Glycogen availability does not affect the TCA cycle or TAN pools during prolonged, fatiguing exercise

Jacinta Baldwin,1 Rodney J. Snow,2 Martin J. Gibala,3 Andrew Garnham,2 Krista Howarth,3 and Mark A. Febbraio1,4

1Department of Physiology, University of Melbourne, Parkville 3010, Victoria; 2School of Health Sciences, Deakin University, Burwood 3125, Victoria; 3Skeletal Muscle Research Laboratory, School of Medical Sciences, Royal Melbourne Institute of Technology, Bundoora 3083, Victoria, Australia; and 4Exercise Metabolism Research Group, Department of Kinesiology, McMaster University, Hamilton, Ontario, Canada L8S4K1

Submitted 20 September 2002; accepted in final form 24 November 2002

Although it is well established that fatigue during prolonged exercise coincides with low intramuscular glycogen stores (for review, see Ref. 21), the functional significance of this association remains unclear. A number of studies (20, 22, 23, 27) have suggested that a net increase in the total concentration of tricarboxylic acid (TCA) cycle intermediates (TCAI) is necessary to establish and maintain a high rate of flux throughout the TCA cycle. “Expansion” of the TCAI pool is dependent on various anaplerotic pathways, which feed carbon into the TCA cycle and utilize pyruvate for a substrate (11). It has been proposed that, during prolonged exercise, when the content of muscle glycogen decreases, there is a gradual decline in muscle TCAI, which compromises TCA cycle flux and ATP regeneration through oxidative phosphorylation (20, 22, 23). The subsequent decrease in ATP provision from carbohydrate oxidation may lead to a transient increase in ADP content, stimulating the myokinase reaction and resulting in a decrease in the total adenine nucleotide (TAN) pool (TAN = ATP + ADP + AMP) and the formation of IMP (22, 25). However, the literature pertaining to the relation between glycogen availability, TCAI, and TAN pool size as a cause of fatigue is inconclusive. Some (1, 4, 17, 18, 20), but not all (1, 9, 16), studies have demonstrated that low intramuscular glycogen stores correlate with a reduction in TCAI, a decrease in the TAN pool, and/or an increase in IMP content at fatigue during prolonged exercise. In addition, the relation between glycogen availability, TCAI pool size, and oxidative energy provision is unclear. Studies have demonstrated that expansion of the TCAI pool at the beginning of exercise is subsequently reduced at fatigue, when low intramuscular glycogen stores, a decrease in the TAN pool, and an increase in IMP accumulation have also been observed (20, 22, 23). Although these studies support a relation between glycogen availability, TCAI pool size, and oxidative energy provision, more recent studies challenge this relation. Sahlin et al. (19) observed a similar increase in TCAI pool size in glycogen phosphorylase-deficient patients exercising at the same absolute exercise intensity as healthy control subjects. In addition, Bruce et al. (5) demonstrated that increasing the TCAI pool

Address for reprint requests and other correspondence: M. A. Febbraio, Skeletal Muscle Research Laboratory, School of Medical Sciences, RMIT University, PO Box 71, Bundoora 3083, Victoria, Australia (E-mail: markfebbraio@rmit.edu.au).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
expansion at the onset of exercise did not result in an increase in energy provision from oxidative sources, whereas Gibala et al. (11) recently observed that a lower preexercise glycogen content actually enhanced, rather than impaired, the TCAI pool expansion at the onset of exercise.

Clearly, the findings from the literature examining the relation between glycogen availability, TCAI content, IMP accumulation, and fatigue remain equivocal. Surprisingly, no previous studies have determined whether glycogen content per se affects TCAI or the TAN pool size and/or IMP accumulation during fatiguing exercise, and this was the aim of the present study. By altering preexercise glycogen content via dietary intervention, we hypothesized that glycogen availability would not affect TCAI or the TAN pool during prolonged, fatiguing exercise.

METHODS

Subjects. Seven endurance-trained men [28 ± 2 yr, 76.4 ± 4.0 kg body wt, 182 ± 3 cm, 64 ± 2 ml·kg⁻¹·min⁻¹ peak O₂ uptake (V̇O₂peak)] volunteered to participate in the study after being fully informed of the experimental procedures and giving informed consent. The Human Research Ethics Committees at the University of Melbourne and Deakin University approved the experiment.

Preexperimental protocol. On the basis of our previous study (2), we chose to determine a workload relative to the onset of blood lactate accumulation [i.e., lactate threshold (LT)], rather than V̇O₂peak. Hence, V̇O₂peak and LT were initially determined as previously described (2), and a work rate corresponding to 95% LT (equivalent to ~70% V̇O₂peak) was calculated from this test. For manipulation of preexercise glycogen content in the vastus lateralis, subjects were provided a low-carbohydrate (9,500 kJ, 6.5% carbohydrate; LG) diet, which they consumed for the remainder of the day. On the second occasion, subjects consumed a high-carbohydrate (9,500 kJ, 84% carbohydrate; HG) diet after the pretrial exercise protocol. The trials were ordered such that all subjects performed the LG trial first. This was necessary so that a muscle sample could be obtained during the HG trial at the same time the fatigue sample was obtained in the LG trial. Subjects consumed 250 ml of distilled water every 20 min throughout exercise. The laboratory was maintained at 20–22°C, and an electric fan was used to limit exercise-induced thermal stress. Before the trials, the subjects arrived at the laboratory after an overnight fast, having refrained from exercise, alcohol, tobacco, and caffeine for 24 h. Heart rate, rating of perceived exertion, and expired pulmonary gases were recorded or collected at rest, 5 min, and 20 min and at 20-min intervals thereafter until fatigue, as previously described (1, 8).

Tissue sampling and analysis. Muscle samples were obtained as previously described (1). In LG and HG, samples were collected at rest, at 15 min of exercise, and at the time of fatigue in HG (F1). An additional sample was collected at fatigue in HG (F2). Subjects ceased cycling for ~40 s while the muscle biopsies were taken. The samples were frozen in liquid nitrogen 26 ± 3 s after the cessation of exercise, and the time taken to freeze the samples was not different (P > 0.50) between sampling points. Muscle samples were freeze-dried, dissected free of any blood and connective tissue, powdered, and analyzed for glycogen, creatine phosphate (CP), creatine (Cr), lactate, ATP, ADP, AMP, IMP, and hypoxanthine, as outlined by Baldwin et al. (1). A further portion of freeze-dried muscle (~6–12 mg) was extracted and analyzed for citrate, isocitrate, fumarate, malate, alanine, glutamate, and pyruvate using methods that we previously described (11). Because citrate, isocitrate, fumarate, malate [sum of 4 TCAI (Σ4 TCAI)] account for >70% of the TCAI pool size in humans (10, 11, 20), the majority of the TCAI pool has been accounted for in this study. To correct for differences in blood and/or connective tissue between samples, muscle metabolites other than lactate, pyruvate, and hypoxanthine (because of their extracellular appearance) were adjusted to the peak total creatine (Cr + CP) content for each subject. Blood samples were collected at rest, 5 min, and 20 min and every 20 min until fatigue and analyzed for plasma hypoxanthine, glucose, and lactate, as previously described (1).

Statistics. Student’s paired t-test was used to compare LG and HG time to exhaustion and all the fatigue data points: F1 (LG) vs. F2 (HG). A one-way analysis of variance [ANOVA; Biomedical Data Processing (BMDP) statistical software] was used to compare the time between cessation of exercise and freezing of muscle samples at each sampling time. A one-way ANOVA was also used to compare a particular metabolite at all the time points within a trial. Muscle and plasma metabolite concentrations and cardiorespiratory data were compared using a two-way ANOVA for repeated measures (BMDP). Simple main effects analyses and Newman-Keuls post hoc tests were used to locate specific differences. Significance was accepted at P ≤ 0.05. Values are means ± SE. To maintain a sample size of seven, appropriate analyses include data points from the start of exercise until the last common time point for all subjects and then at fatigue.

RESULTS

Cardiorespiratory and performance data. Time to exhaustion was 34% longer (P < 0.001) in HG than in LG. Heart rate was not different between the trials; respiratory exchange ratio was higher (P < 0.05) throughout exercise in HG than in LG, and rating of perceived exertion was lower (P < 0.05) at 40, 60, and 80 min in HG than in LG (Table 1). O₂ uptake averaged 44.3 ± 1.8 ml·kg⁻¹·min⁻¹, which was equivalent to 70% of maximum O₂ uptake. No differences were observed when trials were compared (Table 1).

Muscle glycogen. Resting muscle glycogen content was nearly twofold greater (P < 0.005) in HG than in LG. Glycogen content decreased (P < 0.05) throughout exercise in both trials, and it was lower (P < 0.05) at fatigue than at rest in HG and LG. Furthermore, glycogen content was higher (P < 0.01) at F1 and F2 in
Table 1. RPE, RER, heart rate, and \( \dot{V}O_2 \) during exercise in endurance-trained men

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>5 min</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
<th>80 min</th>
<th>Fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \dot{V}O_2, \text{ml-kg}^{-1}\text{-min}^{-1} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HG</td>
<td>43.6±2.3</td>
<td>46.1±1.6</td>
<td>43.3±0.4</td>
<td>42.6±2.7</td>
<td>42.6±2.5</td>
<td>43.9±1.3</td>
<td></td>
</tr>
<tr>
<td>LG</td>
<td>44.6±2.1</td>
<td>46.3±2.0</td>
<td>46.4±1.4</td>
<td>43.4±1.6</td>
<td>43.5±1.8</td>
<td>44.9±1.8</td>
<td></td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HG</td>
<td>0.85±0.02</td>
<td>0.82±0.01</td>
<td>0.80±0.01</td>
<td>0.82±0.02</td>
<td>0.80±0.02</td>
<td>0.80±0.01</td>
<td></td>
</tr>
<tr>
<td>LG</td>
<td>0.80±0.02*</td>
<td>0.79±0.02*</td>
<td>0.76±0.02*</td>
<td>0.75±0.01*</td>
<td>0.75±0.01*</td>
<td>0.76±0.02*</td>
<td></td>
</tr>
<tr>
<td>RPE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HG</td>
<td>10.9±0.7</td>
<td>11.6±0.6</td>
<td>12.4±0.6</td>
<td>13.7±0.6</td>
<td>14.9±0.6</td>
<td>18.1±0.5</td>
<td></td>
</tr>
<tr>
<td>LG</td>
<td>11.3±1.3</td>
<td>12.0±0.9</td>
<td>14.1±0.5*</td>
<td>14.8±0.3*</td>
<td>16.6±0.7*</td>
<td>18.1±0.4</td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HG</td>
<td>57±2</td>
<td>137±5</td>
<td>143±6</td>
<td>146±6</td>
<td>146±5</td>
<td>145±5</td>
<td>155±6</td>
</tr>
<tr>
<td>LG</td>
<td>56±3</td>
<td>142±5</td>
<td>148±5</td>
<td>148±5</td>
<td>149±4</td>
<td>142±3</td>
<td>148±5</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; \( n = 7 \). \( \dot{V}O_2 \), \( O_2 \) uptake; HR, heart rate; HG, glycogen-loaded; LG, glycogen-depleted; RPE, rating of perceived exertion; RER, respiratory exchange ratio. *Different (\( P < 0.05 \)) from LG.

HG that at F1 in LG. Although there was a tendency for glycogen to decline when F1 was compared with F2 in HG, these results were not statistically significant (Fig. 1A).

Muscle adenine nucleotides, IMP, and hypoxanthine. Although IMP tended to increase from rest to 15 min in LG, these results were not statistically significant. Moreover, IMP was not elevated in either trial at fatigue or at any time during exercise (Fig. 1B), nor were there differences in IMP content at F1 in HG compared with LG. IMP accumulation was not greater at any time in LG. Although there were no differences in the TAN pool between trials, muscle ADP was higher (\( P < 0.01 \)) in LG at fatigue (Table 2). Although the ATP content was lower (\( P < 0.05 \)) than at rest in F1 in HG, it returned to resting levels at F2 (Table 2). There were no differences in muscle hypoxanthine, a degradation product of IMP, at any time between HG and LG, and there was no change in hypoxanthine content as a result of exercise (Table 2). To examine the relation between IMP and glycogen content, a correlation analysis was performed on the corresponding values for IMP and glycogen in HG and LG. No relation was observed for HG (\( r = 0.10, P > 0.05 \)) or LG (\( r = 0.26, P > 0.05 \); Fig. 2).

TCAI, pyruvate, and muscle amino acids. \( \Sigma \text{TCAI} \) (>70% of the total TCAI pool) was elevated (\( P < 0.05 \)) at 15 min in both trials and at fatigue in HG (Fig. 1C). There were no differences in \( \Sigma \text{TCAI} \) between LG and HG at any time. The TCAI pool size was very similar at F1 and F2 in both trials. There was a time (\( P < 0.05 \)) and treatment (\( P < 0.05 \)) effect for pyruvate, but there was no interaction between these factors (Table 2). Pyruvate was greater (\( P < 0.05 \)) in HG than in LG (Table 2). Pyruvate was higher (\( P < 0.05 \)) than at rest and fatigue (F2) at 15 min in HG (Table 2). No differences in muscle pyruvate content were observed during the LG trial. Alanine increased (\( P < 0.05 \)) with exercise in LG (Table 2), but there were no differences between trials. Glutamate was higher (\( P < 0.05 \)) at rest in LG than in HG, and it decreased (\( P < 0.05 \)) with exercise in both trials (Table 2).

Lactate, CP, and Cr. Although there was a small exercise-induced increase (\( P < 0.05 \)) in muscle lactate content in LG, there were no differences between trials. Similarly, there was an exercise-induced increase in Cr (\( P < 0.05 \)) and a mirror-image decrease (\( P < 0.05 \)) in CP in LG. Furthermore, Cr was lower (\( P < 0.05 \)) and CP content was higher (\( P < 0.05 \)) in HG than in LG at fatigue (Table 2).

Plasma metabolites. The resting concentrations of all the measured plasma metabolites were not different between HG and LG. There was a small exercise-induced increase (\( P < 0.05 \)) in plasma hypoxanthine (Fig. 3A) and lactate (Fig. 3B) concentrations in both trials. Although plasma hypoxanthine was greater (\( P < 0.05 \)) in LG at 20 and 60 min, it was not different at fatigue compared with HG (Fig. 3A). Plasma lactate concentration was higher (\( P \leq 0.05 \)) in HG at 20 min and fatigue (Fig. 3B). There was a significant exercise-induced reduction (\( P < 0.05 \)) in plasma glucose concentration for both trials, and the glucose concentration at fatigue was lower (\( P < 0.05 \)) than at rest in HG and LG (Fig. 3C). Although there was a steady decline from 20 min in plasma glucose in the LG trial (\( P < 0.05 \)), glucose concentration did not fall in the HG trial until after 60 min of exercise (Fig. 3C). Additionally, plasma glucose was greater (\( P \leq 0.05 \)) in HG at 20, 40, and 60 min and at fatigue (Fig. 3C).

DISCUSSION
This is the first study to examine the effect of reduced muscle glycogen content on TCAI pool size and muscle energy metabolism in humans during prolonged exercise to fatigue. The main findings of this study were that the magnitude of TCAI expansion was the same in LG and HG and that IMP did not significantly accumulate in either trial: <0.15 mmol/kg dry wt at fatigue in both trials. Furthermore, the TCAI pool size was very similar at fatigue in the LG trial, as well as at F1 and F2 in the HG trial, yet subjects cycled for much longer in the latter trial. These findings demonstrate that altered glycogen availability does not
impair the net increase in TCAI at the start of exercise, nor does it lead to a decrease in the TCAI or TAN pool or an increase in IMP during prolonged exercise to fatigue. In addition, the results demonstrate dissociation between the contents of TCAI and IMP and the point of volitional fatigue. Therefore, our results do not support the hypothesis that links glycogen availability with fatigue via impaired aerobic energy provision (20, 22, 23, 27).

Gibala et al. (11) recently observed a larger increase in TCAI content but a lower pyruvate dehydrogenase activity after 10 min of exercise when comparing a glycogen-depleted with a control trial. These previous data demonstrate an uncoupling between glycogen content, aerobic energy contribution, and net increase in TCAI. Although in the present study we did not observe a greater expansion of TCAI in LG, there was no difference in TCAI content between LG and HG. Hence, our data also refute the hypothesis that reducing the supply of substrate prevents a net increase in TCAI at the onset of exercise (21). The initial rapid expansion of the TCAI pool is generally attributed to an increased flux through the alanine aminotransferase (AAT) reaction (10, 20). Because the muscle glutamate content decreased and alanine increased at the beginning of exercise (Table 2), the AAT reaction was probably active at the start of exercise in the present study. This finding is consistent with the findings of others (10, 11, 20) who concluded that resting muscle glutamate (provided flux of pyruvate is sufficient) might determine the net increase in TCAI. Although resting muscle glutamate was higher in LG than in HG in the present study, there was no difference in TCAI content between trials. This contrasts with recent findings (11) that the increased resting glutamate in LG acted via the AAT reaction to increase TCAI more in LG. This discrepancy might be explained in a number of ways: 1) the initial muscle sample in the present study was obtained at 15 min, compared with 10 min in the previous study, and it is, therefore, possible that a small amount of TCAI “drainage” confounded the comparison; 2) in the previous study, the pretrial exercise and dietary regimen was 48 h, rather than 24 h, before the experiment; and 3) the results could be due to differences in subject training status.

One previous study attempted to test the hypothesis that glycogen availability causes fatigue during prolonged exercise by decreasing the initial TCAI pool expansion, thereby inhibiting aerobic energy provision and resulting in a decrease in the TAN pool and an increase in IMP. Spencer et al. (23) observed an increase in IMP at fatigue after exercise in a glycogen-depleted state and a greater increase in TCAI during exercise in a glycogen-loaded state. This study provided credibility to and wide acceptance for the theory that fatigue is generally considered to arise from insufficient glycogen mediated through a limiting supply of substrate to the TCA cycle or limitations in TCA cycle activity due to reduced TCAI. However, it is difficult to interpret these previous results because of several methodological concerns. In this previous study, mus-

**Fig. 1.** Muscle glycogen (A), IMP (B), and sum of 4 tricarboxylic acid (TCA) cycle intermediates (ΣTCAI, >70% of total TCAI pool; C) at rest (0) and during a cycling bout to fatigue in endurance-trained glycogen-loaded (HG) or glycogen-depleted (LG) men (n = 7). Values are means ± SE in mmol/kg dry wt. *Different (P < 0.05) from LG; †different (P < 0.05) from rest.
F2, HG fatigue (155/H11006) Fig. 2. IMP vs. glycogen content (in glycosyl units/kg dry wt).

Table 2. Muscle metabolite contents at rest and during exercise in HG or LG

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Rest</th>
<th>15 min F1</th>
<th>24.8 ± 1.6</th>
<th>24.1 ± 1.3</th>
<th>25.4 ± 0.1</th>
<th>26.0 ± 0.2</th>
<th>25.2 ± 1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>26.6 ± 2</td>
<td>24.3 ± 1.5</td>
<td>23.1 ± 1.6a</td>
<td>24.1 ± 1.3</td>
<td>25.4 ± 0.1</td>
<td>26.0 ± 0.2</td>
<td>25.2 ± 1.6</td>
</tr>
<tr>
<td>ADP</td>
<td>2.5 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>2.2 ± 0.2a</td>
<td>2.4 ± 0.2a</td>
<td>2.4 ± 0.1</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>AMP</td>
<td>0.09 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>0.014 ± 0.005</td>
<td>0.019 ± 0.01</td>
<td>0.01 ± 0.004</td>
<td>0.011 ± 0.005</td>
<td>0.004 ± 0.003</td>
<td>0.061 ± 0.041</td>
<td>0.01 ± 0.006</td>
</tr>
<tr>
<td>Lactatea</td>
<td>4.2 ± 0.3</td>
<td>6.9 ± 1.5</td>
<td>5 ± 0.7</td>
<td>5.5 ± 1</td>
<td>3 ± 0.3</td>
<td>9.9 ± 2.9a</td>
<td>5.1 ± 0.7</td>
</tr>
<tr>
<td>CPT</td>
<td>85.7 ± 5.5</td>
<td>74.2 ± 8.4</td>
<td>74.9 ± 7.6</td>
<td>77.1 ± 7.3a</td>
<td>86 ± 5.5</td>
<td>70.5 ± 8.8*</td>
<td>68.3 ± 6.9*</td>
</tr>
<tr>
<td>Creatinea</td>
<td>46.5 ± 1.4</td>
<td>58.8 ± 5.3</td>
<td>57.3 ± 6.1</td>
<td>55.1 ± 6.7a</td>
<td>46.2 ± 4.4</td>
<td>61.7 ± 5.1*</td>
<td>63.9 ± 4.9*</td>
</tr>
<tr>
<td>Pyruvatea,b</td>
<td>0.12 ± 0.02</td>
<td>0.24 ± 0.04a</td>
<td>0.16 ± 0.02</td>
<td>0.12 ± 0.02f</td>
<td>0.10 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>Glutamata</td>
<td>14.4 ± 0.6a</td>
<td>7.4 ± 0.3a</td>
<td>8.2 ± 0.7a</td>
<td>8.7 ± 0.9a</td>
<td>17.8 ± 0.8</td>
<td>8.1 ± 0.8</td>
<td>6.7 ± 0.5a</td>
</tr>
<tr>
<td>Alaninea</td>
<td>3.5 ± 0.7</td>
<td>6.4 ± 1.1</td>
<td>6.4 ± 0.3</td>
<td>6.4 ± 0.7</td>
<td>2 ± 0.5</td>
<td>5.5 ± 2.4a</td>
<td>5.7 ± 0.9a</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry wt; n = 7, except for pyruvate, glutamate, and alanine, where n = 5. F1, LG fatigue (103 ± 15 min); F2, HG fatigue (155 ± 16 min); CP, creatine phosphate. aTime effect (P < 0.05); b treatment effect (P < 0.05); c different (P < 0.05) from LG; d different (P < 0.05) from F1 (LG); e different (P < 0.05) from rest; f different (P < 0.05) from 15 min.

Muscle was sampled in the glycogen-loaded trial only at the time corresponding to fatigue in the glycogen-depleted trial; therefore, fatigue was reached in only one trial. In addition, in this trial, muscle samples were only obtained before and at the cessation of exercise. Therefore, it could not be determined whether the differences in TCAI pool size or IMP accumulation were related to compromised glycogen stores and/or fatigue. Indeed, the differences between trials may have persisted if muscle was sampled at fatigue in the high-glycogen trial. Likewise, it is also possible that the differences in TCAI pool size and IMP accumulation occurred earlier during exercise, because the largest expansion in TCAI pool size occurs at the onset of exercise (10–12, 20), whereas IMP can significantly accumulate after 30 min in skeletal muscle during cycling exercise (13, 16). Indeed, in this previous study (23), the increase in IMP was likely to have been affected by a glycogen-independent component, because at the termination of exercise in the high-glycogen trial the IMP content of the skeletal muscle increased almost ninefold compared with rest in the presence of an intramuscular glycogen content of >350 mmol/kg dry wt. The present study is, therefore, the first to adequately test the hypothesis that glycogen availability is associated with fatigue during prolonged exercise by decreasing the initial TCAI pool expansion, thereby inhibiting aerobic energy provision and resulting in a decrease in the TAN pool and an increase in IMP. Our results refute this hypothesis. Even when preexercise carbohydrate availability was reduced and the muscle glycogen content was attenuated during exercise, neither the TAN pool nor the IMP content was significantly affected at any time. Even though there was a small increase in plasma hypoxanthine in both trials, accumulation of this IMP degradation product was minor (<2 µM) and occurred, in essence, at the onset of exercise, demonstrating that this accumulation was not related to glycogen availability or fatigue. It is plausible to suggest that we saw no increase in IMP because glycogen content at fatigue was not <100 mmol/kg dry wt, and IMP does not appear to be increased until glycogen is lowered to these values (21, 22). However, if this were the case, we should have observed at least a tendency for IMP to accumulate at fatigue in LG when glycogen was lowered to ~150 mmol/kg. However, no such tendency was observed (Fig. 1). In addition, we observed no correlation between glycogen content and IMP accumulation, and IMP did not accumulate, even in subjects whose glycogen content at fatigue was <100 mmol/kg dry wt (Fig. 2). Finally, in our previous study (9), where subjects exercised at a workload similar to that employed in the present study, IMP levels at fatigue were not significantly elevated compared with rest in the presence of glycogen levels of ~30 mmol/kg dry wt. Taken together, these observations make the possibility that we saw no increase in IMP because the glycogen content at fatigue was not depleted most unlikely.

The glycogen content at fatigue was consistent with some (6, 14, 15) but in contrast with other (1, 9, 18, 22) previous studies. In the previous studies in which glycogen content at fatigue was comparable to that in the present study, altering carbohydrate availability by dietary interventions also altered exercise duration. Nonetheless, these discrepancies raise the following question: What absolute value constitutes “glycogen depletion” at fatigue? It is likely that factors such as the fiber type phenotype, exercise intensity, and fiber type recruitment play a role in determining the mixed muscle glycogen content at fatigue. In the present study, we were careful to choose a workload that involved a large portion of the energy being derived from

![Fig. 2. IMP vs. glycogen content (in glycosyl units/kg dry wt).](http://jap.physiology.org/Downloaded from 10.1152/jappl.00218.2003 by 122.205.35.0 on July 10, 2017)
oxidation of carbohydrate, as evidenced by the respiratory exchange ratio values (Table 1), without increasing metabolic factors such as cellular acidosis or ADP accumulation, which may have impaired excitation-contraction coupling independent of glycogen (Table 2). Because the subjects cycled for an additional 52 min after manipulation of the preexercise glycogen content, we are confident that fatigue was related, at least in part, to carbohydrate availability. It is possible, however, that fatigue was associated with hypoglycemia and not intramuscular glycogen content, because glucose values declined to low levels in both trials (Fig. 3), values consistent with previous observations (6). We were unable to determine the precise cause(s) of fatigue during prolonged exercise, but this was not the aim of the study.

In the present study, we did not measure skeletal muscle aerobic energy provision. However, given that CP did not decline when content at 15 min was compared with that at fatigue and that neither intramuscular lactate nor the TAN pool was different from rest at the point of fatigue in either trial, we have no evidence to suggest that aerobic energy provision was compromised in the presence of reduced glycogen availability at the point of fatigue in either trial. The moderate fall in CP and lack of increase in lactate are in contrast with some (21, 22), but not all (1, 9), previous studies. It was no surprise that lactate did not significantly accumulate, given the relative workload and the training status of the subjects. We expected the CP values to decline to a greater magnitude, and the fact that they remained relatively high may be a function of the delay between the cessation of exercise and the freezing of the muscle samples. Nonetheless, there were no differences in biopsy freeze time when any sampling points were compared. Therefore, although the absolute CP values may be higher than at the time at which exercise ceased at each point, we are confident that CP does not decline at the moment of fatigue, an observation consistent with studies that have compared CP values at fatigue with those obtained throughout exercise (9, 16).

In the present study, although there was a tendency for attenuation of TCAI pool expansion after prolonged exercise in both trials, the reduction was small and insignificant (Fig. 1C). These data are in contrast to previous studies, in which a small reduction in TCAI pool size was observed at fatigue (12, 20). Irrespective of whether the pool size is slightly attenuated at fatigue, the important finding from this study was that TCAI pool size was identical between HG and LG at F1 in the presence of a difference in glycogen of \(200 \text{ mmol/kg dry wt}\) when both trials were compared (Fig. 1). Moreover, in HG, subjects were able to continue to exercise for an additional 52 min without a further change in TCAI pool size, but with a trend toward a further decline in glycogen content. Hence, our data demonstrate dissociation between glycogen content and TCAI pool size.

The lack of 1:1 stoichiometry between the fall in ATP and the increase in IMP was notable because ATP was significantly lower at F1 in HG (Table 2) in the presence of an unaltered IMP content at this time (Fig. 1). Although it is generally accepted that the relation between the decrease in ATP and the increase in IMP

---

Fig. 3. Plasma hypoxanthine (A), lactate (B), and glucose (C) concentrations at rest (0) and during a cycling bout to fatigue in endurance-trained glycogen-loaded (HG) or glycogen-depleted (LG) men (n = 7). Values are means ± SE. a Different \((P < 0.05)\) from LG; b different \((P < 0.05)\) from rest; * different \((P < 0.05)\) from all other points within treatment; † different \((P < 0.05)\) from preceding points within treatment.

---
is stoichiometric (25), we (7) and others (3, 24) previously observed that ATP degradation exceeded IMP formation. The reason for this relatively consistent observation is unclear, but the existence of an acid-insoluble purine derivative that is an oligomer of ATP and phosphoglycerate has been suggested (26). Because acid extraction is used for the adenine nucleotides, this oligomer may have been left in the acid-insoluble portion. This scenario is, however, speculative, because the presence of this oligomer in human skeletal muscle has never been experimentally confirmed.

In summary, our findings demonstrate that altering glycogen availability before the onset of exercise does not impair a net increase in TCAI (TCAI pool expansion), even at fatigue, or lead to a decrease in the TAN pool or an increase in IMP during prolonged exercise to fatigue, despite the fact that such a manipulation has a major effect on exercise duration. In addition, the results demonstrate dissociation between the contents of TCAI and IMP and the point of volitional fatigue. Therefore, although glycogen availability influences exercise duration, our results refute the hypothesis that links attenuated glycogen availability with fatigue via impaired aerobic energy provision, impaired adenine nucleotide metabolism, or TCAI pool size.

We thank the subjects for their extraordinary efforts in participating in this study.

REFERENCES