Adaptations in skeletal muscle exercise metabolism to a sustained session of heavy intermittent exercise

H. GREEN, R. TUPLING, B. ROY, D. O'TOOLE, M. BURNETT, AND S. GRANT
Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Green, H., R. Tupling, B. Roy, D. O'Toole, M. Burnett, and S. Grant. Adaptations in skeletal muscle exercise metabolism to a sustained session of heavy intermittent exercise. Am. J. Physiol. Endocrinol. Metab. 278:E118–E126, 2000.—The purpose of this study was to investigate the hypothesis that a single, extended session of heavy exercise would be effective in inducing adaptations in energy metabolism during exercise in the absence of increases in oxidative potential. Ten healthy males [maximal aerobic power (VO₂peak) = 43.4 ± 2.2 (SE) ml·kg⁻¹·min⁻¹] participated in a 16-h training session involving cycling for 6 min each hour at ~90% of maximal oxygen consumption. Measurements of metabolic changes were made on tissue extracted from the vastus lateralis during a two-stage standardized submaximal cycle protocol before (Pre) and 36–48 h after (Post) the training session. At Pre, creatine phosphate (PCr) declined (P < 0.05) by 32% from 0 to 3 min and then remained stable until 20 min of exercise at 60% VO₂peak before declining (P < 0.05) by a further 35% during 20 min of exercise at 75% VO₂peak. Muscle lactate (mmol/kg dry wt) progressively increased (P < 0.05) from 4.59 ± 0.64 at 0 min to 17.8 ± 2.7 and 30.9 ± 5.3 at 3 and 40 min, respectively, whereas muscle glycogen (mmol glucosyl units/kg dry wt) declined (P < 0.05) from a rest value of 336 ± 24 to 276 ± 31 and 178 ± 36 at similar time points. During exercise after the training session, PCr and glycogen were not as depressed (P < 0.05), and increases in muscle lactate were blunted (P < 0.05). All of these changes occurred in the absence of increases in oxidative potential as measured by the maximal activities of citrate synthase and malate dehydrogenase. These findings are consistent with other studies, namely, that muscle metabolic adaptations to regular exercise are an early adaptive event that occurs before increases in oxidative potential.

oxidative potential; enzymes; metabolites; metabolic control

IT IS BECOMING INCREASINGLY APPARENT that a variety of adaptations both in the skeletal muscle cell and in the vascular system occur soon after the onset of regular, contractile activity. In the muscle cell, as an example, mitochondrial oxidative potential is rapidly upregulated, resulting in increases in the capacity for both oxidative phosphorylation and β-oxidation (6, 42). These adaptations are also accompanied by an increased expression of proteins involved in glucose transport and disposal within the cell, namely hexokinase (35, 44), the enzyme involved in glucose phosphorylation, and GLUT-4, the glucose transporter protein (32, 34, 35). Rapid upregulation is also observed in other proteins such as the sarcoplasmic Na⁺-K⁺-ATPase, the cation pump which is involved in Na⁺ and K⁺ transport (15, 22), and in the monocarboxylate transporter 1 (MCT1), which appears to function as a lactate transporter (3). One of the most conspicuous vascular adaptations is an increase in muscle capillarization, which also may occur within days after the onset of training (41).

Not unexpectedly, profound alterations also occur in muscle energy metabolism during moderate exercise early in training. These adaptations include a pronounced reduction in the rate of glycogen depletion and a lower lactate accumulation, two events which appear at least partly dependent on reductions in glycolysis and glycolysis (5, 6, 14, 34) and, in the case of lactate, an increase in removal from the muscle (30).

The purpose of this study was to investigate the effects of a single session of intermittent exercise on the metabolic adaptations that occur in the working muscle. We have hypothesized that muscle metabolic adaptations may be elicited with a single training session and occur independently of increases in oxidative potential. Unlike previous work, in which we have employed a single, sustained session of low-intensity exercise as the stimulus, in this study we have employed relatively brief periods of heavy exercise. Our rationale for employing this protocol was that the adaptations appear to depend on the exercise intensity and the cellular distur-

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
balances that occur in energy metabolism (45). We have used an extended protocol of intermittent exercise because of previous work (12, 25) that suggested time-dependent changes in metabolism and substrate utilization consistent with what would be expected after regular training.

METHODS

Subjects. Ten healthy, male volunteers, who were active but not exercising on a regular basis (i.e., less than once per week), were recruited for the study. The physical characteristics of the subjects included age, 21 ± 0.5 (SE) yr; height, 179 ± 1.9 cm; and weight, 78.6 ± 3.0 kg. Maximal aerobic power (VO2peak), as determined during a progressive cycle test to fatigue, was 3.36 ± 0.14 l/min and 43.4 ± 2.2 ml·kg−1·min−1. As required, the study was approved by the Office of Human Research and Animal Care, and all volunteers were made fully aware of all procedures before written consent was obtained.

Experimental design. The experimental design was similar to that employed in our short-term training studies (13) with the exception of the training stimulus. Briefly, each subject reported to the laboratory on five occasions, beginning −2 wk before the training session. On the first visit, VO2peak was measured. On the second visit, the subjects cycled for a brief period at the individual workloads that were to be used during a standardized cycling protocol, administered before and after the training session. During the third and fifth visits, the subjects performed the standardized tests. The standardized test was used to evaluate the effect of the single training stimulus. On the fourth visit, the training session was performed. The standardized tests were performed at least 24 h before and 36–48 h after the training session.

The standardized tests consisted of cycling for 20 min at each of two work intensities, namely, 60 ± 0.9 and 75 ± 1.0% of pretraining VO2peak. The same absolute workload was used on both occasions. Before each exercise test, the vastus lateralis of each subject was prepared for needle biopsy sampling (2) by four incisions, two on each leg, made after local anesthesia. In all, eight different sites were selected for biopsies. These incisions were used to extract tissues immediately before the exercise, after the subject had been sitting quietly on the cycle for ~15 min, and at 3, 20, and 40 min of exercise. Two biopsies were performed at each site. Before the start of exercise and immediately after the end of exercise, a tissue sample was rapidly extracted and immediately plunged into liquid N2. This sample was later analyzed for high-energy phosphates, glycogen, and selected metabolites. A second biopsy, extracted from the same site, was used for analyses of muscle enzyme activities. This sample was quickly extracted from the biopsy needle and frozen in liquid N2. This sample was later analyzed for high-energy phosphates, glycogen, and selected metabolites. A second biopsy, extracted from the same site, was used for analyses of muscle enzyme activities. This sample was quickly extracted from the biopsy needle and frozen in liquid N2. On average, exercise was not interrupted for any longer than 30 s when samples were obtained. Muscle samples were stored at −80°C until analyses.

Before and during the exercise, respiratory gas collection was performed according to previous published methods (26) over 4- to 5-min segments beginning at 15 and 35 min of exercise. These measurements were used for determinations of VO2, VCO2, and ventilation (Ve). Heart rates were also recorded during the gas collection periods by standard electrocardiographic techniques. For all tests, an electronically braked cycle ergometer (Quinton 870), calibrated before each test, was used.

The controlled exercise test was performed at approximately the same time of day for each subject and 3–4 h after the ingestion of a liquid supplement consisting of one can of Ensure (1.045 kJ, 14.8% protein, 3.15% fat, and 53.7% carbohydrates; Ross Laboratories, Montréal, PQ, Canada). All subjects were requested to refrain from any other supplement, including coffee, before testing. Testing was conducted at a controlled room temperature (24°C) and at a relative humidity of between 50 and 60%.

The training session consisted of 6 min of cycling performed once per hour for 16 h at ~90% maximal oxygen consumption (VO2max). We have published previously on this protocol (25). All subjects reported to the laboratory at ~7 AM for preliminary preparation. At selected repetitions, determinations of gas exchange kinetics were performed, including respiratory and cardiovascular measurements (to be published elsewhere). Before reporting to the laboratory, each subject was instructed to consume only a light snack consisting of juices. During the first 8 h of the training session, no supplements, with the exception of water, were permitted. After 8 h, the subjects were also allowed to consume Poweraid (Coca Cola) and selected fruits (oranges, bananas). These were permitted ad libitum. During the interval between exercise sessions, the subjects remained in the laboratory area, preoccupied with reading, watching television, or sleeping. The exercise sessions were performed under the same environmental conditions as the controlled exercise tests. All participants were requested to maintain their normal diet over the course of the experimental period.

Analytical techniques. Muscle metabolites including glycogen, a range of glycolytic intermediates, ATP, PCR, Pi, and creatine (Cr) were analyzed fluorometrically after extraction from freeze-dried tissue according to procedures previously published (19, 20). The adenine nucleotides (ATP, ADP, AMP) and IMP concentrations were determined on the same homogenate by use of ion-pair reversed-phase HPLC procedures described by Ingebritsen et al. (27) as modified by our group (19). We have also calculated the free concentrations of ADP (ADPf) and AMP (AMF) on the basis of the near-equilibrium constants that have been published for creatine kinase (Kdss = 1.66 × 109 M) and adenylate kinase (Kdss = 1.05 M) (10). The pH and H+ concentrations were estimated from the concentrations of muscle pyruvate (PYR) and lactate (LAC) according to the regression equation established by Sahlin et al. (37) for dynamic work. The concentration of free Mg2+ was assumed to be 1.0 mM (10). It should be emphasized that the calculation of ADPf and AMPf depends on several assumptions (36), which, along with additional details of the calculation, have been provided in an earlier publication (14).

The average of the total creatine concentration (TCr) for each individual was used to correct the raw values. This procedure allows the contaminating effect of blood and connective tissue to be minimized (14). Three of the metabolites, glucose, pyruvate, and lactate, also exist in the extracellular space. However, they were not corrected for extracellular concentrations because of the uncertainty of the concentrations of these metabolites in this space. It should be emphasized that correction of TCr is based on the stability of TCr before and after the training session. Before the training session, TCr was 106.7 ± 0.31 (SE), and after training, TCr was 107.2 ± 0.65. The difference in TCr was not significant (P > 0.05).

The maximal activities of a number of enzymes that were representative of the major metabolic pathways and segments were also measured. The enzymes selected were used to represent glycolysis (phosphofructokinase, PFK), glucose phosphorylation (hexokinase, HEX), oxidative phosphorylation (citrate synthase, CS; malate dehydrogenase, MDH), and β-oxidation (3-hydroxy-CoA dehydrogenase, 3-HAD). Enzyme activities were performed from muscles hand homogenized
(0–4°C) in a phosphate buffer (pH 7.4) containing 5 mM β-mercaptoethanol, 0.5 mM EDTA, and 0.2% BSA. Homogenates were diluted in 20 mM imidazole buffer with 0.2% BSA. Enzyme measurements were performed at 24–25°C according to the procedures of Henriksson et al. (21). With the exception of PFK, which was performed in fresh homogenates, all other enzyme measurements were assayed from frozen homogenates. Protein was determined by use of the Lowry technique as modified by Schacterle and Pollock (38).

On a given analytical day, all samples for a given subject, either specific metabolites or enzymes, were assayed together. For both metabolites and enzymes, samples were analyzed in duplicate.

Statistical procedures. The data were analyzed using two-way ANOVA for repeated measures, with the training session and exercise as the independent variables. Where significant differences were found, Newman-Kuels techniques were employed to determine which means were different. Significance was set at the 0.05 level.

RESULTS

Respiratory gas exchange. As expected, progressive increases in V\textsubscript{O}2 were observed in response to the two intensities of exercise that were employed during the non-steady-state (Fig. 1). The training session depressed the increase that occurred at this time point.

Table 1. Respiratory gas exchange during exercise before (Pre) and after (Post) a single training session

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.44 ± 0.04</td>
<td>1.99 ± 0.09</td>
</tr>
<tr>
<td>20</td>
<td>1.96 ± 0.12</td>
<td>1.96 ± 0.12</td>
</tr>
<tr>
<td>40</td>
<td>2.46 ± 0.13</td>
<td>2.46 ± 0.13</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10. VO\textsubscript{2}, oxygen consumption; V\textsubscript{CO}2, carbon dioxide production; VE, ventilation; HR, heart rate; RER, respiratory exchange ratio. Main effects of exercise were observed for all variables (P < 0.05). For VO\textsubscript{2}, V\textsubscript{CO}2, VE and HR, 0 < 20 < 40 min; for RER, 0 < 20 = 40 min.

Table 2. Effects of exercise and a single training session on adenine nucleotides

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>3</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>2.72 ± 0.91</td>
<td>2.28 ± 0.98</td>
<td>2.21 ± 0.74</td>
<td>2.17 ± 0.61</td>
</tr>
<tr>
<td>ADP</td>
<td>4.17 ± 0.17</td>
<td>4.22 ± 0.27</td>
<td>4.16 ± 0.17</td>
<td>4.28 ± 0.20</td>
</tr>
<tr>
<td>AMP</td>
<td>0.182 ± 0.04</td>
<td>0.175 ± 0.04</td>
<td>0.177 ± 0.03</td>
<td>0.158 ± 0.02</td>
</tr>
<tr>
<td>TAN</td>
<td>2.72 ± 1.3</td>
<td>2.72 ± 1.2</td>
<td>2.65 ± 0.88</td>
<td>2.61 ± 0.77</td>
</tr>
<tr>
<td></td>
<td>25.1 ± 0.85</td>
<td>26.1 ± 0.87</td>
<td>25.3 ± 0.85</td>
<td>26.5 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry wt; n = 10. Pre, pretraining session; Post, posttraining session; TAN, total adenine nucleotides.

The concentration of PCr was decreased at 3 min of exercise and decreased further at 40 min of exercise (Fig. 2). After the training session, higher PCr values were observed that were not specific to a particular time point. Similar to PCr, both Pi and Cr were altered with exercise and a training session (Fig. 2). However, unlike PCr, the values for both parameters increased with exercise and decreased with the single training session.

The calculated ADP\textsubscript{i} levels were increased, as expected, at 3 min of exercise and increased further at 40 min (Fig. 3). The training session decreased the concentrations of ADP, at 20 and 40 min of exercise. For AMP\textsubscript{i}, an increase was observed at 40 min. In general, AMP\textsubscript{i} levels were lower after the training session.

Glycogen and glycolytic intermediates. Glycogen was reduced by 3 min of exercise and further reduced by 40 min (Fig. 4). In general, glycogen levels were higher after the session of training. All of the glycolytic intermediates were increased by exercise and decreased by the training session (Table 3). For glucose 1-phosphate (G-1-P) and glucose 6-phosphate (G-6-P), increases were observed during the non-steady-state
adjustment to exercise (3 min) and remained elevated for the remainder of exercise. A generalized depression in both of these metabolites occurred after the training session. For fructose 6-phosphate (F-6-P), the specific changes that occurred with exercise depended on the training session. Before the training session, F-6-P was increased at 3 and 20 min and then regressed to a value that remained elevated over rest but not from 3 min. After the single training session, the elevation observed at 3 min of exercise persisted throughout. At 3 and 20 min of exercise, the concentrations of F-6-P were greater before the training session than after it. In the case of fructose 1,6-diphosphate (F-1,6-P2), the concentration was higher at 40 min compared with rest, was unaffected by a single training session (Table 3).

Pyruvate was elevated by exercise but only at 40 min (Table 4). In the case of the terminal metabolite of glycolysis, lactate, initial increases were observed at 3 min and additional increases at 40 min before the training session (Table 4). After the training session, lactate was lower at all exercise time points than before
the single training session. The lactate-to-pyruvate ratio was altered by both exercise and the single training session. Exercise resulted in an increase in the ratio, but only at 40 min, whereas the training session induced a general reduction in the ratio.

None of the maximal activities of the enzymes examined were altered by the single session of training (Table 5). These included two representative enzymes of the citric acid cycle, CS and MDH; a β-oxidation enzyme, 3-HAD; the enzyme for glucose phosphorylation, HEX; and an enzyme used to represent the glycolytic potential, PFK. Protein concentration was also similar before and after the single training session.

Changes in blood lactate concentration were observed with both exercise and the training session (Fig. 5). With exercise, both before and after the training session, lactate was progressively increased at 20 and 40 min. After the single training session, lower lactate values were found at both 20 and 40 min compared with the pretraining session.

DISCUSSION

In this study, we have shown that a single training session, involving 6 min of exercise each hour at ~90% \( \text{VO}_{2\text{peak}} \) for 16 h, elicited a range of metabolic adaptations characteristically reported for training extending over considerably longer periods. To highlight these adaptations, we have employed a standardized cycle exercise protocol that involved 20 min of exercise at each of two step increases in power output. In response to this exercise protocol, the single training session resulted in a more protected energy state, as indicated by the higher levels of ATP (as measured by IMP) and PCr and lower levels of ADP\(_i\) and AMP\(_i\). In addition, muscle glycogen levels were higher and lactate levels were lower. With the exception of lactate, these adaptations were not specific to a particular work intensity, but rather were a generalized effect of the single training session. We have also found that the single session of training was without effect on the maximal activities of the mitochondrial and cytosolic enzymes examined. Collectively, these results support our hypothesis, namely, that metabolic adaptations can occur independently of changes in oxidative potential.

It should be emphasized that the enzymes selected to represent oxidative potential, namely CS and MDH, may not be rate limiting. However, they have been shown to exist in constant proportion with other enzymes of the citric acid cycle and with markers of the electron transport potential during adaptation to contractile activity (43).

This study represents a continuing contribution to numerous studies that we have published in recent years showing a dissociation between the metabolic adaptations that occur with training and the increases in oxidative potential (5, 11, 14, 18, 31). This study is perhaps the most dramatic demonstration, given that the metabolic effects could be induced with one extended training session and without any indication of an altered patterning of the energy metabolic systems, that we have published to date. Indeed, it is possible that similar results could have been obtained using fewer repetitions than the 16 employed in this study. Other investigators, using a 6- to 7-day model of daily cycle exercise performed at a similar intensity and duration (60–65% \( \text{VO}_{2\text{peak}} \) for 120 min) as we have characteristically employed in our earlier studies (5, 11, 14), have reported (6) or inferred (42) similar metabolic adaptations.

Table 3. Effects of exercise and a single training session on selected glycolytic intermediates and F-6-P-to-F-1,6-P\(_2\) ratios

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0</th>
<th>3</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glc Pre</td>
<td>2.04±0.25</td>
<td>3.55±0.28</td>
<td>2.97±0.46</td>
<td>4.13±0.75</td>
</tr>
<tr>
<td>Post</td>
<td>2.20±0.34</td>
<td>3.08±0.32</td>
<td>3.22±0.42</td>
<td>4.20±1.0</td>
</tr>
<tr>
<td>G-1-P Pre</td>
<td>0.185±0.03</td>
<td>0.339±0.04</td>
<td>0.342±0.04</td>
<td>0.348±0.04</td>
</tr>
<tr>
<td>Post</td>
<td>0.162±0.03</td>
<td>0.273±0.04</td>
<td>0.256±0.04</td>
<td>0.302±0.04</td>
</tr>
<tr>
<td>G-6-P Pre</td>
<td>2.57±0.30</td>
<td>4.06±0.39</td>
<td>4.35±0.52</td>
<td>3.91±0.64</td>
</tr>
<tr>
<td>Post</td>
<td>1.91±0.30</td>
<td>2.66±0.31</td>
<td>3.33±0.40</td>
<td>3.90±0.76</td>
</tr>
<tr>
<td>F-6-P Pre</td>
<td>0.184±0.03</td>
<td>0.368±0.07*</td>
<td>0.431±0.10*</td>
<td>0.317±0.06†</td>
</tr>
<tr>
<td>Post</td>
<td>0.160±0.03</td>
<td>0.271±0.06‡</td>
<td>0.305±0.06‡</td>
<td>0.308±0.06*</td>
</tr>
<tr>
<td>F-1,6-P(_2) Pre</td>
<td>0.404±0.07</td>
<td>0.484±0.04</td>
<td>0.556±0.09</td>
<td>0.643±0.10</td>
</tr>
<tr>
<td>Post</td>
<td>0.351±0.07</td>
<td>0.437±0.06</td>
<td>0.437±0.06</td>
<td>0.464±0.07</td>
</tr>
<tr>
<td>F-6-P/F-1,6-P(_2) Pre</td>
<td>0.627±0.17</td>
<td>0.809±0.18</td>
<td>1.03±0.35</td>
<td>0.567±0.15</td>
</tr>
<tr>
<td>Post</td>
<td>0.606±0.16</td>
<td>0.734±0.22</td>
<td>0.807±0.21</td>
<td>0.854±0.26</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry wt; n = 10. Glc, glucose; G-1-P, glucose 1-phosphate; G-6-P, glucose 6-phosphate; F-6-P, fructose 6-phosphate; F-1,6-P\(_2\), fructose 1,6-diphosphate. For Glc, G-1-P, G-6-P, and F-1,6-P\(_2\), and F-6-P/F-1,6-P\(_2\), a main effect (\( P < 0.05 \)) of exercise was found. For Glc and F-1,6-P\(_2\), 0 < 40 min. For G-1-P and G-6-P, 0 < 3 = 20 = 40 min. For F-6-P/F-1,6-P\(_2\), 0 < 20 min. For G-1-P, G-6-P, and F-1,6-P\(_2\), a main effect (\( P < 0.05 \)) of condition was found; Pre > Post. *Significantly different from 0 min (\( P < 0.05 \)); †significantly different from 20 min (\( P < 0.05 \)); ‡significantly different from Pre (\( P < 0.05 \)).

Table 4. Muscle pyruvate, lactate, and the lactate-to-pyruvate ratio with exercise and a single training session

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0</th>
<th>3</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYR Pre</td>
<td>0.146±0.04</td>
<td>0.186±0.04</td>
<td>0.204±0.04</td>
<td>0.283±0.07</td>
</tr>
<tr>
<td>Post</td>
<td>0.110±0.03</td>
<td>0.155±0.02</td>
<td>0.152±0.02</td>
<td>0.199±0.03</td>
</tr>
<tr>
<td>LAC Pre</td>
<td>4.67±0.68</td>
<td>19.8±3.2*</td>
<td>18.3±3.1*</td>
<td>34.1±5.5†‡</td>
</tr>
<tr>
<td>Post</td>
<td>3.98±0.72</td>
<td>10.9±1.7§</td>
<td>10.2±2.4§</td>
<td>23.5±5.2‡§</td>
</tr>
<tr>
<td>LAC/PYR Pre</td>
<td>52.5±15</td>
<td>132±25</td>
<td>114±24</td>
<td>182±67</td>
</tr>
<tr>
<td>Post</td>
<td>54.1±14</td>
<td>72.0±12</td>
<td>60.2±23</td>
<td>149±51</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry wt; n = 10. PYR, pyruvate; LAC, lactate. For PYR, a main effect (\( P < 0.05 \)) was shown for exercise; 0 < 40 min. For LAC/PYR, main effects (\( P < 0.05 \)) were shown for both exercise and the training session. For exercise, 0 < 40 min; for training session, Pre > Post. *Significantly different from 0 min (\( P < 0.05 \)); †significantly different from 3 min (\( P < 0.05 \)); ‡significantly different from 20 min (\( P < 0.05 \)); §significantly different from Pre (\( P < 0.05 \)).
adaptations to our current findings. However, in contrast to our findings, the metabolic changes occurred in conjunction with increases in oxidative potential, as measured by the maximal activities of one (6) or more (42) mitochondrial enzymes. Not surprisingly, our conclusions were rejected, and the primacy of the mitochondrial-metabolic coupling was reasserted (42). The reasons for the underlying contradiction between the results of these investigators (6, 42) and of our studies, which have employed similar daily training sessions for an approximately equal period (5, 11, 31) or longer (18), remain unclear.

The elevated high-energy phosphates and glycogen levels and lower lactate observed in muscle during exercise after the single training protocol are all characteristic of what has been observed with regular training known to induce increases in mitochondrial oxidative potential (24). The increase in oxidative potential has been viewed as being central to the metabolic adaptations that occur (24). According to this hypothesis, increases in oxidative potential serve to protect the high-energy phosphate system, resulting in lower concentrations of ATP, ADP, and P. The lower levels of these putative modulators downregulate glycogenolytic and glycolytic flux rates by allosteric control of key enzymes, namely phosphorylase and PFK. In the absence of increases in oxidative potential, as has been observed with our single training session, how can the metabolic adaptations that we have observed be explained?

The reduced disturbance that was observed in the phosphate energy system is consistent with the notion that the training session created a better balance between ATP-utilizing and ATP-supplying mechanisms (23). This is clearly demonstrated by the more protected PCR concentration that occurred during repetition of the same exercise protocol after the single training session. As well, the concentration of IMP, used as a more sensitive measure of the changes in ATP (23), was depressed after the training session. It should be emphasized that the decrease that occurs in ATP is relatively minor and not detectable statistically. However, the decrease that occurs when IMP is used as a more sensitive measure is important in the calculated changes in ADP and AMP. Ostensibly, the more protected energy state could occur consequent to an improved oxidative phosphorylation or an accelerated anaerobic glycolytic flux that might supply additional ATP. However, the latter mechanism is entirely inconsistent with current evidence, given the near-equilibrium nature of the creatine kinase reaction (28) and the metabolic signals thought to regulate glycogenolysis and glycolysis (7). As shown, the improved high-energy phosphate content with exercise after the training session results in a substantially reduced concentration of one or more of the specific allosteric signals, such as ADP, AMP, and P, which are involved in the regulation of key enzymes of glycogenolysis and glycolysis.

Increases in oxidative phosphorylation remain as the most viable mechanism to explain the more protected energy state. Using a 4-day model of training, we have found that when prolonged moderate-intensity cycle exercise is used as the test protocol, increases in VO2 occur not during the steady-state period but during the non-steady-state period, when mean response time (time to reach 63% of steady-state VO2) is decreased (29). We have also found, using a short-term training model (14), that at similar relative exercise intensities, the changes in the high-energy phosphates are fully manifested during the non-steady-state period of exercise and that adaptations in metabolic behavior can at least partly be explained within this time frame. Such an appear to be the case with the present study. In this study, we have used a two-step protocol to induce a more severe metabolic challenge, with the expectation that we could obtain more convincing evidence of the metabolic adaptations that occur with the single session of training. In the current study, the initial segment of exercise was conducted at approximately the same relative percentage of VO2peak, as previously used (14), and tissue samples were secured at the end of the non-steady-state period (3 min) and during the steady-state period (20 min). As in the previous study, there is clear evidence that at least some of the adjustments in the high-energy phosphate response to the training session occur during the non-steady-state period. Performance of an additional 20 min of exercise at a higher intensity failed to potentiate the response. The finding

### Table 5. Maximal activities of selected muscle enzymes before and after a single, intermittent exercise training session

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>CS</th>
<th>MDH</th>
<th>3-HAD</th>
<th>HEX</th>
<th>PFK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>176±6.8</td>
<td>5.33±0.28</td>
<td>14.7±0.74</td>
<td>7.45±0.21</td>
<td>0.53±0.04</td>
<td>10.7±0.48</td>
</tr>
<tr>
<td>Post</td>
<td>188±5.4</td>
<td>5.22±0.53</td>
<td>14.9±1.2</td>
<td>7.49±0.28</td>
<td>0.54±0.03</td>
<td>10.3±0.64</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10. Protein units are in mg/g; all others are in mol·kg protein⁻¹·h⁻¹. CS, citrate synthase; MDH, malate dehydrogenase; 3-HAD, 3-hydroxyacyl-CoA dehydrogenase; HEX, hexokinase; PFK, phosphofructokinase.
that adaptive changes occur during the initial non-steady-state period in this study, similar to an earlier study (14), suggests that increases in VO2 also occurred with the single training session model.

The increases in VO2 could be mediated by increases in oxygen delivery secondary to increases in blood flow or to an increase in O2 extraction across the working muscles. Changes in blood flow dynamics remain as an inviting mechanism, given the results of an earlier study that showed faster femoral arterial blood velocity kinetics after 10 days of cycle training (39). However, increased availability of one or more of the other substrates needed for oxidative phosphorylation, namely ADP, Pi, H+, and citric acid cycle intermediates, could also be important (7). Because reductions in ADP, Pi, and H+ occurred with the single training session, the increased availability of these substrates would have to be mediated by other adaptations, such as an altered mitochondrial membrane transport.

In this study, as in others (5, 11, 31), we have also found pronounced decreases in muscle lactate during exercise after the training session. This finding could indicate a reduction in glycolytic flux, which we have previously postulated to occur during the non-steady-state period (14), or in increased removal of lactate from the contracting muscle (8, 9). The reduction in one or more of the putative allosteric modulators of PFK, mediated as a consequence of the higher phosphate energy content (7), is consistent with a depression in activity of the rate-limiting enzyme in glycolysis (PFK) and a reduction in lactate production. We have also found reductions in the glycolytic intermediates examined, G-6-P, F-6-P, and F-1,6-P2, during exercise after the training session, also supportive of a decreased flux rate. To examine specifically the role of PFK, as a potential rate-limiting site, mediating the apparent inhibition of glycolysis after the training session, we have calculated the F-6-P-to-F-1,6-P2 ratio. Because we were unable to detect increases in this ratio during exercise after the training session, it would appear that reductions in PFK activity are not involved in the lower lactate accumulation that was observed.

It must be emphasized that, although a reduction in glycolysis remains inviting to explain the lower muscle lactate accumulation, other mechanisms may be involved. It has been proposed that activation of pyruvate dehydrogenase may divert some of the pyruvate to the mitochondria, resulting in the increased formation of acetyl-CoA, a strategy that has received little experimental support (34). It is also possible that increased lactate oxidation could have occurred in the mitochondria, either in the contracting muscle cell or neighboring cells. Recently, Brooks et al. (4) reported that skeletal muscle mitochondria contains an internal lactate dehydrogenase pool that facilitates oxidation of lactate. Additionally, evidence exists that enhanced clearance rate of the lactate from the muscle may occur, a hypothesis which is supported by the early adaptive increases observed in the lactate transporter MCT1, which occurs with training (3), and the findings from studies using stable isotopes, which indicate increased clearance (8, 9, 30). Indeed, we have been able to show that induced hypervolemia by itself (16), which we have also found with this study, results in lower muscle lactate concentrations during submaximal exercise. Interestingly, with induced hypervolemia, the rate at which glycogen was depleted during exercise was unaltered (16).

Higher exercise glycogen levels were also found in this study after the single training session. However, the higher glycogen levels could be explained by the supercompensation that occurred in the resting level. When glycogen values were standardized to 100% at rest before and after the training session, no differences in depletion rates could be detected. This adaptation is different from that observed with longer training studies, in which the higher glycogen levels observed during exercise are due, in large part, to a reduced rate of depletion during the exercise itself, suggesting that glycogenolysis is attenuated (17, 18). The present findings could be explained by proposing that glycogenolysis rates are not altered and that the reduced lactate occurs primarily by enhanced removal or increased oxidation. Alternatively, it is possible that the single session of training did promote a decreased glycogenolysis, and that glycogen depletion rates were not altered because of a reduced muscle glycogen synthesis, a process which is known to occur during the exercise itself (33). The reduction that we have observed in G-1-P and in ADP, AMP, and Pi is supportive of an altered allosteric regulation of phosphorylase and depressions in glycogenolysis (6, 7).

It is conceivable that the metabolic adaptations that we have observed during cycling after the single training session could have resulted from an altered recruitment strategy. Although we have not examined alterations in recruitment in this study, we have done so in a previous short-term training study (40). Activation profiles, determined using full wave rectified electromyographic techniques of seven different muscles including the vastus lateralis, were not altered with training. In addition, phasic behavior, determined by cross-correlation, was also unaltered. These findings suggest that alterations in motor unit recruitment, both within and between the muscles used in cycling, cannot explain the metabolic adaptations.

In summary, the major finding of this study is that an improved balance between ATP-synthesizing and ATP-utilization processes in working muscle can be induced by a single, extended training session consisting of repeated bouts of heavy exercise, known to disturb energy homeostasis (1). This adaptation, characterized by an improved energy state, is also accompanied by a lower muscle lactate concentration and unchanged glycogen depletion rates and steady-state VO2. These results, in addition to suggesting that other strategies may be operative in promoting metabolic adaptation, question existing concepts regarding metabolic control. When considered in conjunction with other studies using the short-term training model, which also induce a tighter metabolic control, resulting in less perturbation in "high-energy" phosphate metabolites at a con-
stant level of oxidative phosphorylation, it can be concluded that increases in oxidative potential are not a prerequisite. The apparent enhancement in respiratory control sensitivity does not appear to depend on increases in the potential for oxidative phosphorylation. Collectively, these results serve to emphasize what has been claimed by others, namely, that there is much to learn regarding the regulation of muscle metabolism and its organization (23).

This study was supported by the Natural Sciences and Engineering Research Council, Canada.

Address for reprint requests and other correspondence: H. J. Green, Dept. of Kinesiology, University of Waterloo, Waterloo, ON, Canada N2L 3G1 (E-mail: green@healthy.uwaterloo.ca).

Received 4 January 1999; accepted in final form 15 September 1999.

REFERENCES

15. Green, H. J., E. R. Chin, M. Ball-Burnett, and D. Ranney. Increases in human skeletal muscle Na+-K+-ATPase concentra-

E125
EXERCISE METABOLISM AND SINGLE-SESSION TRAINING

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on June 25, 2017


