Initial aerobic power does not alter muscle metabolic adaptations to short-term training

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Green, H., S. Grant, E. Bombardier, and D. Ranney. Initial aerobic power does not alter muscle metabolic adaptations to short-term training. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E39–E48, 1999.—To investigate the hypothesis that training-induced increases in muscle mitochondrial potential are not obligatory to metabolic adaptations observed during submaximal exercise, regardless of peak aerobic power (V\textsubscript{O\text{2peak}}) of the subjects, a short-term training study was utilized. Two groups of untrained male subjects (n = 7/group), one with a high (HI) and the other with a low (LO) V\textsubscript{O\text{2peak}} (means ± SE; 51.4 ± 0.90 vs. 41.0 ± 1.3 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}; P < 0.05), cycled for 2 h/day at 66–69% of V\textsubscript{O\text{2peak}} for 6 days. Muscle tissue was extracted from vastus lateralis at 0, 3, and 30 min of standardized cycle exercise before training (0 days) and after 3 and 6 days of training and analyzed for metabolic and enzymatic changes. During exercise after 3 days of training in the combined HI + LO group, higher (P < 0.05) concentrations (mmol/kg dry wt) of phosphocreatine (40.5 ± 3.4 vs. 52.2 ± 4.2) and lower (P < 0.05) concentrations of Pi (61.5 ± 4.4 vs. 53.3 ± 4.4), inosine monophosphate (0.520 ± 0.19 vs. 0.151 ± 0.05), and lactate (37.9 ± 5.5 vs. 22.8 ± 4.8) were observed. These changes were also accompanied by reduced levels of calculated free ADP, AMP, and P\textsubscript{i}. All adaptations were fully expressed by 3 min of exercise and by 3 days of training and were independent of initial V\textsubscript{O\text{2peak}} levels. Moreover, maximal activity of citrate synthase, a measure of mitochondrial capacity, was only increased with 6 days of training (5.71 ± 0.29 vs. 7.18 ± 0.37 mol·kg protein\textsuperscript{-1}·h\textsuperscript{-1}; P < 0.05). These results demonstrate that metabolic adaptations to prolonged exercise occur within the first 3 days of training and during the non-steady-state period. Moreover, neither time course nor magnitude of metabolic adaptations appears to depend on increases in mitochondrial potential or on initial aerobic power.

ENZYMATIC, ADAPTATION, CYCLING, OXIDATIVE PHOSPHORYLATION

IN PREVIOUS STUDIES, we have been able to demonstrate that extensive adaptations, both cardiovascular and metabolic, occur within the first several days of prolonged, submaximal cycle training in untrained males (13). In skeletal muscle, as an example, a standardized protocol of moderate exercise elicits less of a reduction in cellular energy state and glycogen and less of an increase in lactate after the training (13). All of these adaptations are at least qualitatively similar to what has been reported for training extending over several weeks and months (22).

What is potentially unique about these short-term training studies is not that extensive adaptations occur but that they appear to occur in the absence of changes in mitochondrial enzyme potential (13). Increase in the potential for oxidative phosphorylation has long been accepted as fundamental, at least for the muscle metabolic adaptations that occur (11, 22).

However, recently, our findings have been challenged. Spina et al. (38) have reported that a training protocol similar to one that we have employed, namely 2 h of exercise per day at between 60 and 70% of peak aerobic power (V\textsubscript{O\text{2peak}}), resulted in an increase in the maximal activities of a number of mitochondrial enzymes within 7 days. The increase in mitochondrial enzyme potential was also accompanied by a decrease in respiratory exchange ratio and blood lactate concentration during submaximal exercise (38). Spina et al. have assumed that the muscle metabolic adaptations, although not measured, would be consistent with what we have reported for short-term training. In a subsequent study, Chesley et al. (6), with a training program involving a similar daily training stimulus and for a similar number of days, reported adaptations in both mitochondrial enzyme potential and in the metabolic response to submaximal exercise. The results of these two studies support the hypothesis that metabolic adaptations are mechanistically linked to increases in the potential for oxidative phosphorylation.

Such a conclusion may be premature because neither study examined time-dependent adaptations, namely the adaptations resulting during the 7-day period of training. Conceivably, the metabolic adaptations could have preceded the increases in mitochondrial enzyme potential during this period. Indeed, we have shown that changes in muscle metabolic behavior during submaximal exercise may occur as early as 3–4 days of training (14). In addition, it is not at all clear what the importance of the initial aerobic power (V\textsubscript{O\text{2peak}}) is, in the time course of adaptations, even when training at the same percentage of V\textsubscript{O\text{2peak}}. Although a prerequisite for entry into our studies was that the subjects not be regularly active, the range of V\textsubscript{O\text{2peak}} has been substantial (5, 14, 15, 17). In spite of the fact that the initial V\textsubscript{O\text{2peak}} for the males in the study by Spina et al. (38) cannot be determined because of a failure to separate the males from the females, the average V\textsubscript{O\text{2peak}} was substantially lower than that observed in several of our studies (5, 15, 17). It is possible that the subjects with the lower V\textsubscript{O\text{2peak}} might demonstrate both a larger and earlier onset adaptation in V\textsubscript{O\text{2peak}} and mitochondrial potential.

In this study, our objective was to examine the time-dependent adaptations to short-term training as modified by initial V\textsubscript{O\text{2peak}}. We have hypothesized that the group with the lower V\textsubscript{O\text{2peak}} would demonstrate the more rapid adaptation in the metabolic...
response to exercise and that this adaptation would occur in the absence of increases in mitochondrial enzymatic potential. In contrast, in the group with the higher VO$_2$peak, both the mitochondrial and metabolic adaptations would occur coincidentally and not until later in the training.

METHODS

Subjects. Male subjects were recruited for the study and divided into two groups based on the VO$_2$peak that they were able to attain during a progressive cycle task to fatigue. Subjects in the low VO$_2$peak group (LO) achieved a VO$_2$peak (means $\pm$ SE) of 41.0 $\pm$ 1.3 ml·kg$^{-1}$·min$^{-1}$ and subjects in the high VO$_2$peak group (HI) achieved a VO$_2$peak of 51.4 $\pm$ 0.90. For the HI group ($n = 7$), mean age, height, and weight were 21.6 $\pm$ 1.0 yr, 176 $\pm$ 3.0 cm, and 72.3 $\pm$ 3.9 kg, respectively. Comparable values for the HI group ($n = 7$) were 21.0 $\pm$ 0.63 yr, 175 $\pm$ 2.2 cm, and 71.6 $\pm$ 1.9 kg. All subjects were healthy (as determined by questionnaire), and none were engaged in physical exercise on a regular basis. Subdivision into HI and LO groups was accomplished on the basis of testing a large group of subjects who displayed widely varying VO$_2$peak values. Written consent was obtained from all volunteers as required after approval of the study by the Office of Human Research.

Experimental design. To investigate the time-dependent adaptations to short-term training as modified by pretraining measures of VO$_2$peak, subjects trained for 6 consecutive days. To determine the training-induced effects, the responses to a standardized cycle ergometer task were examined before the training (0 days), at 3 days, and after 6 days of training. The standard-ized ergometer task were performed for 30 min at power levels or the subjects who displayed widely varying VO$_2$peak values.

Analyses of enzymatic activities. The order of sampling from the nine sampling sites was selected randomly and distributed over both legs.

The initial cycle test was conducted 1–2 days before training, the test at 3 days was performed $\sim$24–30 h after the third training session, and the test at 6 days was performed $\sim$24–36 h after training. Testing was conducted in the same environmental conditions used for the training. At least 4 h before the exercise tests, subjects consumed a liquid supplement consisting of one can of Ensure (1.045 kJ; 14.8% protein, 31.5% fat, and 53.7% carbohydrate; Ross Laboratories, Montreal, PQ, Canada). All subjects were requested to refrain from any other supplement, including coffee before the testing. During the experimental period, the subjects were requested to maintain their normal dietary practices.

Measurements of VO$_2$peak were obtained at least 1 wk before the start of training and between 36 and 48 h after the final submaximal exercise test.

Analytical procedures. VO$_2$peak was determined as described previously with an electronic cycle (Quinton 870) and an open-current gas collection system (23). The values used for peak were the highest values obtained over a 30-s period. The submaximal tests were conducted with the same ergometer and collection system. During the submaximal exercise, the gas collection measurements were performed over a 3-4 min period immediately after the submaximal exercise, which were performed at 3 and 30 min.

For analyses of the muscle metabolic changes, the tissue was freeze-dried and analyzed with fluorometric techniques (18, 19). Each muscle sample was analyzed for the concentration of high-energy phosphate compounds [ATP and phosphocreatine (PCr)] and their metabolites [inorganic phosphate (Pi) and creatine (Cr)], glycogen, and a range of glycolytic intermediates. The adenine nucleotides (ATP, ADP, AMP) and inosine monophosphate (IMP) were measured with ion-pair reversed-phase high-performance liquid chromatography techniques (24). The free concentrations of ADP (ADP$_f$) and AMP (AMP$_f$) were estimated on the basis of the near-equilibrium nature of the Cr phosphokinase reaction and the adenylate kinase reaction, respectively, as previously described (15). Free Pi concentration during exercise was estimated as the difference between resting and exercise Cr concentrations plus the resting free Pi concentration. Resting concentration of free Pi was assumed to be 2.5 or 10.8 mmol/kg dry wt (3). The total Cr content (TCr), obtained for each individual by averaging the results from all nine biopsies, was used to adjust individual metabolite concentrations to minimize the effect of blood and connective tissue. As in previous studies, we have found that training has no systematic effect on TCr (14, 17). Muscle glucose, pyruvate, and lactate were not corrected for extracellular water content because of the uncertainty of water content in this space. It should also be emphasized that the calculation of ADP$_f$ and AMP$_f$ involves making a number of assumptions. These have been discussed in an earlier paper (16).

Maximal activities were determined on a number of enzymes designed to represent glycolysis [phosphofructokinase (PFK)], glucose phosphorylation [hexokinase (HEX)], and the citric acid cycle [citrate synthase (CS) and succinic dehydrog-enase (SDH)]. Enzymatic activities were performed from muscles hand homogenized in a phosphate buffer (pH 7.4) containing 5 mM $\beta$-mercaptoethanol, 0.5 mM EDTA, and 0.02% BSA. Homogenates were diluted in 20 mM imidazole buffer with 0.2% BSA. With the exception of PFK and SDH, in which the measurements were performed on fresh homogenates, all other enzymes were assayed with frozen homoge-nates (20). The enzyme assays were performed at 24–25°C after the procedures of Henriksson et al. (20) as modified in...
Effects of short-term training on \( \dot{V}O_{2\text{peak}} \)

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LO</strong></td>
<td>2.08 ± 0.13</td>
<td>2.08 ± 0.13</td>
<td>2.08 ± 0.13</td>
</tr>
<tr>
<td>0 days</td>
<td>0.36 ± 0.01</td>
<td>2.05 ± 0.11*</td>
<td>2.05 ± 0.11*</td>
</tr>
<tr>
<td>3 days</td>
<td>0.30 ± 0.04</td>
<td>1.99 ± 0.11*</td>
<td>1.99 ± 0.11*</td>
</tr>
<tr>
<td>6 days</td>
<td>0.38 ± 0.04</td>
<td>2.06 ± 0.13*</td>
<td>2.06 ± 0.13*</td>
</tr>
<tr>
<td><strong>HI</strong></td>
<td>2.08 ± 0.13</td>
<td>2.08 ± 0.13</td>
<td>2.08 ± 0.13</td>
</tr>
<tr>
<td>0 days</td>
<td>0.39 ± 0.02</td>
<td>2.44 ± 0.06*</td>
<td>2.44 ± 0.06*</td>
</tr>
<tr>
<td>3 days</td>
<td>0.36 ± 0.03</td>
<td>2.46 ± 0.08*</td>
<td>2.46 ± 0.08*</td>
</tr>
<tr>
<td>6 days</td>
<td>0.40 ± 0.04</td>
<td>2.48 ± 0.09*</td>
<td>2.48 ± 0.09*</td>
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</table>

Values are means ± SE; \( n = 7 \)/group. \( \dot{V}O_{2\text{peak}} \), peak oxygen consumption; LO, low \( \dot{V}O_{2\text{peak}} \); HI, high \( \dot{V}O_{2\text{peak}} \).

RESULTS

Maximal exercise. The \( \dot{V}O_{2\text{peak}} \) obtained during a progressive cycle exercise to fatigue was 41.0 ± 1.3 and 51.3 ± 0.90 ml·kg\(^{-1}\)·min\(^{-1}\) for the LO and HI groups, respectively (Table 1). Six days of training was without effect in increasing \( \dot{V}O_{2\text{peak}} \) in either group.

Submaximal exercise. No effect of either exercise duration or training duration was found for \( \dot{V}O_{2\text{peak}} \), independent of \( \dot{V}O_{2\text{peak}} \) level (Table 2). For both the LO and HI groups, steady-state \( \dot{V}O_2 \) was attained by 15 min of exercise, regardless of the state of training. As expected, the \( \dot{V}O_2 \) was persistently higher during exercise in the HI group compared with the LO group. Before training, the LO and HI groups were exercising at a \( \dot{V}O_2 \) that represented 69 and 66% of \( \dot{V}O_{2\text{peak}} \), respectively.

Blood lactate was altered both by exercise and by training (Fig. 1). With exercise, lactate increased during the first 15 min and then remained stable over the remaining 15 min of exercise. Lactate at both 15 and 30 min of exercise was reduced with training, an effect that was fully manifested within 3 days. No differences in the time course of the response was observed between groups.

Of the high-energy phosphates, ATP and PCR, ATP was not affected by exercise and training (Table 3). In contrast, PCR was altered (Fig. 2). These effects were independent of initial \( \dot{V}O_{2\text{peak}} \) levels. Before training, PCR was reduced ~44% by 3 min of exercise with no further reductions noted during the remainder of the exercise. Training attenuated the decrease in PCR, an effect that was observed at 3 min of exercise and persisted until 30 min. Only 3 days of training were needed for the adaptation in PCR to be fully expressed, regardless of group. The concentrations of IMP were also altered by exercise and training (Fig. 2). Before training, IMP was elevated at both 3 and 30 min of exercise. At both 3 and 6 days of training, no elevation in IMP was observed with exercise. Only 3 days of training were needed for the adaptation in IMP to be fully expressed, regardless of group.
training were needed to elicit reductions in exercise IMP levels.

As expected, changes in P\textsubscript{i} and Cr, two metabolites of high-energy phosphate metabolism, were also observed (Fig. 3). By 3 min of exercise, the increases in both P\textsubscript{i} and Cr were maximal. Reductions were observed in both metabolites at 3 and 30 min of exercise, after 3 and 6 days of training. However, only in the case of P\textsubscript{i} did a further reduction occur (3 min) with an additional 3 days of training.

Before training, glucose 6-phosphate (G-6-P) increased by 3 min of exercise and then declined by 30 min, the value remaining higher than at rest (Fig. 4). Training blunted the increase that was observed at 3 min of exercise, an effect that was apparent by 3 days of training and persisted with the additional 3 days of training. Muscle glucose concentration was altered by exercise but not by training. With exercise, glucose increased at 3 min of exercise and remained elevated throughout the exercise. As with G-6-P, glucose 1-phosphate (G-1-P) was elevated at both 3 and 30 min of exercise with the peak value observed early in exercise (Fig. 5). After 6 days of training, the increase in G-1-P was blunted but only at 3 min of exercise. Only exercise altered fructose 6-phosphate (F-6-P) concentrations. With exercise, F-6-P was elevated at both 3 and 30 min. No group differences in the response to training were observed for any of the glycolytic metabolites examined.

The increase in muscle lactate observed with exercise was fully realized by 3 min (Fig. 6). Thereafter, lactate remained elevated. Decreases in exercise lactate concentration occurred during the first 3 days of training, with no further decreases observed thereafter. The decreases were observed at both the 3- and 30-min time points examined. No changes were observed in pyruvate with training. However, exercise did elevate pyruvate at 30 min. The lactate-to-pyruvate ratio, a measure of the cytosolic redox potential, increased maximally at 3 min of exercise before training and then declined over the remaining period of exercise (Fig. 7). At both 3 and 6 days of training, the lactate-to-pyruvate ratio was reduced both early and late in exercise. Training beyond 3 days did not exaggerate the adaptation.

Changes in calculated ADP\textsubscript{f}, AMP\textsubscript{f}, and free P\textsubscript{i} concentration were also found (Fig. 8). For ADP\textsubscript{f} and AMP\textsubscript{f}, a main effect was found for both exercise and training. For exercise, both ADP\textsubscript{f} and AMP\textsubscript{f} increased at 3 min and continued to increase at 30 min. By 3 days of training, both metabolites were reduced during exercise regardless of duration. Training for an additional 3 days was without further effect. The concentration of free P\textsubscript{i} was also increased by exercise and decreased by training. In the case of this metabolite, further increases were not observed beyond 3 min of exercise regardless of training duration. Progressive reductions occurred in exercise free P\textsubscript{i} concentration at both 3 and
6 days of training. Time course adaptations in ADP_F, AMP_F, and free Pi were independent of initial VO2_peak. Muscle glycogen levels were altered by exercise and training but not by initial VO2_peak levels. For both the LO and HI groups, a progressive reduction in glycogen levels was observed at 3 and 30 min (Fig. 9). Three days of training resulted in overall elevations in glycogen levels at rest and at 3 and 30 min of exercise, effects that persisted throughout the remainder of the training. At 6 days of training, glycogen levels were higher than at 3 days both before exercise and at 3 min of exercise.

Muscle enzymes. Changes in the maximal activities of two enzymes were observed with training (Table 4). Of the two marker enzymes selected as a measure of mitochondrial potential, SDH and CS, both were elevated and only at 6 days of training. The enzyme of glucose phosphorylation, HEX, although increased, failed to reach significance (P < 0.05). The two training groups, LO and HI, did not display any time-dependent differences in the enzymatic response. However, in general, the activity of both SDH and CS was higher in the HI group. The enzyme used to represent glycolytic potential, PFK, remained unchanged by training regardless of the group. No effect of training was observed in protein concentration.

DISCUSSION
This study has clearly demonstrated that a range of metabolic adaptations, typical of the trained state,
occurs in response to a short period of daily, prolonged exercise. The adaptations are extremely rapid, occurring within 3 days of the onset of training. An additional 3 days of training failed to produce additional effects. The initial metabolic adaptations occur in the absence of increases in mitochondrial potential, as indicated by the maximal activities of two representative mitochondrial enzymes. Moreover, the initial or pretraining V˙O₂peak failed to alter both the time courses and magnitude of the metabolic adaptations that were observed. On the basis of these results, we conclude that mechanisms other than an increase in the potential for oxidative phosphorylation must be involved in inducing the early training responses in muscle metabolism.

We have attempted to provide some insight into the nature of the mechanisms involved through our exercise and muscle sampling protocol. A submaximal exercise protocol was selected, which is known to result in a steady-state V˙O₂ by 3 min (39). Muscle biopsies performed at 3 and 30 min of exercise allowed us to determine the time at which the metabolic adaptations occurred. As in a previous study (14), we have found that the metabolic changes to both exercise and training were fully expressed by 3 min of exercise. The increases in IMP (used as a measure of the change in ATP), the decreases in PCr, and near stoichiometric increases in Cr and Pi and increases in lactate occurred during the non-steady-state period. It was also during the non-steady-state period that the reductions in IMP, Cr, Pi, and lactate and more preserved PCr were observed after training. Sustaining the exercise for 30 min failed to result in further changes in either the high-energy phosphates or lactate. The most probable reason for the metabolic imbalance, observed during the non-steady-state period, relates to an inability of oxidative phosphorylation to satisfy the ATP requirements of the contracting muscle and the need to recruit the high-energy phosphates (12). Short-term training appears to attenuate the discrepancy that occurs between the ATP-synthesizing pathways and ATP utilization processes during the early period.

We have proposed, based on previous observations of increases in V˙O₂ kinetics during exercise after short-term training (31), that increases in oxidative phosphor-
NADH-to-NAD observed in the lactate-to-pyruvate ratio early in exercise. Femoral artery blood flow kinetics occur relatively early because we have previously shown increases in working muscle is an inviting possibility (12), particularly with oxidizable substrates. Theoretically, all of the factors regulating oxidative phosphorylation could be implicated in the increase in V˙O2 kinetics that was observed, namely O2, H+, ADP, P, and oxidizable substrates. Training-induced increases in O2 delivery to the working muscle is an inviting possibility (12), particularly because we have previously that increases in femoral artery blood flow kinetics occur relatively early in training (37). The depression that we have observed in the lactate-to-pyruvate ratio early in exercise after training is consistent with a reduction in the NADH-to-NAD+ ratio and alleviation of cellular hypoxia (35).

Increased availability of citric acid cycle intermediates mediated via increases in pyruvate or free fatty acid entry into the mitochondria also appears possible. Increased pyruvate entry would not be expected, however, because in previous work with a 5-day training model, we found reductions in carbohydrate oxidation (32). Increased fat oxidation could be involved given the results of Odland et al. (28), who found that during exercise of a comparable intensity with that used in this study, infusion of an Intralipid-heparin solution, which resulted in a substantial increase in blood free fatty acids, induced some of the adaptations observed with short-term training. With Intralipid-heparin, an improved cellular energy state, increased fat utilization, and glycogen sparing occurred (28). The 5-day training model results in a small but significant increase in total fat oxidation as a result of increases in intramuscular triglyceride oxidation (32). However, different mechanisms appear to be involved with a short-term training model because, unlike the Intralipid experiments, muscle lactate is depressed in addition. The training model demonstrates that the metabolic adaptations occur during the nonsteady state and not later in exercise as observed with Intralipid (9).

As has been previously observed (13), reductions in both blood and muscle lactate concentration during exercise also accompany the adaptations in the phosphate energy system and also occur early in exercise. The association between the two events suggests a coupling between high-energy phosphate metabolism and glycogenolysis-glycolysis. It has been proposed that the more protected energy state may dampen the activation of phosphorylase and PFK as a result of reduced concentrations of one or more of the allosteric activators free P, ADP, and AMP (6, 22). Interestingly, we have found reductions in both blood and muscle lactate during exercise of a comparable intensity with that used in this study, infusion of an Intralipid-heparin solution, which resulted in a substantial increase in blood free fatty acids, induced some of the adaptations observed with short-term training. With Intralipid-heparin, an improved cellular energy state, increased fat utilization, and glycogen sparing occurred (28). The 5-day training model results in a small but significant increase in total fat oxidation as a result of increases in intramuscular triglyceride oxidation (32). However, different mechanisms appear to be involved with a short-term training model because, unlike the Intralipid experiments, muscle lactate is depressed in addition. The training model demonstrates that the metabolic adaptations occur during the nonsteady state and not later in exercise as observed with Intralipid (9).

The changes in the glycolytic intermediates, G-1-P and G-6-P, both of which were observed to accumulate during exercise after training, are also consistent with an enhanced inhibition of glycogenolysis and/or glucose phosphorylation (25). Because no training-induced increase in F-6-P was observed during exercise, PFK would not appear to be a principal control site in the apparent reduction in glycolytic flux that occurs. Reductions in PFK activity may have occurred, consistent with allosteric control by AMPf and free Pi concentration (25), both of which were reduced with training; however, the reduction would not appear to be rate limiting.

Glycogen depletion results provide additional support for phosphorylase as being the regulatory site that is altered with training. The training program resulted in higher glycogen levels during exercise after training. However, the higher glycogen levels can be explained, in part, by the higher training-induced resting levels. When the initial levels are adjusted for, a reanalysis of the data indicates a reduced depletion rate after training. At 30 min, as an example, glycogen loss amounted to 175 mmol · glucosyl U−1 · kg dry wt−1 before training and 112 and 113 mmol · glucosyl U−1 · kg dry wt−1 at 3 and 6 days of training (P < −0.05). There are clear indications that a substantial fraction of the reduced depletion occurs early in exercise. We have previously found a reduced rate of glycogen depletion with short-term training (5, 14, 15) as have others (6) who have also concluded that because phosphorylase (total and phosphorylase a) was unaltered early in exercise after training, that posttranslational control of phosphorylase, mediated by reductions in free P concentration and AMP, is responsible for the blunted glycogenolysis.

It must be emphasized that at present it can only be speculated that a reduced glycolytic flux has occurred.

### Table 4. Effects of short-term training on the maximal activities of selected enzymes

<table>
<thead>
<tr>
<th></th>
<th>Protein, mg/g</th>
<th>CS</th>
<th>SDH</th>
<th>HEX</th>
<th>PFK</th>
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<tr>
<td><strong>LO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0 days</td>
<td>182 ± 7.6</td>
<td>5.31 ± 0.32</td>
<td>3.77 ± 0.44</td>
<td>0.366 ± 0.04</td>
<td>10.2 ± 1.4</td>
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<tr>
<td>3 days</td>
<td>174 ± 10</td>
<td>5.72 ± 0.31</td>
<td>3.95 ± 0.36</td>
<td>0.416 ± 0.05</td>
<td>9.64 ± 1.9</td>
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<tr>
<td>6 days</td>
<td>168 ± 4.6</td>
<td>6.43 ± 0.31</td>
<td>4.58 ± 0.19</td>
<td>0.436 ± 0.02</td>
<td>9.99 ± 1.9</td>
</tr>
<tr>
<td><strong>HI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 days</td>
<td>160 ± 4.0</td>
<td>6.10 ± 0.45</td>
<td>4.49 ± 0.22</td>
<td>0.382 ± 0.03</td>
<td>11.4 ± 1.3</td>
</tr>
<tr>
<td>3 days</td>
<td>170 ± 4.9</td>
<td>6.99 ± 0.52</td>
<td>4.57 ± 0.19</td>
<td>0.417 ± 0.19</td>
<td>12.5 ± 1.2</td>
</tr>
<tr>
<td>6 days</td>
<td>171 ± 5.1</td>
<td>7.92 ± 0.57</td>
<td>5.08 ± 0.28</td>
<td>0.456 ± 0.04</td>
<td>12.5 ± 0.65</td>
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<td><strong>LO-HI</strong></td>
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<td>0 days</td>
<td>171 ± 5.1</td>
<td>5.71 ± 0.29</td>
<td>4.13 ± 0.26</td>
<td>0.37 ± 0.02</td>
<td>10.8 ± 0.93</td>
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<td>3 days</td>
<td>172 ± 5.5</td>
<td>6.42 ± 0.37*</td>
<td>4.26 ± 0.21</td>
<td>0.42 ± 0.03</td>
<td>11.1 ± 1.2</td>
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<tr>
<td>6 days</td>
<td>170 ± 3.3</td>
<td>7.18 ± 0.37*</td>
<td>4.83 ± 0.18*</td>
<td>0.45 ± 0.02</td>
<td>13.3 ± 1.0</td>
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</table>

Values are means ± SE in mol · kg protein−1 · h−1; n = 7/group. LO, low V˙O2peak; HI, high V˙O2peak. CS, citrate synthase; SDH, succinic dehydrogenase; HEX, hexokinase; PFK, phosphofructokinase. *Significantly different from 0 days (P < 0.05).
because only muscle lactate concentration was measured. Evidence exists based on lactate exchange across the working muscle (34) and stable isotope measurements with 13C lactate tracer (7, 33) that increases in lactate removal may be important. This possibility is further supported by the observation that a lactate transporter, monocarboxylate transporter 1, is also upregulated soon after the onset of training (4). Unfortunately, given the questionable assumptions inherent in both procedures, it is not possible to unequivocally argue for either adaptations in production or removal as the predominant mechanism (10).

The adaptations that we have observed in muscle metabolism during moderate intensity exercise in response to short-term training clearly occur during the early phase of the exercise. It is during this phase that recruitment of high-energy phosphate metabolism occurs and glycogenolysis and glycolysis are rapidly accelerated. When exercise is sustained, no further alteration in phosphorylation potential occurs. The metabolic adaptations expressed early in the exercise persist as exercise is continued. During the steady-state period, at the exercise intensity that we have used, which is below the lactate threshold, oxidative phosphorylation appears to supply essentially all of the ATP requirements of the contracting muscle (12). The results of this study and others (6, 14, 15) indicate that in steady state, whole body VO2 and probably VO2 leg (12) remain unaltered. Consequently, if muscle VO2 is unaltered in the face of a training-induced decrease in ADP and free Pi concentration, respiratory control sensitivity must be enhanced (8). The enhancement would appear to occur during the non-steady-state adjustment to moderate exercise. The critical question is what mediates the enhanced sensitivity?

We have reported that the adaptive effects in muscle metabolism occur in conjunction with increased VO2 kinetics (31) accompanied by an increased blood flow (Q) (37) and ostensibly oxygen delivery (QCaO2) to the working muscle. However, for this hypothesis to be viable, it must be demonstrated that events outside the muscle cell, rather than events within the muscle cell, dictate the level of oxidative phosphorylation during the non-steady-state adjustment period. Grassi et al. (12) have examined muscle VO2 kinetics (VO2 leg) during moderate exercise and concluded that there are two phases during the on transition: an initial phase, phase I, which lasts for 10–15 s, followed by a second phase, phase II, which extends for 1–2 min. Phase I is characterized by a large increase in blood flow and only modest increases in VO2 leg, suggesting that during this period constraints within the muscle limit oxidative phosphorylation. During phase II, a monoexponential increase in VO2 leg occurred with approximately similar time courses observed for QCaO2 leg and arteriovenous O2 content across the leg. Our experimental design did not permit us to determine if the metabolic adaptations were specific to phase I or phase II. In previous studies with a short-term training model, we have been able to determine that the increase in blood flow kinetics occurs with the first 1–2 min (37), suggesting that a CaO2 limitation occurs during this period.

It has been shown that during phase II, a single reaction with first-order kinetics controls VO2 muscle (27, 29). This single reaction is dependent on changes in the energy state. Previous work has shown that the decrease in PCr is also nonexponential (1, 26). Based on this association, the more conserved PCr level that we have observed during exercise with training would suggest a decrease in VO2 kinetics, not an increase, as we have observed (31). For an increase in VO2 kinetics to have occurred in the face of a more protected high-energy phosphorylation potential, mitochondrial control sensitivity would have to be altered during the non-steady-state period.

It has been hypothesized that the increase in respiratory control sensitivity occurs as a result of increases in mitochondrial or oxidative capacity (8, 22, 29). However, our results do not support this proposition. We have found that after 3 days of training, the metabolic adaptations that occurred were not accompanied by increases in the maximal activities of CS or SDH, the two enzymes selected to represent oxidative potential. After 6 days of training, oxidative potential increased but no further exaggeration occurred in the metabolic response. These results suggest that other factors are involved in the regulation of mitochondrial metabolism (21).

The changes that we have observed in the marker enzymes of oxidative capacity with 6 days of training are similar to what has been recently reported (6, 38) but contradict what we reported previously (15, 17, 34). Because, in previous studies, we have employed subjects with widely different VO2peak values, it was hypothesized that both the enzymatic and metabolic adaptations may occur with a different time frame. We have found no evidence that there is a time-dependent effect of training in the present study, because the group with the low VO2peak and the group with the high VO2peak showed the same response at both 3 and 6 days of training. Consequently, it is possible that our earlier studies failed to find an increase in mitochondrial potential when it did in fact occur (15–17). However, it should be emphasized that a recent study (34) has also failed to find increases in CS activity, in the face of extensive metabolic adaptations, after 7 days of training with a similar protocol to the one we have employed (14, 15, 17).

In this regard, a potential limitation must be acknowledged. It is possible that the enzymes selected to represent mitochondrial potential in our studies (16) and others (6, 34, 38) may not have been appropriate. The enzymes have been selected because they exist in constant proportion with other mitochondrial enzymes (30). Controversy continues to exist as to what the rate-limiting enzyme is.

In summary, we have been able to demonstrate that metabolic adaptations occur during moderate exercise within the first 3 days after the onset of training. These adaptations, which are expressed during the non-steady-state adjustment period, are characterized by a more
protected phosphorylation state (less recruitment of high-energy phosphates) and an apparent reduction in glycolgenolysis as indicated by lower muscle lactate levels. The improved energy state with training is unaccompanied by increases in mitochondrial potential and dissociated from the initial aerobic power (VO2peak of the subjects). Although the mechanisms remain elusive, it is strongly suspected that the metabolic adaptations are a consequence of a reduction in cellular hypoxia during the onset of exercise, secondary to improved blood flow and oxygen delivery to working muscles.

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