Time-dependent effects of short-term training on muscle metabolism during the early phase of exercise

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Green HJ, Bombardier E, Burnett ME, Smith IC, Tupling SM, Ranney DA. Time-dependent effects of short-term training on muscle metabolism during the early phase of exercise. Am J Physiol Regul Integr Comp Physiol 297: R1383–R1391, 2009. First published August 26, 2009; doi:10.1152/ajpregu.00203.2009.—In this study, we investigated the hypothesis that the metabolic adaptations observed during steady-state exercise soon after the onset of training would be displayed during the nonsteady period of moderate exercise and would occur in the absence of increases in peak aerobic power (V\textsubscript{O\textsubsymbol{2peak}}) and in muscle oxidative potential. Nine untrained males [age = 20.8 ± 0.70 (SE) yr] performed a cycle task at 62% V\textsubscript{O\textsubsymbol{2peak}} before (Pre-T) and after (Post-T) training for 2 h/day for 5 days at task intensity. Tissue samples extracted from the vastus lateralis at 0 min (before exercise) and at 10, 60, and 180 s of exercise, indicated that at Pre-T, reductions (P < 0.05) in phosphocreatine and increases (P < 0.05) in creatine, inorganic phosphate, calculated free ADP, and free AMP occurred at 60 and 180 s but not at 10 s. At Post-T, the concentrations of all metabolites were blunted (P < 0.05) at 60 s. Training also reduced (P < 0.05) the increase in lactate and the lactate-to-pyruvate ratio observed during exercise at Pre-T. These adaptations occurred in the absence of change in V\textsubscript{O\textsubsymbol{2peak}} (47.8 ± 1.7 vs. 49.2 ± 1.7 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) and in the activities (mol·kg\textsuperscript{-1}·h\textsuperscript{-1}) of succinic dehydrogenase (3.48 ± 0.21 vs. 3.77 ± 0.35) and citrate synthase (7.48 ± 0.61 vs. 8.52 ± 0.65) but not cytochrome oxidase (70.8 ± 5.1 vs. 79.6 ± 6.6 U/g protein; P < 0.05). It is concluded that the tighter metabolic control observed following short-term training is initially expressed during the nonsteady state, probably as a result of increases in oxidative phosphorylation that is not dependent on changes in V\textsubscript{O\textsubsymbol{2peak}} while the role of oxidative potential remains uncertain.

adaption; exercise; muscle; non-steady state; metabolism; enzymes

IT IS WELL ESTABLISHED THAT REGULAR EXERCISE PROMOTES EXTENSIVE METABOLIC ADAPTATIONS IN WORKING MUSCLE. IT IS ALSO CLEAR THAT SOME OF THESE ADAPTATIONS CAN BE OBSERVED WITHIN THE FIRST DAYS (6, 17, 18, 20, 23, 49, 51) AND EVEN HOURS (25) OF TRAINING, DEPENDING ON THE CHALLENGE IMPOSED BY THE TRAINING REGIMES. THIS EARLY ADAPTATION, WHICH IS PARTLY DUE TO AN INCREASE IN THE NUMBER AND SIZE OF MITOCHONDRIA, IS INTIMATELY INVOLVED IN THE TRAINING-INDUCED ENZYMATIC RESPONSE (13, 32). THAT SUCH ADAPTATIONS CAN OCCUR IS NOT IN DISPUTE, AT LEAST DURING EXTENDED TRAINING REGIMES, NOR IS THERE ANY DISPUTE THAT THE MITOCHONDRIAL CHANGES RESULT IN INCREASES IN THE POTENTIAL MAXIMAL FLUX RATES IN OXPHOS, RESULTING IN LESS DEPENDENCY ON HIGH-ENERGY PHOSPHATE TRANSFER REACTIONS AND GLYCOLYSIS. BECAUSE STEADY-STATE PHOSPHATE TRANSFER REACTIONS (17, 20, 46) OR EVEN DECREASED (6) FOLLOWING TRAINING, IT WOULD APPEAR THAT, IF INCREASED OXPHOS IS INVOLVED, INCREASES IN THE KINETICS OF V\textsubscript{O\textsubsymbol{2}} WOULD HAVE TO OCCUR. THIS IS ESSENTIALLY WHAT WE HAVE FOUND, NAMELY A FASTER MEAN RESPONSE TIME (TIME TAKEN TO REACH 63% OF STEADY STATE) IN V\textsubscript{O\textsubsymbol{2}} DETECTED AS EARLY AS 4 DAYS AFTER THE ONSET OF TRAINING USING BREATH-BY-BREATH DATA AND ANALYZED USING A TWO-COMPONENT MODEL (46). TWO RECENT STUDIES HAVE ALSO CONFIRMED THAT INCREASES IN V\textsubscript{O\textsubsymbol{2}} KINETICS OCCUR SOON AFTER THE ONSET OF TRAINING (2, 43). THE INCREASE IN V\textsubscript{O\textsubsymbol{2}} KINETICS CANNOT BE EXPLAINED BY INCREASES IN PEAK AEROBIC POWER (V\textsubscript{O\textsubsymbol{2peak}}) SINCE NO CHANGES OCCUR DURING THIS TIME FRAME (6, 17, 18, 46).

FOR INCREASES IN OXPHOS TO BE IMPLICATED IN THE ALTERED RESPONSE, PROGRESSIVE CHANGES IN METABOLISM SHOULD BE DISCERNABLE DURING THE NON-STADY-STATE TIME FRAME, WHICH IS WHEN A 3-MIN INTERVAL IS COMPLETE. HOWEVER, FEW STUDIES HAVE ADDRESSED THIS ISSUE. IN PREVIOUS STUDIES BY OUR GROUP, WE HAVE SHOWN THAT, AT 3 MIN OF EXERCISE, THE PRIMARY TIME INTERVAL TO SAMPLE METABOLIC CHANGES, THE METABOLIC MODIFICATIONS WERE NEARLY COMPLETE WHEN CONTRASTED TO THE STEADY-STATE RESPONSE (17, 20), SUPPORTING THE TIGHT COUPLING BETWEEN V\textsubscript{O\textsubsymbol{2}} KINETICS AND HIGH-ENERGY PHOSPHATE METABOLISM (52, 53). NO STUDIES HAVE EXAMINED THIS ISSUE DURING THE PERIOD BEFORE 3 MIN WHERE A BLUNITING OF THE REDUCTION IN ENERGETIC STATE WOULD BE EXPECTED, GIVEN THE INCREASE IN OXPHOS THAT OCCURS DURING THIS PERIOD. UNCLEAR ARE THE MECHANISMS ELICITING THE APPARENT INCREASE IN OXPHOS DURING THE EARLY TRANSIENT PERIOD FOLLOWING TRAINING. TWO THEORIES EXIST, ONE BASED ON CENTRAL AND THE OTHER BASED ON PERIPHERAL LOCI. THE PERIPHERAL THEORETISTS WOULD POSITULATE THAT THE CRITICAL CHANGES OCCUR WITHIN THE RECRUITED MUSCLE CELL AND THAT THE INCREASE IN V\textsubscript{O\textsubsymbol{2}} IS SECONDARY, OCCURRING AS A RESULT OF CHANGES THAT ALLOW THE MITOCHONDRIA TO USE THE AVAILABLE O\textsubsymbol{2} AS A SUBSTRATE TO INCREASE MITOCHONDRIAL RESPIRATION (15). THE PROPONENTS OF THE CENTRAL THEORY WOULD SUGGEST THAT, DURING THE TRANSITION TO STEADY-STATE EXERCISE OF MODERATE INTENSITY, THE DELIVERY OF O\textsubsymbol{2} IS LIMITING AND THAT THE INCREASE IN V\textsubscript{O\textsubsymbol{2}} KINETICS SIMPLY REFLECTS TRAINING-INDUCED INCREASES IN O\textsubsymbol{2} AVAILABILITY TO THE MITOCHONDRIA (34), POSSIBLY MEDIATED BY ELEVATIONS IN FEMORAL ARTERY BLOOD FLOW (58).

A LONG-STANDING AND POPULAR HYPOTHESIS IS THAT PERIPHERAL ADAPTATIONS TO TRAINING, WHICH CONSIST, IN PART, BY INCREASES IN SIZE AND NUMBER OF MITOCHONDRIA, ARE INTIMATELY INVOLVED IN THE TRAINING-INDUCED ENZYMATIC RESPONSE (13, 32). THAT SUCH ADAPTATIONS CAN OCCUR IS NOT IN DISPUTE, AT LEAST DURING EXTENDED TRAINING REGIMES, NOR IS THERE ANY DISPUTE THAT THE MITOCHONDRIAL CHANGES RESULT IN INCREASES IN THE POTENTIAL MAXIMAL FLUX RATES IN OXPHOS, RESULTING IN LESS DEPENDENCY ON HIGH-ENERGY PHOSPHATE TRANSFER REACTIONS AND GLYCOLYSIS.
of the tricarboxylic acid cycle (TAC) and the electron transport chain (ETC), as indicated by the increased maximal activity ($V_{\text{max}}$) of a wide range of enzymes, representative of the pathways (32) and by direct supportive measurements (31). It has been suggested that these adaptations can increase the mitochondrial sensitivity, allowing a blunting of the effectors, such as one or more of the products of high-energy phosphate transfer, needed to attain a given level of OXPHOS (13, 32). According to this theory, $O_2$ to the mitochondrion is not perceived as limiting. What is in dispute is the significance of these mitochondrial changes since a number of studies from our laboratory (17, 18, 25, 49) and others (7, 51) have not been able to detect elevations in the oxidative potential with the short-term exposure to exercise as assessed by changes in the $V_{\text{max}}$ of a range of representative enzymes of the TAC.

Our primary objective in this study was to investigate the time-dependent effects of short-term training on the metabolic changes that occur during the non-steady-state adjustment to submaximal cycle exercise of moderate intensity. We have hypothesized that the metabolic effects, consisting in part of a more conserved PCR and less accumulation of Cr and Pi would occur early, namely during the non-steady-state phase. We also postulate that these changes would occur in the absence of increases in $V_{\text{O}_2\text{peak}}$ and in oxidative potential.

**METHODS**

**Participants.** Nine males, all healthy and not engaged in exercise on a regular basis (as determined by questionnaire), volunteered for the study. On entry into the study, the age, height, and weight were $20.8 \pm 0.70$ yr, $175 \pm 2.8$ cm, and $71.2 \pm 2.7$ kg (means $\pm$ SE), respectively. Before formal acceptance into the study, the volunteers were individually interviewed at which time the study purpose and design were explained and each of the experimental protocols detailed, along with associated risks. The study was approved by the Office of Research Ethics at the University of Waterloo and, as a condition of approval, written consent was obtained from each participant.

**Experimental design.** Because the objective of the study was to determine the effects of short-term training on the muscle metabolic response to submaximal exercise, participants were required to perform a standardized cycle test both before (Pre-T) and following (Post-T) training. This test was performed for 15 min at $\sim 62\% V_{\text{O}_2\text{peak}}$. The Pre-T test was performed $\sim 1$ wk before the beginning of training while the Post-T was performed 24–48 h following the last training session. Both submaximal tests were performed in the morning in the postabsorbent state. The participants reported to the laboratory $\sim 60$ min before the test for preparation. During the preparatory period, a catheter was inserted in a dorsal hand vein for arterialized blood sampling (17), and the vastus lateralis of both legs was prepared for tissue sampling (3). Blood sampling occurred just before the start of cycling and at 5-min intervals of exercise during each test. Four tissue samples were extracted, two from each leg using separate sites, during each of the Pre-T and Post-T sessions. The sampling occurred just before the formal work protocol and at 10, 60, and 180 s of exercise. To minimize the effects of repetitive tissue sampling on the dependent variables, the exercise protocol was completed in two stages. During stage one, the first tissue sample was obtained following warm-up, which consisted of 3 min of cycling at 25 W. The cycling at 25 W was then repeated for 3 min, the power output was increased to the preestablished level, and the second tissue sample was extracted at 60 s of exercise at this power output. The participants were then provided with 60 min of rest before the exercise was initiated for the second time. The 60-min period of rest was selected to minimize the effects on gas exchange and metabolism on the remaining portion of the test (38). During this stage, the 3-min warmup was repeated, and the full 15-min protocol was completed with brief interruptions for tissue sampling at 10 and 180 s of exercise. Full details of the sampling locations, the rapid sampling techniques, and tissue preparation appear in earlier publications from our laboratory (11, 17, 22). Respiratory gas exchange and heart rate (HR) were also measured in the 10- to 15-min window during the second stage of exercise.

To determine the relative workload of each volunteer for the submaximal cycle test and to determine the effect of training, it was necessary to measure $V_{\text{O}_2\text{peak}}$, both before and after training. The assessment of $V_{\text{O}_2\text{peak}}$ was accomplished using a progressive protocol to fatigue (35), which involved 4 min of loadless cycling, followed by a continuous ramp increase in power output until the subject could no longer maintain pedaling rate (60 revolutions/min) when motivated by verbal encouragement. The $V_{\text{O}_2\text{peak}}$ was defined as the highest $V_{\text{O}_2}$ obtained, averaged over a 30-s period. Gas collection was performed continuously throughout the progressive test, using an open-circuit gas collection system as described earlier (36). This system, calibrated on a daily basis, was also employed during the submaximal tests. As with the submaximal tests, HR was determined using standard electrocardiogram techniques. For all tests, an electronically braked cycle ergometer (Quinton 870) was employed with seat height standardized to each individual. All testing was performed in the same setting at temperatures and relative humidities that ranged between 20–22°C and 45–50%, respectively.

The training program consisted of cycling for 2 h/day for 5 consecutive days at 62% $V_{\text{O}_2\text{peak}}$. Where 2 h of continuous cycling could not be performed, as during the first days of exercise, brief rest periods were provided to allow some recovery, so that the exercise could be continued. The training typically occurred in late morning with water provided ad libitum. The participants consumed breakfast 2–4 h before the start of the training session. Environmental conditions were similar to those reported for the maximal and submaximal tests.

**Analytical techniques.** The tissue properties concentrated on the adenine nucleotides (ATP, ADP, and AMP), IMP, PCr, Cr, and Pi, as well as glycogen and selected glycolytic intermediates [glucose 6-phosphate (G-6-P), glucose 1-phosphate (G-1-P), fructose 6-phosphate (F-6-P), fructose 1,6-diphosphate], pyruvate, and lactate. The concentrations of the adenine nucleotides were obtained using HPLC procedures (37) while the concentrations of the other metabolites were measured fluorometrically (41). The measurements were made on tissue that was contained in the biopsy needle, that was immediately plunged in liquid N$_2$ following extraction from the vastus lateralis muscle, and that was stored a low temperature (−80°C) until analyses. All measurements were made on homogenates prepared from freeze-dried tissue that had been cleaned of visible connective tissue and fat. Details of both the tissue sampling protocols and the assessment techniques as employed in our laboratory appear in earlier papers (17, 18, 49).

Because the measurements of ADP and AMP represent total concentration, it was necessary to calculate the free concentration of both metabolites (ADP$^f$ and AMP$^f$). These calculations were possible since the reactions determining their free concentrations [creatinine phosphokinase (CPK) and adenylate kinase (AK)] are based on near-equilibrium reactions. The near-equilibrium constants for CPK and AK have been reported as 1.66 $\times 10^9$ mol/L and 1.05 mol/L, respectively (10). The pH and $H^+$ concentrations needed to estimate the free concentrations of ADP$^f$ and AMP$^f$ were calculated from the concentrations of pyruvate and lactate (54) while the concentration of free Mg$^{2+}$, also needed in the calculation, was assumed to be 1.0 mmol/L (10).

To determine the effects of training on the potential of selected metabolic pathways and segments, we have measured the activities of several representative enzymes in resting muscle Pre-T and Post-T. The enzymes selected for the TAC included succinic dehydrogenase
Correlated were employed to assess the effects of our treatment conditions. To locate differences between means. Significance was set at 0.05. Where significant differences were found, the Tukey technique was used to all comparisons. Where differences between means are indicated in the text, significance is implied.

RESULTS

Maximal exercise responses. \( V_{O2\text{peak}} \), measured during progressive cycle exercise to fatigue, was not altered by the training program whether expressed as liters per minute or as milliliters per kilogram per minute (Table 1). Similarly, no differences were observed before and following training in the maximal responses of \( V_E \), respiratory exchange ratio (RER), and HR.

Submaximal exercise responses. At steady state, before training, the submaximal cycling protocol resulted in a \( V_{O2} \) of 2.10 ± 0.12 l/min (Table 2), representing 62% \( V_{O2\text{peak}} \). The \( V_{O2} \) was unchanged by training. Training also failed to alter \( V_{CO2}, V_E \), and RER during exercise. Training did result in a lower HR and blood lactate concentration during steady-state exercise. Reductions in resting Hct were observed with training (45.9 ± 0.53 vs. 44.8 ± 0.54%), an adaptation that probably reflects the increase in plasma volume, typically observed early in training (21).

Total adenine nucleotides (TAN) in vastus lateralis were unaffected both by exercise and training (Table 3). Exercise and training also failed to affect the concentrations of the individual components of TAN, namely ATP, ADP, and AMP. The intensity of exercise was insufficient to induce an increase in IMP both before and following training.

As expected, changes were observed in high-energy phosphate metabolism with exercise, with the magnitude dependent on the exposure time (Fig. 1). For PCr, Cr, and Pi, no changes were found during the initial 10 s of exercise, regardless of training state. After 60 s of exercise, initial reductions in PCr were observed that persisted without further reduction, for the remaining 120 s. Training blunted the depletion in PCr at 60 but not at 180 s of cycling. Essentially the same pattern was observed in Cr, except that exercise elevated the concentrations. Training also resulted in a reduction in Cr concentration, a main effect that was not specific to a time point. In the case of Pi, the effect of exercise Pre-T was similar to Cr, namely an increase at 60 s that persisted over the remaining duration. As with PCr, different concentrations of Pi were noted before training at 60 but not at 180 s of exercise. Increases in both ADP and AMP were observed during exercise before training at both 60 and 180 s of exercise compared with rest and 10 s of exercise (Fig. 2). Both metabolites also responded similarly to training, namely lower concentrations at 60 s of effort only.

For the glycolytic intermediates, exercise increased the concentrations of G-6-P, G-1-P, and F-6-P (Table 4). In the case of F-6-P and G-1-P, the increases were specific to 60 s only. For G-6-P, the initial increase in concentration noted at 10 s persisted at 60 and 180 s of exercise. Only in the cases of G-6-P and F-6-P was an effect of training observed. For both metabolites, a general decrease in concentration was observed post-T. Training also resulted in a reduction in lactate but not pyruvate concentration that was not specific to a time point (Fig. 3). In general, increases in lactate concentration were noted at 60 and 180 s of cycling compared with rest while pyruvate concentration was unaffected by exercise. The lactate-to-pyruvate ratios, while increasing at 60 s of effort and by additional increases at 180 s before training, were not altered during exercise following training, regardless of exercise duration. At 180 s of exercise but not at the other time points, the ratios were lower following the training protocol. No effects of either exercise or training were observed at 10 s for pyruvate, lactate, or the pyruvate-to-lactate ratio.

For both muscle glucose and glycogen, main effects of training were observed, which resulted in higher concentrations for both substrates (Fig. 4). In the case of glucose, an

<table>
<thead>
<tr>
<th>Pre-T</th>
<th>Post-T</th>
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<tr>
<td>( V_{O2\text{peak}} ) l/min</td>
<td>3.39 ± 0.18</td>
</tr>
<tr>
<td>( V_{O2\text{peak}} ) ml-kg(^{-1})-min(^{-1} )</td>
<td>47.8 ± 1.7</td>
</tr>
<tr>
<td>( V_{E\text{peak}} ) l/min</td>
<td>127 ± 5.7</td>
</tr>
<tr>
<td>RER</td>
<td>1.29 ± 0.01</td>
</tr>
<tr>
<td>HRmax, beats/min</td>
<td>200 ± 2.4</td>
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</table>

Values are means ± SE; \( n = 9 \) experiments. \( V_{O2\text{peak}} \), peak oxygen consumption; \( V_{E\text{peak}} \), peak ventilation at \( V_{O2\text{peak}} \); RER, respiratory exchange ratio; HRmax, maximal heart rate; Pre-T, pretraining; Post-T, posttraining.
exercise effect was demonstrated, which resulted in higher levels at 180 s compared with 60 s. This was a main effect, not specific to a condition.

Measurement of the activities of representative enzymes of selected metabolic pathways and segments indicated an effect of training but only for two enzymes, namely HEX and COX (Table 5), suggesting that the potential for glucose phosphorylation and mitochondrial electron transport, respectively, had been increased. No changes were observed in the potential of the TAC (SDH; CS), β-oxidation (β-HAD), or glycolysis (PFK). The difference between Pre-T and Post-T in muscle protein concentration (173 ± 3.3 vs. 167 ± 3.3 mg/g) was not significant.

**DISCUSSION**

The unique finding in the current study is that we have been able to demonstrate that, in response to five consecutive days of submaximal cycling, decreases in metabolic strain (improvements in metabolic stability occur), as shown by a more protected energetic state, within 60 s after the onset of moderate intensity exercise. The improvement in metabolic stability is expressed in the absence of change in the total nucleotide pool or in total Cr. As expected, reductions in lactate concentration were induced by training. The muscle metabolic modifications occurred in the absence of changes in VO2peak and in steady-state VO2 measured during the tissue sampling protocol. We also report that our training protocol elevated HEX, the enzyme involved in glucose phosphorylation, and COX, used as a measure of ETS potential. No changes were found in the potential of the TAC as indicated by the lack of change in the activity of SDH and CS or in the glycolytic potential as measured by PFK.

Insight into the mechanisms underlying the effect of training on the metabolic response depends on a careful reexamination of the effects of exercise in the pretrained state. The relatively low exercise blood lactate concentration and the lack of a progressive increase in blood lactate (unpublished) support the conclusion that the intensity was below the lactate threshold. During this type of protocol, it is known that the reduction in PCr after exercise onset is complete within the first 3 min with little drift as the exercise is extended to steady state (39, 52, 53). We have previously shown that, in moderate-intensity cycling, the attenuation in PCr following short-term training is fully manifested by 3 min compared with steady-state exercise at 15 min (17, 20, 25). Because we now demonstrate that the adaptation can occur within the first 60 s after exercise onset, it would appear that the mechanism responsible for this behavior is triggered very early in exercise. Given that we have previously demonstrated that increases in VO2 kinetics occur soon after training onset (46), as have others (2, 43), a reasonable assumption is that increases in OXPHOS mediate the improvement in metabolic stability.

A curious finding in the present study was the failure to find further reductions in PCr (and increases in Cr and Pi) when the exercise was extended to 3 min. Although only one previous study has appeared to examine early time-course changes in PCr after exercise onset using two-leg cycle ergometry and direct tissue sampling (27), the kinetics have been described using quadriceps exercise and 31P magnetic resonance spectroscopy technology (40, 52, 53). These studies report similar findings, namely that, with moderate- to low-intensity exercise, the reductions in PCr are near complete by 60 s. The above studies also report that only a small change in PCr occurs in the first 10 s of exercise, similar to what we report. At the exercise intensities employed, the increased ATP resynthesis needed during this period that is necessary to sustain a high energetic state, as a result of the demands imposed by the excitation and contraction processes, may occur via OXPHOS, secondary to O2 supplied to the mitochondrion as a result of myoglobin desaturation (15, 26, 50, 61). Our failure to find a training effect in PCr at 180 s, similar to what was observed at 60 s, as would be expected (17, 20), may be a reflection of differences in the relative intensity of the cycling task or, perhaps, more realistically, a statistical anomaly, mediated by the large interindividual variability in the response pattern. Comparison of the Pre-T and Post-T values for PCr at 180 s resulted in a near-significant difference (P = 0.097).

As in previous studies, the training-induced increase in the stability of the energetic state is also accompanied by other metabolic adaptations and, in particular, reductions in muscle

### Table 2. Respiratory gas exchange, blood lactate, and heart rate responses during submaximal cycling before and following short-term training

<table>
<thead>
<tr>
<th></th>
<th>VO2, l/min</th>
<th>VCO2, l/min</th>
<th>VEBTPS, l/min</th>
<th>RER</th>
<th>HR, beats/min</th>
<th>La, mM</th>
</tr>
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<tbody>
<tr>
<td>Pre-T</td>
<td>2.10±0.12</td>
<td>2.06±0.12</td>
<td>52.8±2.9</td>
<td>0.98±0.01</td>
<td>161±4.0</td>
<td>2.81±0.42</td>
</tr>
<tr>
<td>Post-T</td>
<td>2.07±0.12</td>
<td>2.01±0.13</td>
<td>52.9±3.7</td>
<td>0.97±0.01</td>
<td>151±3.7*</td>
<td>2.14±0.26*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9 experiments. VO2, oxygen consumption; VCO2, carbon dioxide production; La, lactate; HR, heart rate. Respiratory gas exchange and HR were measured during 10- to 15-min period of exercise. *Significantly different (P < 0.05) from Pre-T.

### Table 3. Concentrations of adenine nucleotides and IMP in vastus lateralis during exercise pre- and posttraining

<table>
<thead>
<tr>
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<th>Time, s</th>
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<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>ATP</td>
<td>24.1±0.75</td>
</tr>
<tr>
<td>ADP</td>
<td>23.7±0.79</td>
</tr>
<tr>
<td>AMP</td>
<td>5.83±0.47</td>
</tr>
<tr>
<td>IMP</td>
<td>5.74±0.39</td>
</tr>
<tr>
<td>TAN</td>
<td>0.18±0.03</td>
</tr>
<tr>
<td>TAN/IMP</td>
<td>0.25±0.07</td>
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</tbody>
</table>

Values are means ± SE; n = 9 experiments. Units are mmol/kg dry wt. TAN, total adenine nucleotides. Time, duration of exercise.
Based on conventional regulatory theory, the reduction in concentration following training would be expected to result of reduced flux via improvement in cellular energy status, resulting in less activation of phosphorylase and/or glycolysis (9, 44). It is probable that increases in lactate removal from the cell also occurred (5), particularly since we have shown that increases in the monocarboxylate transporters occur early in training (16, 24), in association with increased clearance of lactate (48).

As expected, exercise before training increased the cytosolic redox potential as indicated by the lactate-to-pyruvate ratio. This was an expected finding, although the increases are rather modest when compared with more intense exercise (55). The lactate-to-pyruvate ratio was also depressed during exercise following our training protocol, an adjustment that was mediated by reductions in lactate concentration, since no change in pyruvate was observed. We have also reported a similar effect of training previously with the short-term training model (17, 18, 20, 25).

Training-induced increases in muscle glycogen concentration, as we have reported in this study, are a common observation early in training that appear to be mediated by both an increased resting level in combination with a reduced rate of depletion during exercise (6, 17, 18, 20, 49). Our results indicate that the higher glycogen level posttraining was mediated by the higher resting level only, probably because of the abbreviated period of exercise employed, which does not promote large reductions in concentration.

To gain some insight into whether the mechanism responsible for the metabolic modifications we have observed were related to alterations at the level of the mitochondria in the working muscle cell, we have assessed the catalytic activity of several representative enzymes involved in OXPHOS. It has
been postulated that increases in oxidative potential with regular exercise occur as a result of increases in the number of respiratory chains that, in turn, reduce the flux rate per respiratory chain required to attain a given level of OXPHOS (14, 32). As a result, mitochondrial sensitivity in enhanced and less of a disturbance in energetic state (and specific activators) are needed to achieve a desired overall flux rate. As a consequence, it is expected that metabolic inertia or the time taken for activation of OXPHOS would be reduced.

With our training regime, we found no increases in the two representative enzymes, namely SDH and CS, used to estimate TAC potential, a finding reported on several previous occasions using a similar training model (18, 20, 23, 25). However, we did find that COX, a key complex of the ETS, and not measured in our earlier studies, was increased. At present, it is difficult to assess the importance of this finding given that the rate-limiting enzyme in OXPHOS is controversial (45). Because COX is the terminal complex in the ETC that catalyzes the oxidation of reduced cytochrome c by oxygen, it is viewed as a key process regulating OXPHOS (12, 56). However, the activity of COX is believed to exist in excess and not to limit maximal flux rates (12). In regard to the adaptations in enzyme activity, it is important to emphasize that our 5- to 7-day training models may be on the threshold of inducing an increase in mitochondrial potential, depending on the training intensity and initial fitness level of the participants, since increases have been reported (17, 59). It should be noted that the regulation of mitochondrial respiration also depends on a variety of other factors (64) that presumably could effect changes in metabolic inertia. In addition, the supply of select substrates and cofactors to the mitochondria may be limiting, as a result in the delay in pyruvate dehydrogenase activation (27), which might also delay the increase in OXPHOS.

The increase in HEX activity was not surprising. Even though we have found conflicting results in earlier studies with a comparable training stimulus (17, 18, 47), increases soon after the onset of training have been commonly reported (30).

This increase in HEX in combination with the increase in the glucose transporters that occurs within the first days of exercise (16) would appear to be involved either in supplying glucose (via G-6-P) as a substrate during exercise or in glyconeogenesis, leading to the elevated muscle glycogen concentration observed following training (30).

Table 4. Concentrations of selected glycolytic intermediate in vastus lateralis muscle during the early phase of submaximal cycle pre- and posttraining

<table>
<thead>
<tr>
<th></th>
<th>Time, s</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>G-6-P</td>
<td></td>
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</tr>
<tr>
<td>Pre-T</td>
<td>1.97±0.21</td>
<td>3.57±0.58</td>
<td>3.46±0.74</td>
</tr>
<tr>
<td>Post-T</td>
<td>1.62±0.17</td>
<td>2.28±0.51</td>
<td>2.72±0.45</td>
</tr>
<tr>
<td>G-1-P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-T</td>
<td>0.11±0.01</td>
<td>0.16±0.03</td>
<td>0.14±0.04</td>
</tr>
<tr>
<td>Post-T</td>
<td>0.12±0.02</td>
<td>0.12±0.03</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>F-6-P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-T</td>
<td>0.28±0.03</td>
<td>0.49±0.07</td>
<td>0.52±0.10</td>
</tr>
<tr>
<td>Post-T</td>
<td>0.24±0.03</td>
<td>0.34±0.07</td>
<td>0.39±0.07</td>
</tr>
<tr>
<td>F-1,6-P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-T</td>
<td>0.26±0.03</td>
<td>0.32±0.04</td>
<td>0.28±0.03</td>
</tr>
<tr>
<td>Post-T</td>
<td>0.22±0.05</td>
<td>0.35±0.04</td>
<td>0.29±0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9 experiments. Units are mmol/kg dry wt. G-6-P, glucose 6-phosphate; G-1-P, glucose 1-phosphate; F-6-P, fructose 6-phosphate; F-1,6-P, fructose 1,6-diphosphate. For G-6-P and F-6-P, main effects (P < 0.05) of both condition and time were found. For condition and both metabolites, Pre-T < Post-T. For time, G-6-P, 0 < 10, 60, and 180 s; for F-6-P, 0 < 60 s. For G-1-P, a main effect (P < 0.05) of time was found. For time, 0 < 60 s. No significance, either for condition or time was found for F-1,6-P.
that hypoxia is known to exaggerate the metabolic instability known to occur with the model employed (17, 49) and given responses were observed. Because metabolic modifications are normoxia before training, no differences in the metabolic in hypoxia following short-term training and compared with glucose, 60 s

Recently, we found that if moderate exercise was performed in hypoxia following short-term training and compared with normoxia before training, no differences in the metabolic responses were observed. Because metabolic modifications are known to occur with the model employed (17, 49) and given that hypoxia is known to exaggerate the metabolic instability (19) and to decrease Vo2 kinetics (8, 33), it would appear that training simply neutralized the mechanisms whereby hypoxia increased the metabolic stress. Because it appears that the hypoxic-induced increase in metabolic stress occurs secondary to centrally mediated reductions in O2 availability to the mitochondria, it is speculated that the training-induced metabolic responses that we have observed reflect increases in OXPHOS.

Perspectives

This paper adds to the growing body of literature that demonstrates that rapid changes occur in muscle metabolic behavior soon after the onset of training, allowing moderate exercise to be performed with less of a disturbance in energetic state. These results have both theoretical and practical importance. From a theoretical perspective, these early adaptive responses challenge current wisdom regarding metabolic regulation during exercise and suggest swift strategies can be invoked to protect energy homeostasis. As such, the model employed offers another opportunity to distinguish between the relative roles of central vs. peripheral factors in meeting the demand for rapid increases in OXPHOS. Not to be forgotten as well is the need to address the behavior of the energy-consuming processes, namely the excitation and contraction processes and the specific ATPases, given the rapid changes that can also be induced at some of these sites with exercise and training. It would be naïve to conclude that energy homeostasis during increased demand could be efficiently realized without a close integration between the processes involved in generating and in consuming energy.

The current study should be viewed as a catalyst, designed to stimulate further investigation. Studies are needed to determine the compatibility between the different work models employing different muscle masses. The use of 31P nuclear magnetic resonance techniques offers the opportunity to perform kinetic studies on selected aspects of metabolism, which unfortunately is limited to relatively small muscle groups. The use of the biopsy technique to extract tissue is limited by the few sites that can be sampled but allows for large muscle group tasks like cycling and a much more comprehensive evaluation of the properties affected. The question remains, namely, does the amount of muscle mass activated alter the relative roles of peripheral vs. central factors in regulating OXPHOS during the early transient period of exercise? In this regard, future studies should also incorporate measures of V02 kinetics, a limitation in the current study.

Finally, identifying mechanisms offers the opportunity to deliberately craft strategies to improve metabolic stability in both health and disease. Many of the tasks involved in daily living and in work and sport are intermittent in nature, involving relatively brief periods of effort and recovery. The need to protect energy homeostasis by optimizing OXPHOS is important, in general, and particularly so in compromised populations such as chronic heart failure and chronic obstructive pulmonary disease where challenges in O2 delivery occur.

Table 5. Activities of selected mitochondrial and cytosolic enzymes in resting vastus lateralis muscle before and after training

<table>
<thead>
<tr>
<th></th>
<th>SDH</th>
<th>CS</th>
<th>COX</th>
<th>β-HAD</th>
<th>HEX</th>
<th>PFK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-T</td>
<td>3.48±0.21</td>
<td>7.48±0.61</td>
<td>70.8±5.1</td>
<td>3.34±0.29</td>
<td>0.40±0.02</td>
<td>6.82±0.51</td>
</tr>
<tr>
<td>Post-T</td>
<td>3.77±0.35</td>
<td>8.52±0.65</td>
<td>79.6±6.8*</td>
<td>3.84±0.44</td>
<td>0.46±0.03*</td>
<td>7.44±0.52</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9 experiments. Units are mol·kg protein⁻¹·h⁻¹. SDH, succinic dehydrogenase; CS, citrate synthase; COX, cytochrome c oxidase; β-HAD, β-hydroxyl CoA-dehydrogenase; HEX, hexokinase; PFK, phosphofructokinase. *Significantly different (P < 0.05) from Pre-T.
REFERENCES


