr>
<th></th>
<th>Pre-Bout 1</th>
<th>Pre-Bout 2</th>
<th>Pre-Bout 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ATP (E), mmol/kg dry weight</strong></td>
<td>25.6 ± 1.3</td>
<td>14.0 ± 1.1*</td>
<td>21.8 ± 0.8†</td>
</tr>
<tr>
<td><strong>ATP, (HPLC), mmol/kg dry weight</strong></td>
<td>26.3 ± 1.3</td>
<td>15.8 ± 1.1*</td>
<td>23.0 ± 0.8†</td>
</tr>
<tr>
<td><strong>ADP, mmol/kg dry weight</strong></td>
<td>2.87 ± 0.15</td>
<td>2.76 ± 0.09</td>
<td>2.74 ± 0.36</td>
</tr>
<tr>
<td><strong>AMP, mmol/kg dry weight</strong></td>
<td>0.10 ± 0.00</td>
<td>0.11 ± 0.03</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td><strong>TAN, mmol/kg dry weight</strong></td>
<td>29.3 ± 1.3</td>
<td>18.7 ± 1.1*</td>
<td>25.9 ± 1.03†</td>
</tr>
<tr>
<td><strong>IMP, mmol/kg dry weight</strong></td>
<td>0.14 ± 0.01</td>
<td>7.00 ± 1.17*</td>
<td>0.26 ± 0.09†</td>
</tr>
<tr>
<td><strong>Hypoxanthine, mmol/kg dry weight</strong></td>
<td>&lt;0.01</td>
<td>0.07 ± 0.02*</td>
<td>0.16 ± 0.06†</td>
</tr>
<tr>
<td><strong>Inosine, mmol/kg dry weight</strong></td>
<td>&lt;0.01</td>
<td>1.03 ± 0.02*</td>
<td>0.80 ± 0.06*</td>
</tr>
<tr>
<td><strong>Creatine phosphate, mmol/kg dry weight</strong></td>
<td>90.6 ± 3.6</td>
<td>55.6 ± 2.4*</td>
<td>100.9 ± 2.7‡</td>
</tr>
<tr>
<td><strong>Creatine, mmol/kg dry weight</strong></td>
<td>36.9 ± 2.0</td>
<td>71.9 ± 8.4*</td>
<td>26.6 ± 4.1‡</td>
</tr>
<tr>
<td><strong>Lactate, mmol/kg dry weight</strong></td>
<td>4.8 ± 0.7</td>
<td>104.7 ± 7.3*</td>
<td>6.2 ± 2.4†</td>
</tr>
<tr>
<td><strong>Glycogen, mmol/kg dry weight</strong></td>
<td>537 ± 64</td>
<td>286 ± 43*</td>
<td>350 ± 49*</td>
</tr>
<tr>
<td><strong>[H(^+)], nM</strong></td>
<td>69.4 ± 4.4</td>
<td>223 ± 18.4*</td>
<td>68.8 ± 2.3†</td>
</tr>
<tr>
<td><strong>SR Ca(^{2+}) uptake, mmol·min(^{-1})·mg wet weight(^{-1})</strong></td>
<td>23.4 ± 1.4</td>
<td>16.9 ± 1.5*</td>
<td>24.1 ± 1.5†</td>
</tr>
<tr>
<td><strong>SR Ca(^{2+}) uptake, mmol·min(^{-1})·mg protein(^{-1})</strong></td>
<td>339 ± 25</td>
<td>272 ± 18</td>
<td>374 ± 39</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. Muscle ATP was measured by using enzymatic, fluorometric (E) and HPLC methods. TAN, total adenine nucleotides; IMP, inosine 5'-monophosphate; SR, sarcoplasmic reticulum. *Significantly different from Pre-Bout 1, P < 0.05; †significantly different from Pre-Bout 3, P < 0.05.
significantly lower than resting values, an observation that has been made previously (5, 6, 26), suggesting a relatively slow rate of CP resynthesis. It is possible that the passive recovery between bouts 2 and 3 contributed to this slow rate, as has been suggested previously (26). In contrast, immediately before bout 4 (i.e., after ~90 min of recovery that included 30 min of low-intensity exercise), muscle CP was in fact higher than values at rest (Table 3). This “overshoot” above resting muscle CP levels has been observed previously in type II fibers after intense electrical stimulation (25), and we have no explanation for the phenomenon. It is possible that an increased mitochondrial activity, resulting in increased ATP resynthesis, may have resulted in enhanced production of CP via mitochondrial creatine kinase during recovery.

Increases in muscle \([\text{H}^+]\) may also contribute to impaired high-intensity exercise performance. Studies in skinned muscle fibers have demonstrated inhibition of tension development under conditions of acidosis (22), although during recovery from fatiguing isometric contractions in humans, there is recovery of force production despite the likelihood of a low pH in muscle (24). Similarly, no relationship has been observed between muscle pH and peak power restoration during recovery from intense, dynamic exercise (6). Of greater significance may be inhibition of glycolysis by acidosis, mediated via \([\text{H}^+]\) effects on phosphorylase and phosphofructokinase (26). Although it has been suggested that the negative effects of \([\text{H}^+]\) on these enzymes can be overcome by increases in AMP, IMP, and P_i, induced alkalosis is associated with increased muscle glycolysis and enhanced high-intensity-exercise performance (29).

Furthermore, the recovery of isometric endurance after a fatiguing isometric contraction more closely follows the assumed recovery of muscle pH (24). This result suggests an inhibitory effect of increased \([\text{H}^+]\) on ATP-generating processes. It is possible, therefore, that the increases and decreases in muscle \([\text{H}^+]\) that we have observed (Table 3) contributed to the impaired and restored exercise performance seen in bouts 3 and 4, respectively (Table 1). Muscle lactate was also elevated before bout 3. Recently, it has been suggested that an increase in [lactate], in the absence of acidosis, can reduce tension development in canine skeletal muscle (18). This suggests a potential role for this metabolite. In addition, although we did not measure P_i in our muscle samples, it is possible that alterations in the level of this metabolite may also have influenced exercise performance (9). Electrolyte shifts, particularly \([\text{K}^+]\), across contracting skeletal muscle have been implicated in the fatigue process. In the present study, the plasma \([\text{K}^+]\) changes reflect the alterations in work output during the four exercise bouts (Table 2); however, we are unable to assess their role in the development of fatigue.

In recent years, it has become apparent that reduced SR \(\text{Ca}^{2+}\) release and impaired excitation-contraction coupling are major causes of muscle fatigue (see Ref. 1 for review). Reduced substrate (i.e., ATP, glycogen) availability may reduce SR \(\text{Ca}^{2+}\) release (11, 23), and metabolic end products, such as \([\text{H}^+]\), lactate, and \(\text{Mg}^{2+}\), have been shown to reduce \(\text{Ca}^{2+}\) release from SR vesicles (14), although the effect of increased \([\text{H}^+]\) is not seen in skinned fiber preparations (19). Thus, the metabolic alterations resulting from the sprints may have impaired SR function and contributed to the reduced exercise performance we have observed in the present study. Indeed, SR \(\text{Ca}^{2+}\) uptake was reduced before bout 3 (Table 3), suggesting impaired SR function after intense exercise, as observed previously (15). This must represent some prolonged alteration in SR function, because the assay was conducted under optimal temperature and substrate conditions. One possibility is a temperature-induced alteration in SR \(\text{Ca}^{2+}\) uptake and \(\text{Ca}^{2+}\)-ATPase activity (7, 31). It has been shown previously that 30 min of recovery from intense exercise were not sufficient for full restoration of SR function (15). However, in the present study, 90 min of recovery resulted in a return of SR \(\text{Ca}^{2+}\) uptake to preexercise values (Table 3). In contrast, after prolonged exercise to fatigue, SR \(\text{Ca}^{2+}\) uptake remains depressed for several hours (7).

The first two bouts of exercise resulted in a substantial fall in TAN and an increase in the degradation products IMP, hypoxanthine, and inosine (Table 3). Of note, although IMP concentrations had returned to resting levels before bout 4 of exercise, the TAN content was still significantly reduced. In addition, when the sum of the degradation products (IMP, inosine, and hypoxanthine) was added to the TAN, the content was lower compared with the resting TAN (Table 3). These data indicate that acute, intense exercise results in substantial purine loss from active skeletal muscle. In addition, because the sum of the degradation products and TAN still resulted in a shortfall compared with resting muscle, these data indicate that neither purine nucleotide cycling nor purine salvage could compensate for the reduction in TAN. Rather, these data indicate that the reduction in TAN that is characteristic of this type of exercise necessitates restoration by the de novo synthesis pathway, which is a slow and energy-consuming pathway (30).

In summary, high-intensity intermittent exercise results in large decreases in muscle ATP, CP, and glycogen, with concomitant increases in \([\text{H}^+]\), lactate, and ATP degradation products. The decline in exercise performance with repeated bouts does not appear to be related to a reduction in muscle glycogen. Rather, it may be caused by reduced CP availability, increased \([\text{H}^+]\), impairment in SR function, or some other fatigue-inducing agent, but our experimental protocol could not separate their relative importance.

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This study was supported by the Australian Sports Commission. Address for correspondence: M. Hargreaves, School of Human Movement, Deakin University, Burwood 3125, Australia (E-mail: mharg@deakin.edu.au).

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REFERENCES