Time-dependent Effects of Short-Term Training on Muscle Metabolism During the Early Phase of Exercise

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Short title: Muscle metabolism and training

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Abstract

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 non-steady period of moderate exercise and would occur in the absence of increases in peak aerobic power (\(\dot{V}O_{2peak}\)) and in muscle oxidative potential. Nine untrained males (age =20.8±0.70 yr; \(\bar{x} \pm SE\)) performed a cycle task at 62\% \(\dot{V}O_{2peak}\) 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 (prior to exercise) and at 10, 60 and 180 s of exercise, indicated that at Pre-T, reductions (P<0.05) in phosphocreatine (PCr) and increases (P<0.05) in creatine (Cr), inorganic phosphate (P_i), calculated free ADP (ADP_i) and free AMP (AMP_i) occurred at 60 s 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/pyruvate ratio observed during exercise at Pre-T. These adaptations occurred in the absence of change in VO2peak (47.8±1.7 versus 49.2±1.7 ml. kg\(^{-1}\)·min\(^{-1}\)) and in the activities (mol·kg\(^{-1}\)·prot\(^{-1}\)·h\(^{-1}\)) 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 (COX; 70.8±5.1 vs 79.6±6.6 Units g prot\(^{-1}\); P<0.05). It is concluded that the tighter metabolic control observed following short-term training is initially expressed during the non-steady state, probably as a result of increases in oxidative phosphorylation that is not dependent on changes in \(\dot{V}O_{2peak}\) while the role of oxidative potential remains uncertain.

Keywords: Adaptation, exercise, muscle, non-steady state, metabolism, enzymes
Introduction

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), following the onset of contractile activity, depending on the challenge imposed by the training stimulus. These alterations, typically demonstrated during steady-state submaximal exercise, serve to reduce metabolic strain during increased demands by reducing the decrease in the content of high energy compounds such as ATP and creatine phosphate (PCr), both of which are important in energetic state (1, 9), decreasing the rate of depletion of endogenous carbohydrate reserves (glycogen) and depressing the accumulation of metabolic by-products produced from both high-energy phosphate transfer reactions (Cr, Pi) and glycolysis (H^+, lactate).

An appealing possibility is that the tighter metabolic control that we have observed with our brief training protocol is due to increases in oxidative phosphorylation (OXPHOS), resulting in less dependency on high-energy phosphate transfer reactions and glycolysis. Since steady-state $\dot{V}O_2$ is unchanged (17, 18, 20, 46) or even decreased (6) following training, it would appear that if increased OXPHOS is involved, increases in the kinetics of $\dot{V}O_2$ 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 $\dot{V}O_2$, detected as early as 4 days after the onset of training using a breath-by-breath data and analyzed using a 2-component model (46). Two recent studies have also confirmed that increases in $\dot{V}O_2$ kinetics occur soon after the onset of training (2, 43). The
increase in $\dot{V}O_2$ kinetics cannot be explained by increases in $\dot{V}O_2_{\text{peak}}$ 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-steady state time frame, which in the case of moderate intensity exercise, should occur during the primary phase which is essentially complete by 3 min of exercise (62). However, few studies have addressed this issue. In previous studies by our group, we have shown that at 3 min of exercise, the earliest time sampled, the metabolic modifications were nearly complete when contrasted to the steady-state response (17, 20), supporting the tight coupling between $\dot{V}O_2$ kinetics and high-energy phosphate metabolism (52, 53). No studies have examined this issue with training during the period prior to 3 min where a blunting 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 theorists would postulate that the critical changes occur within the recruited muscle cell and that the increase in $\dot{V}O_2$ is secondary, occurring as a result of changes which allow the mitochondria to use the available O$_2$ 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$_2$ is limiting and that the increase in $\dot{V}O_2$ kinetics simply reflects training-induced increases in O$_2$ 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 metabolic 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 of the citric acid cycle (CAC) 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 maximal activity \( V_{\text{max}} \) of a range of representative enzymes of the citric acid cycle.

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 P\(_i\), would occur early, namely during the non-steady state phase. We also postulate that these changes would occur in the absence of increases in \( \dot{V}O_{2,\text{peak}} \) 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±0.70 yr, 175±2.8 cm and 71.2±2.7 kg (\(\bar{x} \pm SE\)), 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. Since 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 prior to (Pre-T) and following (Post-T) training. This test was performed for 15 min at ~62% \(\dot{VO}_2\) peak. The Pre-T test was performed ~ one week prior to 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 post-absorbent state. The participants reported to the laboratory ~ 60 min prior to the test for preparation. During the preparatory period, a catheter was inserted into 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 prior to the start of cycling and at 5 min intervals of exercise during each test. Four tissue samples were extracted, 2 from each leg using separate sites, during each of the Pre-T and Post-T sessions. The sampling occurred just prior to the formal work protocol and at 10 s, 60 s and 180 s of exercise. To minimize the effects of repetitive tissue sampling on the
dependent variables, the exercise protocol was completed in 2 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 increased to the pre-established level and the second tissue sample 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 in order 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 s 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 were also measured in the 10-15 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 $\dot{V}O_2^{\text{peak}}$, both before and after training. The assessment of $\dot{V}O_2^{\text{peak}}$ 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 per min) when motivated by verbal encouragement. The $\dot{V}O_2^{\text{peak}}$ was defined as the highest $\dot{V}O_2$ 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, heart rate was determined using standard ECG 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 which ranged between 20-22°C and 45-50%, respectively.

The training program consisted of cycling for 2 h per day for 5 consecutive days at 62% \( \dot{V}O_2 \text{peak} \). Where 2 h of continuous cycling could not be performed, as during the first days of exercise, brief rest periods were provided in order 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 prior to 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, F-1,6-P), 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 which was contained in the biopsy needle and which was immediately plunged into liquid N\(_2\) following extraction from the vastus lateralis muscle and stored a low temperature (-80°C) until analyses. All measurements were made on homogenates prepared from freeze dried tissue which 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).

Since 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 (creatine phosphokinase, CPK and adenylate kinase, AK) are based on near-equilibrium reactions. The near-equilibrium constants (K$_{obs}$) for CPK and AK have been reported as 1.66 x 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 CAC included succinic dehydrogenase (SDH) and citrate synthase (CS), for the ETS, cytochrome C oxidase (COX), for β-oxidation, β-hydroxyl-CoA dehydrogenase (β-HAD), for glycolysis, phosphfructokinase (PFK) and for glucose phosphorylation, hexokinase (HEX). With the exception of COX, the enzyme activities were determined using fluorometric techniques at 24-25°C according to the procedures of Henriksson et al. (29) which is based on an end point, as modified in our laboratory (22). For SDH and PFK, the activities were assessed in fresh homogenates while for CS and HEX, the assays were conducted on homogenates that had been frozen and stored at -80°C pending analyses. It has been shown that for SDH and PFK freezing the homogenates results in significant reduction in activity (29). After cleaning the tissue of visible blood, fat and connective tissue, the homogenate was prepared using hand homogenization (0-4°C) in a phosphate buffer (pH 7.4) containing 5 mM-β-mercaptoethanol, 0.5 mM EDTA, and 0.2% BSA and diluted in 20 mM imidazole buffer with 0.25 BSA.
In the case of COX, a spectrophotometric assay was used (30°C) which was based on the disappearance of reduced cytochrome c absorbance at 550 nm for 3 min (4). For the measurement of COX, the reaction medium consisted of 10 mM potassium phosphate buffer (pH 7.0) and 1 mm solution of reduced cytochrome c. The sample was diluted in 1:10 in the potassium phosphate buffer and the assay started by adding 1 ml of the diluted homogenate to the reaction medium. The units of activity were calculated using the measured slope and the extinction coefficient of reduced cytochrome c and expressed in units per gram protein.

Protein in the homogenates was determined by the Lowry technique as modified by Schacterle and Pollock (57).

Care was taken to ensure that for a given property, all samples for a given individual (4 for Pre-T and 4 for Post-T) were measured in duplicate during the same analytical session.

The blood samples were used for the determination of both hematocrit (Hct) and lactate concentration. The Hct was measured in triplicate by standard techniques and corrected for trapped plasma (0.96) and venous-to-whole-body Hct difference (0.91). For lactate, the blood was immediately deproteinized following sampling by cold perchloric, centrifuged, and the supernatant was frozen and stored until analysis by fluorometric methods (41).

**Data analyses.** Both studentized t-tests and analyses of variance (ANOVA) procedures were employed to assess the effects of our treatment conditions. Correlated t-tests were employed to determine the effect of training where only a single value was available for a given property. Two way ANOVA procedures for repeated measures were applied where exercise time and condition represented the dependent variables. Where significant differences were found, the
Tukey technique were used to locate differences between means. Significance was set at $p < 0.05$ for all comparisons. Where differences between means are indicated in the text, significance is implied.

**Results**

**Maximal exercise responses.** $\dot{V}O_{2\text{peak}}$, measured during progressive cycle exercise to fatigue, was not altered by the training program whether expressed as l.min$^{-1}$ or as ml.kg$^{-1}$.min$^{-1}$ (Table 1). Similarly, no differences were observed prior to and following training in the maximal responses of $V_E$, RER and HR.

**Submaximal exercise responses.** At steady-state, prior to training, the submaximal cycling protocol resulted in a VO$_2$ of 2.10±0.12 l.min$^{-1}$ (Table 2), representing 62% $\dot{V}O_{2\text{peak}}$. The $\dot{V}O_2$ was unchanged by training. Training also failed to alter $\dot{V}CO_2$, $\dot{V}_E$ 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 prior to and following training.

As expected, changes were observed in high-energy phosphate metabolism with exercise, 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. Following 60 s of exercise initial reductions in PCr were observed which 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 P1, the effect of exercise pre-training was similar to Cr, namely an increase at 60 s which persisted over the remaining duration. As with PCr, different concentrations of P1 were noted before training at 60 s but not at 180 s of exercise. Increases in both ADPf and AMPf were observed during exercise before training at both 60 s and 180 s of exercise compared to 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 s 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-training. 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 s and 180 s of cycling compared to 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 prior to 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 was an exercise effect was demonstrated, which resulted in higher levels at 180 s compared to 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 2 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 citric acid cycle (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 5 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 creatine. As expected, reductions in lactate concentration were induced by training. The muscle metabolic modifications, occurred in the absence of changes in $\dot{V}O_2\text{peak}$ and in steady-state $\dot{V}O_2$ 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 CAC 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 re-examination of the effects of exercise in the pre-trained 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). Since we have previously shown that in moderate intensity cycling, the attenuation in PCr following short-term training is fully manifested by 3 min as compared to steady-state exercise at 15 min (17, 20, 25). Since 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 $\dot{V}O_2$ 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 P$_i$) 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 $^{31}$P-MRS 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 re-synthesis needed during this period which 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 O$_2$ 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 lactate concentration(6, 17, 18, 20, 25, 49, 51). 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 prior to training increased the cytosolic redox potential as indicated by the lactate/ pyruvate ratio. This was an expected finding, although the increases are rather
modest when compared to more intense exercise (55). The lactate/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 post-training 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 which, 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 2 representative enzymes, namely SDH and CS, used to estimate CAC 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). Since COX is the terminal complex in the ETC which 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 depend on a variety of other factors (64), which presumably could effect changes in metabolic inertia. In addition, the supply of select substrates and co-factors 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).

Recently, we found that if moderate exercise was performed in hypoxia following short-
term training and compared to normoxia prior to training, no differences in the metabolic responses were observed. Since 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. Since, it appears that the hypoxic-induced increase is 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 oxidative phosphorylation.

**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 versus 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 naive 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 $^{31}$P-NMR 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 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 versus central factors in regulating OXPHOS during the early transient period of exercise? In this regard, future studies should also incorporate measures of $\dot{V}O_2$ kinetics, a limitation in the current study.

Finally, identifying mechanisms offer 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 $O_2$ delivery occur.
Grants

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Table 1. Peak aerobic power and related properties during progressive cycle exercise to fatigue prior to and following short-term training

<table>
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<tr>
<th></th>
<th>$\dot{V}O_2_{\text{peak}}$ (1.min$^{-1}$)</th>
<th>$\dot{V}O_2_{\text{peak}}$ (mℓ. kg$^{-1}$.min$^{-1}$)</th>
<th>VEBTPS (ℓ.min$^{-1}$)</th>
<th>RER</th>
<th>HRmax (b.min$^{-1}$)</th>
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<tr>
<td>Pre-T</td>
<td>3.39±0.18</td>
<td>47.8±1.7</td>
<td>127±5.7</td>
<td>1.29±0.01</td>
<td>200±2.4</td>
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<tr>
<td>Post-T</td>
<td>3.51±0.18</td>
<td>49.2±1.8</td>
<td>134±8.1</td>
<td>1.27±8.1</td>
<td>202±3.0</td>
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Values are $\bar{x} \pm SE$ (n=9); VO$_{2\text{peak}}$, peak oxygen consumption; VEBTPS, peak ventilation at $\dot{V}O_2_{\text{peak}}$; RER, respiratory exchange rate; HR$_{\text{max}}$, maximal heart rate. Pre-T, pre-training; Post-T, post-training.
Table 2. Respiratory gas exchange, blood lactate and heart rate responses during submaximal cycling prior to and following short-term training

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<th></th>
<th>$\dot{VO}_2$ (1.min$^{-1}$)</th>
<th>VCO$_2$ (1.min$^{-1}$)</th>
<th>VEBTPS (1.min$^{-1}$)</th>
<th>RER</th>
<th>HR (b.min$^{-1}$)</th>
<th>La (mM)</th>
</tr>
</thead>
<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 $\bar{x} \pm SE$ (n=9); $\dot{VO}_2$, oxygen consumption; VCO$_2$, carbon dioxide production; La, lactate; VEBTPS, minute ventilation; HR, heart rate; RER, respiratory exchange rate; Pre-T, pre-training; Post-T, post-training. Respiratory gas exchange and heart rate 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 inosine monophosphate in vastus lateralis during exercise pre and post training

<table>
<thead>
<tr>
<th></th>
<th>Time(s)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>60</td>
<td>180</td>
</tr>
<tr>
<td>ATP</td>
<td>24.1±0.75</td>
<td>24.6±0.91</td>
<td>25.0±0.89</td>
<td>24.8±1.1</td>
</tr>
<tr>
<td>Pre-T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-T</td>
<td>23.7±0.79</td>
<td>22.6±1.2</td>
<td>22.8±0.68</td>
<td>22.9±0.84</td>
</tr>
<tr>
<td>ADP</td>
<td>5.83±0.47</td>
<td>5.83±0.43</td>
<td>5.90±0.52</td>
<td>6.06±0.57</td>
</tr>
<tr>
<td>Pre-T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-T</td>
<td>5.74±0.39</td>
<td>5.63±0.37</td>
<td>6.01±0.26</td>
<td>5.79±0.37</td>
</tr>
<tr>
<td>AMP</td>
<td>0.18±0.03</td>
<td>0.26±0.04</td>
<td>0.24±0.04</td>
<td>0.24±0.03</td>
</tr>
<tr>
<td>Pre-T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-T</td>
<td>0.25±0.07</td>
<td>0.22±0.06</td>
<td>0.24±0.06</td>
<td>0.19±0.03</td>
</tr>
<tr>
<td>TAN</td>
<td>29.8±0.92</td>
<td>30.7±1.0</td>
<td>31.2±1.0</td>
<td>31.1±1.5</td>
</tr>
<tr>
<td>Pre-T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-T</td>
<td>29.7±1.0</td>
<td>28.4±1.5</td>
<td>29.0±0.86</td>
<td>28.9±1.2</td>
</tr>
<tr>
<td>IMP</td>
<td>0.03±0.01</td>
<td>0.05±0.03</td>
<td>0.05±0.01</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>Pre-T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-T</td>
<td>0.04±0.01</td>
<td>0.03±0.01</td>
<td>0.03±0.01</td>
<td>0.05±0.02</td>
</tr>
</tbody>
</table>

Values are $\bar{x} \pm SE$ (n=9) in mmol.kg$^{-1}$ dw; Pre-T, pre-training; Post-T, post-training. TAN, total adenine nucleotides; IMP, inosine monophosphate. Time(s), duration of exercise in s.
Table 4. Concentrations of selected glycolytic intermediate in vastus lateralis muscle during the early phase of submaximal cycle pre and post training.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Time(s)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>60</td>
<td>180</td>
</tr>
<tr>
<td>G-6-P</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-T</td>
<td>1.97±0.21</td>
<td>3.57±0.58</td>
<td>3.46±0.74</td>
<td>3.34±0.26</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>
<td>2.49±0.53</td>
</tr>
<tr>
<td>G-1-P</td>
<td></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>
<td>0.15±0.02</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>
<td>0.11±0.02</td>
</tr>
<tr>
<td>F-6-P</td>
<td></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>
<td>0.45±0.04</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>
<td>0.42±0.12</td>
</tr>
<tr>
<td>F-1, 6-P</td>
<td></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>
<td>0.28±0.09</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>
<td>0.28±0.07</td>
</tr>
</tbody>
</table>

Values are \( \bar{x} \pm SE \) (n=9) in \( \mu mol.kg^{-1} \) dw; Time(s), duration of exercise in s. Pre-T, pre-training; Post-T, post-training. 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, Pe-Tand Post-T. For time, G-6-P < 10, 60, 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.
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±6.6*</td>
<td>3.84+_0.44</td>
<td>0.46±0.03*</td>
<td>7.44±0.52</td>
</tr>
</tbody>
</table>

Values are \( \bar{x} \pm SE \) (n=9) in mol.kg\(^{-1}\) prot\(^{-1}\). h\(^{-1}\). Pre-T, pre-training; Post-T, post-training. SDH, succinic dehydrogenase; CS, citrate synthase; COX, cytochrome c oxidase; β-HAD, β-hydroxyl Co A-dehydrogenase; HEX, hexokinase; PFK, phosphofructokinase. *Significantly different (P<0.05) from Pre-T.
List of figures

Figure 1. Phosphocreatine (A), creatine (B) and inorganic phosphate (Pi) in vastus lateralis muscle during the early phase of submaximal cycling prior to and following training. Values are $\bar{x} \pm SE$ (n=9). Time, duration of exercise in seconds. PCr, phosphocreatine; Cr, creatine; Pi, inorganic phosphate. *Significantly different (P<0.05) from Pre-T; †Significantly different (P<0.05) from 0 s; ‡Significantly different (P<0.05) from 10 s. For Cr, a main effect (P<0.05) of training was found. For training, Pre-training > Post-training.

Figure 2. The response of free adenosine diphosphate and free adenosine monophosphate to submaximal cycling after exercise onset prior to and following training. Values are $\bar{x} \pm SE$ (n=9). Time, duration of exercise in seconds. ADP, free adenosine diphosphate; AMP, free adenosine monophosphate. * Significantly different (P<0.05) from Pre-T; † Significantly different (P<0.05) from 0 s; ‡Significantly different (P<0.05) from 10 s.

Figure 3. Concentrations of pyruvate and lactate and lactate/pyruvate ratios in vastus lateralis muscle during the early phase of submaximal cycling prior to and following training. Values are $\bar{x} \pm SE$ (n=9). Time, duration of exercise in seconds; For lactate, main effects (P<0.05) of both condition and time were found. For condition, Pre-T > Post-T. For time, 0<60 <180 s. * Significantly different (P<0.05) from Pre-T; † Significantly different (P<0.05) from 0 s. ‡Significantly different (P<0.05) from 10 s.

Figure 4. Glucose and glycogen concentrations in vastus lateralis muscle during the early phase
of submaximal cycling prior to and following training. Values are $\bar{x} \pm SE$ (n=9). Time, duration of exercise in seconds. For both glucose and glycogen, main effects (P<0.05) of condition were found. For both properties, Post-T > Pre-T. For glucose, a main effect (P<0.05) of time was found. For glucose, 60 s < 180 s.
Figure 1

A

Pre-Training
Post-Training

PCr (mol·kg⁻¹ dry weight)

Time (s)

0 10 60 180

B

Cr (mol·kg⁻¹ dry weight)

Time (s)

0 10 60 180

C

Pi (mol·kg⁻¹ dry weight)

Time (s)

0 10 60 180
Figure 2:

A

[Graph showing ADP$_r$ (μmol·kg$^{-1}$ dry weight) over time (s) for Pre-Training and Post-Training.]

B

[Graph showing AMP$_r$ (μmol·kg$^{-1}$ dry weight) over time (s) for Pre-Training and Post-Training.]
Figure 3:

A. Pyruvate (mmol·kg⁻¹ dry weight)

B. Lactate (mmol·kg⁻¹ dry weight)

C. Lactate/Pyruvate
Figure 4:

A

Glucose (mmol • kg⁻¹ dry weight)

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Pre-Training</th>
<th>Post-Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.0 ± 0.5</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>10</td>
<td>3.5 ± 0.3</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>60</td>
<td>2.5 ± 0.2</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>180</td>
<td>3.0 ± 0.8</td>
<td>4.0 ± 0.7</td>
</tr>
</tbody>
</table>

B

Glycogen (mmol glucosyl units • kg⁻¹ dry weight)

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>300 ± 10</td>
</tr>
<tr>
<td>10</td>
<td>250 ± 5</td>
</tr>
<tr>
<td>60</td>
<td>300 ± 15</td>
</tr>
<tr>
<td>180</td>
<td>250 ± 10</td>
</tr>
</tbody>
</table>