Short-term training attenuates muscle TCA cycle expansion during exercise in women

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Dawson, Kristen D., Krista R. Howarth, Mark A. Tarnopolsky, Nathan D. Wong, and Martin J. Gibala. Short-term training attenuates muscle TCA cycle expansion during exercise in women. J Appl Physiol 95: 999–1004, 2003. First published May 23, 2003; 10.1152/japplphysiol.01118.2002.—Muscle glycogenolytic flux and lactate accumulation during exercise are lower after 3–7 days of “short-term” aerobic training (STT) in men (e.g., Green HJ, Helyar R, Ball-Burnett M, Kowalchuk N, Symon S, and Farrance B. J Appl Physiol 72: 484–491, 1992). We hypothesized that 5 days of STT would attenuate pyruvate production and the increase in muscle tricarboxylic acid cycle intermediates (TCAI) during exercise, because of reduced flux through the reaction catalyzed by alanine aminotransferase (AAT; pyruvate + glutamate ↔ 2-oxoglutarate + alanine). Eight women [22 ± 1 yr, peak oxygen uptake (V̇O2 peak) = 40.3 ± 4.6 ml·kg−1·min−1] performed seven 45-min bouts of cycle exercise at 70% V̇O2 peak over 9 days (1 bout/day; rest only on days 2 and 8). During the first and last bouts, biopsies (vastus lateralis) were obtained at rest and after 5 and 45 min of exercise. Muscle glycogen concentration was ~50% higher at rest after STT (493 ± 38 vs. 330 ± 20 mmol/kg dry wt; P ≤ 0.05), and net glycogenolysis and lactate accumulation were reduced after 5 min of exercise by 59 and 49%, respectively (P ≤ 0.05). The net increase in four measured TCAI was ~40% lower (P ≤ 0.05) during exercise after training (1.68 ± 0.60 vs. 2.71 ± 0.44 mmol/kg dry wt), and the net decrease in glutamate concentration was attenuated (P ≤ 0.05). We conclude that 1) the contraction-induced increase in flux through AAT is reduced after 5 days of aerobic training and 2) the muscle glycogenolytic response during exercise after STT in women is similar to that in men.

metabolic regulation; glycogen; lactate; tricarboxylic acid cycle

DURING THE INITIAL MINUTES of a bout of moderate to intense exercise, there is a net increase in the total concentration of tricarboxylic acid (TCA) cycle intermediates (TCAI) in skeletal muscle (9, 27). Expansion of the TCAI pool is attributable to a net increase in carbon flux into the TCA cycle through reactions other than citrate synthase, a phenomenon called “anaplerosis” (23). There are numerous pathways that influence carbon flux into and out of the TCA cycle; however, the near-equilibrium reaction catalyzed by alanine aminotransferase (AAT; pyruvate + glutamate ↔ 2-oxoglutarate + alanine) appears quantitatively most important for the rapid increase in TCAI at the start of exercise (9, 27). The increase in AAT flux appears to be driven by an increase in pyruvate availability, which occurs when the rate of glycolytic flux transiently exceeds flux through the pyruvate dehydrogenase (PDH) reaction. This leads to an increased production of 2-oxoglutarate, which expands the TCAI pool, as the amino group from glutamate is transferred onto pyruvate, forming alanine (2, 9).

The mechanistic explanation for the net increase in muscle TCAI is generally accepted; however, the physiological significance of this phenomenon remains controversial. In 1990, two groups of investigators independently proposed that an increase in TCAI concentration is necessary to optimize aerobic energy provision during exercise (27, 31). This theory remains prevalent in the literature (30), although we are aware of no direct evidence to support it. Indeed, recent studies by one of the present authors (11) and others (4) demonstrated that augmenting the rate of TCAI expansion during the initial phase of exercise did not affect aerobic energy provision. These findings are consistent with an alternative explanation for anaplerosis; namely, changes in TCAI concentration during exercise reflect alterations in carbon flux into and out of the TCA cycle but are not causally related to TCA cycle turnover (8, 10).

It could be argued that a more appropriate method to investigate the physiological significance of anaplerosis would be to attenuate the magnitude of TCAI expansion during exercise and examine the effect on oxidative energy metabolism. Although attempts have been made with pharmacological (12) and nutritional interventions (11), no study has successfully attenuated the expansion of the TCAI pool during exercise. One potential strategy to achieve this goal would be to reduce glycolytic flux during exercise and thus pyruvate availability for the AAT reaction. In this regard, several studies have shown that 3–7 days of aerobic exercise training dramatically reduced muscle glyco-
genolysis and lactate accumulation during submaximal exercise (5, 16). In the present investigation, we used “short-term” training as a model to investigate the physiological significance of reduced TCAI expansion in a group of young women. We hypothesized that 5 days of aerobic training would attenuate muscle TCAI expansion during exercise, because of a better matching between pyruvate production and oxidation; however, this would not compromise muscle oxidative metabolism. In addition, because all comparable short-term training investigations of similar duration examined muscle metabolic adaptations in men (5, 7, 13–16, 26), we sought to determine whether changes in muscle glycogen and lactate metabolism induced by 5 days of aerobic training were similar in women.

METHODS

Subjects. Ten healthy individuals from the McMaster University undergraduate student population took part in the study; however, inadequate muscle biopsy samples were obtained from two subjects, and hence complete data for eight women are described herein. Subject characteristics are presented in Table 1. The women were recreationally active but not specifically trained, and they participated in individual exercise sessions, aerobics, or intramural sports two to three times per week. The experimental procedures and potential risks were explained to the subjects before their participation, and all provided written, informed consent. The project was approved by the McMaster University and Hamilton Health Sciences Research Ethics Board.

Overview of experimental design. Subjects reported to the laboratory at least 3 days before the experiment and performed an incremental exercise test on a cycle ergometer (Excilibur Sport ver. 2.0, Lode, Groningen, The Netherlands) to determine their peak oxygen uptake (V\textsubscript{O\textsubscript{2}}\text{peak}) by using an on-line gas collection system (Moxus modular oxygen uptake system, AEI Technologies, Pittsburgh, PA). The first three stages of the V\textsubscript{O\textsubscript{2}}\text{peak} test consisted of 2-min intervals at 50, 100, and 150 W, respectively, and thereafter workload was increased by 25 W every minute until volitional exhaustion. The value used for V\textsubscript{O\textsubscript{2}}\text{peak} corresponded to the highest value achieved over a 30-s collection period. Subjects were instructed to refrain from exercise or strenuous physical activity on the day before and day of the experiment. They were also advised to consume their habitual diet and to refrain from the consumption of alcohol throughout the duration of the study. All experimental trials were conducted during the morning, ∼2–3 h after the consumption of a light breakfast of the subjects’ choosing. Subjects were instructed to consume the same types and quantities of food during the 24 h before each experimental trial, and all subjects maintained pretrial food diaries. The diaries were subsequently analyzed for nutritional content (Nutritionist 5, ver. 1.7, First DataBank, San Bruno, CA) to confirm that total energy intake and proportion of energy from carbohydrate, fat, and protein were similar before each experimental trial. The women were tested during the early follicular phase of their menstrual cycle, and six of the eight subjects were taking oral contraceptives (OCs).

Experimental protocol. On arrival at the laboratory for the first experimental trial, subjects rested in the supine position, and the area over the lateral aspect of one thigh was prepared for the extraction of needle biopsy samples from the vastus lateralis muscle. After injection of a local anesthetic into the skin and underlying fascia (1% wt/vol lignocaine hydrochloride with epinephrine; Antigen Pharmaceuticals, Roscra, Ireland), three small incisions were made and a resting biopsy sample was obtained. The incision used for the first biopsy was closed with a suture, and the remaining two incisions were covered with sterile gauze and surgical tape. Subjects then mounted an ergometer and cycled at a power output corresponding to ∼70% V\textsubscript{O\textsubscript{2}}\text{peak} (163 ± 24 W) for 45 min. Needle biopsy samples were obtained after 5 and 45 min of exercise. Blood samples and cardiorespiratory measurements were obtained during the 20- to 25-min period of each work bout. On completion of the exercise bout, the remaining two incisions were closed with sutures, and the subjects were instructed to refrain from physical activity for the rest of the experimental day and the following day (day 2). They reported back to the laboratory on the third day and performed the first of five consecutive exercise bouts over 5 days, i.e., the short-term training regime. Each bout of exercise on days 3–7 consisted of 45 min of cycling at 70% V\textsubscript{O\textsubscript{2}}\text{peak}, and all training sessions were supervised by one of the investigators. Subjects were instructed to refrain from exercise on day 8, and then they reported back to the laboratory during the morning of day 9 for the second experimental exercise trial, which was performed at the same absolute work intensity as the first trial. Biopsy samples and cardiorespiratory measurements were obtained as described for the first trial, except that the opposite leg was used for muscle sampling. Two days after the second experimental exercise trial, the subjects reported back to the laboratory and performed a second V\textsubscript{O\textsubscript{2}}\text{peak} test using procedures identical to those employed during the pretraining V\textsubscript{O\textsubscript{2}}\text{peak} test.

Muscle analyses. Each biopsy sample was immediately frozen by plunging the needle into liquid nitrogen, and the samples were removed from the needle while still frozen. All resting samples were subsequently divided into two pieces. One piece from each resting sample was homogenized and analyzed for the maximal activities of citrate synthase and AAT (19) with an Ultrospec 3000 pro UV/Vis spectrophotometer. The remainder of the resting samples, and all exercise samples, were subsequently freeze-dried, powdered to dissect out nonmuscle elements, and stored at −86°C. Aliquots of powdered muscle were extracted and assayed for glycogen, lactate, phosphocreatine, citrate, isocitrate, malate, fumarate, alanine, and glutamate with enzymatic methods (1, 18, 25) adapted for fluorometry (Hitachi model F-2500, Hitachi Instruments, Tokyo, Japan). To correct for differences in blood or connective tissue between samples, muscle metabolites were corrected to the highest total creatine value obtained in all biopsy samples from a given subject.

Statistical analyses. All muscle metabolite data were analyzed by using a two-factor (time × condition) repeated-measures ANOVA. Mean cardiorespiratory data and net changes in muscle metabolites from rest to 5 min of exercise, including the sum total of the four measured TCAI (ΣTCAI), were analyzed by using paired t-tests. Statistical significance

Table 1. Subject characteristics before and after training

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretraining</th>
<th>Posttraining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>21.5 ± 0.8</td>
<td>21.0 ± 0.8</td>
</tr>
<tr>
<td>Mass, kg</td>
<td>67.6 ± 6.3</td>
<td>67.3 ± 6.2</td>
</tr>
<tr>
<td>V\textsubscript{O\textsubscript{2}}\text{peak} l/min</td>
<td>2.72 ± 0.3</td>
<td>2.79 ± 0.3</td>
</tr>
<tr>
<td>ml·kg\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>40.3 ± 4.6</td>
<td>41.5 ± 4.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 women.
changes in glutamate were significant by 40 and 25% respectively, posttraining, but only concentration (Table 2) during exercise were attenuated in muscle glutamate concentration (Fig. 4) and alanine malate concentration, which accounted for expansion was primarily attributable to changes in concentration of TCAI was indeed lower after exercising pyruvate through the AAT reaction. There was no significant difference between trials in the individual concentrations of $\Sigma$TCAI; however, the net increase in the sum total of the TCAI was indeed lower after training (Fig. 3). Consistent with our hypothesis and as discussed further below, muscle glycogenolysis and lactate accumulation were reduced by ~50% during exercise, which suggests there was a reduced rate of pyruvate formation, and the net increase in muscle glutamate was attenuated by ~40%, which is indicative of a reduced flux through AAT. Although the maximal activity of AAT was unchanged after training, the analytic method employed does not permit the determination of “active” enzyme activity at the time of tissue

Table 2. Cardiorespiratory data during exercise before and after training

<table>
<thead>
<tr>
<th></th>
<th>Pretraining</th>
<th>Posttraining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>177 ± 4</td>
<td>171 ± 4</td>
</tr>
<tr>
<td>Oxygen uptake, l/min</td>
<td>1.98 ± 0.1</td>
<td>1.97 ± 0.1</td>
</tr>
<tr>
<td>Expired ventilation, l/min</td>
<td>71.9 ± 7.9</td>
<td>67.6 ± 8.1</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>0.92 ± 0.02</td>
<td>0.91 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 women. Data were collected and averaged over the 20- to 25-min period of exercise.

DISCUSSION

The two major, novel findings from the present work were the following: 1) the net increase in the muscle TCAI pool during moderate-intensity exercise was reduced after 5 days of aerobic training, which is consistent with reduced flux through the AAT reaction; and 2) women responded in a similar manner to that previously shown for men with respect to resting and exercise-stimulated changes in muscle glycogen and lactate concentrations after short-term training.

Effect of short-term training on muscle TCAI. We hypothesized that the balance between pyruvate production and oxidation would be improved after short-term training, and this would attenuate the exercise-induced expansion of the muscle TCAI pool by decreasing pyruvate flux through the AAT reaction. There was no significant difference between trials in the individual concentrations of $\Sigma$TCAI; however, the net increase in the sum total of the TCAI was indeed lower after training (Fig. 3). Consistent with our hypothesis and as discussed further below, muscle glycogenolysis and lactate accumulation were reduced by ~50% during exercise, which suggests there was a reduced rate of pyruvate formation, and the net increase in muscle glutamate was attenuated by ~40%, which is indicative of a reduced flux through AAT. Although the maximal activity of AAT was unchanged after training, the analytic method employed does not permit the determination of “active” enzyme activity at the time of tissue.
harvesting. We also cannot discount the possibility that an increased rate of pyruvate oxidation through the PDH reaction may have contributed to the reduction in TCAI expansion. A previous study, however, showed that the active fraction of PDH was not different during exercise after short-term training (26).

Two previous studies showed that administration of the pharmacological agent dichloroacetate reduced the concentrations of TCAI in resting human muscle (8, 12), presumably by increasing PDH activity and the oxidative disposal of pyruvate, which diverted substrate away from anaerobic pathways. However, this is the first study that has successfully attenuated the expansion of the muscle TCAI pool during exercise. Despite the blunted increase in TCAI concentration, aerobic energy provision was not compromised, as evidenced by the similar rate of phosphocreatine degradation during exercise after training. This finding appears at odds with the hypothesis that links changes in muscle TCAI during exercise primarily reflect changes in substrate availability for the AAT reaction and are not directly related to TCA cycle turnover.

Our laboratory (10) and others (8) have previously observed that the TCA cycle appears to operate as two distinct "spans" during exercise, and, on contraction, intermediates in the "second" span of the cycle (particularly malate) increase disproportionately. Consistent with this phenomenon, the blunted TCAI concentration response during exercise in the present study was primarily due to an attenuated rise in malate concentration. A notable discrepancy in the present study was that the resting concentration of malate was approximately twofold higher than values our laboratory has previously reported in men (9–12). To our knowledge, no study has specifically investigated potential gender differences in muscle TCAI metabolism, and we suspect that the discrepancy might simply reflect individual variability. We did not perform muscle fiber-type analyses, but Hintz et al. (20) showed that the resting concentration of malate varied by sevenfold in various fiber groups from the rat hindlimb, with higher values measured in more oxidative fibers. Although specula-

Table 3. Muscle metabolites at rest and during exercise before and after training

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Pretraining Rest</th>
<th>5 min</th>
<th>45 min</th>
<th>Posttraining Rest</th>
<th>5 min</th>
<th>45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate*</td>
<td>0.58 ± 0.04</td>
<td>0.67 ± 0.04</td>
<td>1.09 ± 0.08</td>
<td>0.79 ± 0.06</td>
<td>0.91 ± 0.11</td>
<td>1.20 ± 0.11</td>
</tr>
<tr>
<td>Isocitrate*</td>
<td>0.14 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.18 ± 0.03</td>
<td>0.14 ± 0.02</td>
<td>0.21 ± 0.02</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Malate†</td>
<td>1.01 ± 0.14</td>
<td>2.96 ± 0.24</td>
<td>1.17 ± 0.12</td>
<td>1.06 ± 0.10</td>
<td>2.18 ± 0.34</td>
<td>1.41 ± 0.11</td>
</tr>
<tr>
<td>Fumarate</td>
<td>0.21 ± 0.04</td>
<td>0.83 ± 0.08</td>
<td>0.30 ± 0.03</td>
<td>0.34 ± 0.03</td>
<td>0.73 ± 0.12</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td>Glutamate*</td>
<td>9.7 ± 0.6</td>
<td>4.6 ± 0.3</td>
<td>5.5 ± 0.3</td>
<td>9.8 ± 0.3</td>
<td>6.8 ± 0.3</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>Alanine*</td>
<td>8.0 ± 0.5</td>
<td>12.2 ± 0.3</td>
<td>11.3 ± 0.4</td>
<td>8.1 ± 0.3</td>
<td>11.3 ± 0.8</td>
<td>11.9 ± 0.5</td>
</tr>
<tr>
<td>PCr*</td>
<td>77.4 ± 6.0</td>
<td>43.9 ± 3.5</td>
<td>53.3 ± 1.8</td>
<td>77.2 ± 8.2</td>
<td>48.6 ± 5.1</td>
<td>56.9 ± 5.6</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry wt; n = 8 women. PCr, phosphocreatine. *Significantly different after 5 and 45 min of exercise compared with rest (main effect for time, P ≤ 0.05). †Significantly different after 5 min of exercise compared with rest (main effect for time, P ≤ 0.05). There were no significant interactions between trials for any metabolite.

Fig. 3. Net increase (Δ) in the total concentration of 4 measured tricarboxylic acid cycle intermediates (TCAI; citrate, isocitrate, fumarate, and malate) from rest to 5 min of exercise before and after 5 days of aerobic training. Values are means ± SE. *P ≤ 0.05 vs. pretraining.

Fig. 4. Net increase in the muscle glutamate concentration from rest to 5 min of exercise before and after 5 days of aerobic training. Values are means ± SE. *P ≤ 0.05 vs. pretraining.
tive, is also possible that a heightened sense of anxiety or nervousness by our subjects before the biopsy procedures may have influenced the resting concentrations of TCAI. We did not measure blood catecholamine concentrations, but epinephrine infusion has previously been shown to increase the resting intramuscular concentration of malate in humans, presumably by stimulating glycogenolytic rate and flux through AAT (28). Irrespective of the absolute resting concentration of malate, which was similar between trials at rest, the net increase in TCAI concentration was attenuated posttraining, and this was largely attributable to a reduction in malate concentration. Moreover, a subsequent study from our laboratory, which has appeared in abstract form (22), confirmed that the exercise-induced expansion of the muscle TCAI pool was attenuated after a more extensive 7-wk aerobic training program, and this was primarily due to a blunted rise in malate concentration.

Muscle glycogen and lactate concentrations after short-term training in women. Several investigations have examined the effect of 3–7 consecutive days of aerobic training at an intensity equivalent to 60–70% $\dot{V}O_2_{\text{peak}}$ for 2 h each day on muscle metabolic adaptations in men (5, 7, 13–16, 26). The present study examined metabolic adaptations in women in response to five consecutive bouts of training at a similar work intensity (70% $\dot{V}O_2_{\text{peak}}$) but shorter bout duration (45 min/day). Consistent with all but one of the studies cited above (16), we observed a significant effect of training on muscle glycogen content, such that glycogen concentration was significantly increased at rest and remained higher during exercise compared with pretraining values. The overall magnitude of increase in resting glycogen concentration in our study (50%) was higher than previously reported for men (average increase: ~20%; range: 13–35%) (5, 7, 13–15, 26). Also consistent with several previous studies that sampled muscle during the initial few minutes of exercise after short-term training (7, 13–16), we observed a marked decrease in muscle glycogenolysis and lactate accumulation after 5 min of exercise posttraining. The potential mechanisms responsible for the blunted glycogenolytic and glycolytic responses after short-term training have been previously discussed (13–16) and appear to involve reductions in the putative modulators of glycogen phosphorylase and phosphofructokinase (i.e., free ADP, free AMP, and Pi). The present study, therefore, confirms that healthy, recreationally active young women respond in a manner similar to men with respect to resting and exercise-induced changes in muscle glycogen and lactate concentrations after several consecutive days of aerobic training.

It should be noted that our subjects commenced the 9-day experimental protocol during the early follicular phase of the menstrual cycle, and six of the eight women were taking OCs. However, data regarding the effect of both menstrual cycle phase (6, 21) and OC use (3, 29) on substrate metabolism during exercise are equivocal and inconsistent. For example, two recent, carefully controlled studies that employed stable-isotopoe tracer techniques to examine the effect of menstrual cycle phase on substrate utilization during moderate-intensity exercise produced conflicting results: Campbell et al. (6) reported slightly higher rates of carbohydrate oxidation during the follicular compared with the luteal phase, whereas Horton et al. (21) reported no effect of menstrual cycle phase on glucose kinetics or whole body fuel oxidation. Similarly, studies that have directly examined the effect of menstrual cycle phase on muscle glycogen utilization during exercise have yielded equivocal data (17, 24). Thus, although we cannot rule out the possibility that fluctuations in the sex steroid environment may have influenced substrate utilization in the present study, it seems unlikely that hormonal changes alone would have produced the dramatic reductions in muscle glycogenolysis and lactate accumulation that were observed posttraining. With respect to OC use, although there may have been subtle differences in the individual responses between the users and nonusers in the present study, each subject served as her own control. Thus our experimental design controlled for between-subject variability, and we nonetheless detected a significant treatment effect.

Conclusion. In summary, the present study showed that the contraction-induced increase in muscle TCAI concentration was lower after 5 days of aerobic training, and this was likely due in part to a lower net flux through AAT during the rest-work transition. However, the attenuated rate of TCAI expansion did not appear to compromise aerobic energy delivery, since there was no difference between trials in phosphocreatine degradation during exercise, and subjects were able to maintain the same power output posttraining despite a 40% decrease in the TCAI pool. Although this is the first study to successfully attenuate muscle TCAI expansion during exercise, our findings are consistent with several recent reports that showed a dissociation between TCAI pool size and indirect markers of mitochondrial respiration (4, 11). Collectively, these data argue against the hypothesis that links changes in TCAI during exercise with the capacity for oxidative metabolism (27, 31). Finally, in addition to changes in TCAI metabolism, we also observed that resting muscle glycogen concentration was increased after training and that net glycogenolysis and lactate accumulation during exercise were reduced. We conclude that young healthy women demonstrate rapid shifts in carbohydrate metabolism after short-term exercise training, similar to those previously reported for men (13–16).

We thank our subjects for their time and effort.

DISCLOSURES

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REFERENCES

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