Endurance training attenuates the decrease in skeletal muscle malonyl-CoA with exercise

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Endurance training attenuates the decrease in skeletal muscle malonyl-CoA with exercise. J. Appl. Physiol. 83(6): 1917–1922, 1997.—Muscle malonyl-CoA has been postulated to regulate fatty acid metabolism by inhibiting carnitine palmitoyltransferase 1. In nontrained rats, malonyl-CoA decreases in working muscle during exercise. Endurance training is known to increase a muscle’s reliance on fatty acids as a substrate. This study was designed to investigate whether the decline in malonyl-CoA with exercise would be greater in trained than in nontrained muscle, thereby allowing increased fatty acid oxidation. After 6–10 wk of endurance training (2 h/day) or treadmill habituation (5–10 min/day), rats were killed at rest or after running up a 15% grade at 21 m/min for 5, 20, or 60 min. Training attenuated the exercise-induced drop in malonyl-CoA and prevented the exercise-induced increase in the constant for citrate activation of acetyl-CoA carboxylase in the red quadriceps muscle of rats run for 20 and 60 min. Hence, contrary to expectations, the decrease in malonyl-CoA was less in trained than in nontrained muscle during a single bout of prolonged submaximal exercise.


Materials and Methods

Animal care. Male Sprague-Dawley rats (Sasco, Omaha, NE) were housed in a temperature-controlled environment (19–21°C) with a 12:12-h light-dark cycle (lights on from 7 AM to 7 PM) and were provided rat chow (Teklad rodent diet, Harlan, Madison, WI) as described below and water ad libitum.

Training, diet, and exercise protocols. Animals were randomly assigned to a training or a nontraining group. The rats in the training group were run for 100–120 min each day (60 min in the morning and 40–60 min in the late afternoon/early evening) 5 days/wk on a rodent treadmill up a 15% grade. The initial training speed was set at 21 m/min and was gradually increased to 27 m/min over a 2-wk period. Training then continued at 27 m/min for another 4–8 wk. The nontraining group was habituated to treadmill running by exercising for 5 min/day, 5 days/wk, at the same gradient and speed as the training group. During the training period, trained animals were allowed to feed ad libitum and nontrained animals were provided 20 g of rat chow per day. At the end of the training period, 3 days before the testing protocol, all animals were run for 1 h (trained) or 5 min (nontrained) and a jugular catheter was installed (under ether anesthesia). The trained rats were run for 2 h and the nontrained rats were run for 5 min on the day after catheterization and were then allowed to rest for 48 h before the test protocol. All animals were fed 20 g of rat chow per day for the 48 h after catheterization and 25 g of rat chow for the 24 h before the testing protocol. On the day of the testing protocol, rats from the trained and nontrained groups were paired by weight as closely as possible (trained rats weighed 316 ± 3 g and nontrained rats weighed 305 ± 4 g) and were anesthetized via the catheter at rest or after running on the treadmill at 21 m/min for 5, 20, or 60 min up a 15% grade. After the animals were anesthetized, the red portion of the left and right quadriceps muscles was rapidly excised (−400 mg from each muscle), clamped in aluminum tongs (precooled in liquid nitrogen), and stored under liquid nitrogen until assayed for the various metabolite concentrations and enzyme activities. The right gastrocnemius muscle and a lobe of the liver were also excised, clamp frozen, and stored at −75 and −95°C, respectively, until assayed for glycogen. A blood sample was withdrawn from the abdominal aorta, an aliquot was heparinized to collect plasma for determination of free fatty acids, and a perchloric acid extract was prepared for measurement of glucose and lactate concentrations. Both blood samples were kept at −20°C until the relevant assay was performed.

Muscle and blood assays. Muscles were ground to powder under liquid nitrogen just before analysis. Neutralized perchloric acid extracts of the muscles were used for determination of malonyl-CoA (19). Fatty acid synthase for malonyl-CoA assays was isolated from livers of fasted-refed rats as described
by Linn (see Ref. 32). ACC activities were determined using ammonium sulfate precipitates from homogenates prepared from the ground muscles, as previously described (33). Muscle citrate synthase activity was measured according to Srere (26). Muscle and liver glycogen were determined by the anthrone method (11). Blood glucose (2) and blood lactate (10) were determined on neutralized perchloric acid extracts, and plasma free fatty acids were determined as described by Novak (21).

Values are means ± SE. Statistical differences (P < 0.05) between treatment groups were determined using analysis of variance and Fisher’s least significant difference (as a post hoc test) or a t-test, where appropriate.

RESULTS

Rats assigned to the training group ran with various degrees of competence. Inasmuch as we were interested in comparing responses of well-trained rats with nontrained rats, data from rats in the “trained group” that demonstrated <30% increase in red quadriceps citrate synthase activity (marker of degree of training) are not included. If data from a “trained” rat were discarded for this reason, data from the paired nontrained rat killed at the same time point were also eliminated. The citrate synthase activity in the red portion of the quadriceps muscle was 66.2 ± 2.2 and 39.1 ± 1.7 (SE) µmol·g⁻¹·min⁻¹ for the trained and nontrained groups, respectively. Malonyl-CoA decreased during the 60 min of running in the nontrained and trained animals (P < 0.05) and was significantly lower (P < 0.05) in the nontrained rats at 20 and 60 min of running (Fig. 1).

Table 1 and Fig. 2 show the effect of running on the citrate dependence of ACC that had been precipitated from muscle homogenates with ammonium sulfate. The Vmax was lower for all exercised groups than for the nonexercised group (P < 0.05) in the nontrained and trained animals. The K0.5 for citrate activation tended to increase (P = 0.06) in the nontrained animals by 5 min of running and continued to remain higher (P < 0.05) in rats run for 20 and 60 min than in nonexercised, nontrained animals. Training lowered (P < 0.05) the K0.5 in muscles from rats run for 20 and 60 min and tended to decrease the K0.5 (P = 0.07) in muscles from rats run for 5 min compared with the nontrained rats.

The activation constant calculated from the Hill equation (Ks) demonstrated a pattern similar to the K0.5, showing an exercise-induced increase (P < 0.05) in the nontrained rats but not in the trained rats. The Ks values for citrate were significantly lower in the trained than in the nontrained rats after 20 and 60 min of exercise. The difference between the trained and nontrained ACC activity at 0.2 mM citrate, which is in the physiological range, was especially pronounced (Fig. 3). All trained, exercised groups showed significantly greater ACC activity at 0.2 mM citrate than did their nontrained counterparts (P < 0.05).

Figure 4 shows the change in muscle and liver glycogen concentration with exercise and due to training. The glycogen content of the red quadriceps muscle was significantly greater (P < 0.05) before exercise in the trained state than in the nontrained state. The rate of utilization of glycogen in this muscle was similar in trained and nontrained rats: 29 and 32 µmol glucose units/g, respectively. The gastrocnemius muscle showed less depletion of glycogen with training: 18 and 28 µmol/g for trained and nontrained groups, respectively.

Liver glycogen utilization in trained rats was markedly different from that in nontrained rats (Fig. 4). In trained rats, liver glycogen was 454 ± 49 and 288 ± 47 µmol glucose units/g at rest and after 60 min of running, respectively. In nontrained rats, corresponding values were 384 ± 28 and 82 ± 22 µmol glucose units/g. Average liver weights for rats killed at these same time points were 12.0 ± 0.4 and 11.7 ± 0.5 g for trained and 12.0 ± 0.5 and 10.2 ± 0.4 g for nontrained rats. The total liver glycogen utilized during the 60-min bout of exercise was 2,079 and 3,772 µmol glucose units for the trained and nontrained rats, respectively. This represents a 45% reduction in the amount of liver glycogen utilized in trained rats compared with the nontrained rats during exercise.

Blood glucose did not change significantly (P > 0.05) with exercise in the nontrained or trained group (Fig. 5). Blood lactate was significantly lower (P < 0.05) in the trained animals that had been run for 60 min than in their nontrained counterparts (Fig. 5). Free fatty acids in the plasma increased (P < 0.05) with exercise for the nontrained group and tended to increase (P = 0.08) by 60 min of running in the trained group.

Table 1. Effect of treadmill running on ACC Vmax and K0.5 for citrate in red quadriceps muscle of trained and nontrained rats

<table>
<thead>
<tr>
<th>Rest</th>
<th>Exercise 5 min</th>
<th>Exercise 20 min</th>
<th>Exercise 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>T</td>
<td>NT</td>
<td>T</td>
</tr>
<tr>
<td>Vmax</td>
<td>3.76 ± 0.25</td>
<td>4.04 ± 0.27</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>K0.5</td>
<td>2.4 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>2.5 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE from 5-7 rats. ACC, acetyl-CoA carboxylase; NT, nontrained; T, trained; Vmax, maximum velocity. *Significantly different from resting group (P < 0.05). †Significantly different from nontrained muscle (P < 0.05).
DISCUSSION

This study confirms earlier studies on nontrained rats (32, 33), in which malonyl-CoA has been demonstrated to decrease in working muscle during exercise. To our knowledge, this is the first report demonstrating a decrease in malonyl-CoA in working muscle of endurance-trained rats during a bout of treadmill exercise. We hypothesize that this decrease in malonyl-CoA is important for relieving inhibition of CPT 1 and allowing increased rates of fatty acid oxidation during exercise in trained and nontrained rats.

Previous studies have clearly demonstrated that endurance-trained human subjects obtain a greater proportion of their energy requirements from fat oxidation during exercise than do nontrained subjects working at the same absolute submaximal work rate (6, 14, 16, 17, 29). Trained and nontrained rats in this study were working at the same absolute work rate. Although we did not measure oxygen consumption, rats of this strain have been reported to utilize 70 ml oxygen/kg body wt when running at this grade and speed (1). If we assume a maximal oxygen consumption of 120 ml·kg\(^{-1}\)·min\(^{-1}\) (1), the nontrained rats were working at \(-58\%\) of maximal oxygen consumption. The trained rats were likely working at a level somewhat \(<58\%\) of maximal oxygen consumption. In RESULTS, we calculated the total liver glycogen utilization during the 60-min exercise bout to be 3,772 µmol glucose units for the nontrained rats and 2,079 µmol glucose units for the trained rats. The amount of oxygen required to oxidize these amounts of glycogen completely to carbon dioxide is 506 ml for the nontrained and 269 ml for the trained rats. If it is assumed the oxygen cost is 21 ml/min for these rats running at this speed, the total oxygen cost for 60 min of exercise would be 1,260 ml. Thus the oxygen requirement for oxidizing glucose derived from liver glycogen in the nontrained rats represents 40% of the total oxygen cost. The corresponding value in the trained rats is 21%. The remaining oxygen is utilized principally to oxidize glucose units derived from muscle glycogen, glucose produced by gluconeogenesis, and fatty acids. Much of the gluconeogenic substrate is derived from glycogen. Lactic acid produced by muscles must ultimately come from muscle or liver glycogen or from dietary carbohydrate. The difference in the amount of lactate in the extracellular fluid volume between trained and nontrained rats represents a relatively minor fraction of the total carbohydrate derived from liver and muscle glycogen. Although lactate is continually being produced during exercise, it is also being utilized for gluconeogenesis in the liver and is oxidized by the active muscle fibers (27).

Because glycogen utilization appears also to be less in the mixed-fiber muscles of the trained rats, it is logical to conclude that the deficit in liver and muscle glycogen utilization must be met principally by fatty acid oxidation.

The purpose of this experiment was to determine whether endurance training alters the malonyl-CoA response to exercise. Malonyl-CoA is an inhibitor of CPT 1, the enzyme responsible for transfer of long-chain fatty acyl-CoA to the mitochondria for oxidation
We hypothesized that endurance training would result in a greater decline in muscle malonyl-CoA during submaximal exercise, thereby allowing an increased rate of oxidation of fatty acids as a source of energy. As can be seen from Fig. 1, however, this hypothesis proved to be incorrect. The muscle malonyl-CoA content was significantly higher after 20 and 60 min of exercise in trained rats than in nontrained rats running at the same treadmill speed and grade. We inferred from liver and muscle glycogen data that the rate of carbohydrate utilization was decreased in the trained rats and that increased fat oxidation compensated for the diminished glycogen utilization. These facts together lead us to conclude that the higher rate of fat oxidation during exercise in response to endurance training cannot be attributed to a lower malonyl-CoA content of the trained muscle.

The mechanism of the training-induced enhancement of fatty acid oxidation during submaximal exercise may thus be related more closely to the increase in muscle mitochondrial oxidative enzymes (12, 13, 20, 25) or perhaps to increased availability of long-chain acyl-CoA to mitochondria of the working muscle. In the present study we noted an ~70% increase in citrate synthase in the red region of the quadriceps muscle in response to the training program. Previous studies have clearly demonstrated that endurance training induces an increase in palmitoyl-CoA synthase, an increase in total CPT, and an increase in capacity of mitochondrial preparations to oxidize fatty acids (20). With this increase in mitochondria, the fatty acid oxidation rate at any fixed malonyl-CoA concentration would be expected to be higher in the trained than in the nontrained muscle, all other regulatory factors being equal. With the malonyl-CoA concentration higher in the trained than in the nontrained muscle during exercise, it is more difficult to predict the effect on the rate of fatty acid oxidation. It depends somewhat on the nature of the malonyl-CoA inhibition curve (plot of malonyl-CoA vs. CPT 1 activity) in trained vs. nontrained rats. Inasmuch as we do not know the characteristics of this inhibition curve under in vivo conditions in working muscle, we cannot come to any certain conclusion regarding the effect of the higher malonyl-CoA in the trained rats. Possible differences between trained and nontrained rats in compartmentalization of the total muscle malonyl-CoA must also be considered. It is conceivable that in the trained muscle a greater propor-

![Fig. 4. Effect of treadmill running on glycogen contents of red quadriceps, gastrocnemius muscle, and liver in trained and nontrained rats. Values are means ± SE from 5–7 rats/group. *Significantly different from resting muscle (P < 0.05). **Significantly higher than nontrained muscle at same time point (P < 0.05).](#)

![Fig. 5. Effect of treadmill running on blood glucose, blood lactate, and plasma free fatty acids (FFA) in trained and nontrained rats. Values are means ± SE from 5–7 rats/group. *Significantly different from resting value (P < 0.05). **Significantly different from nontrained value at same time point (P < 0.05).](#)
tion of the total muscle malonyl-CoA is bound to protein or is not in the compartment exposed to the malonyl-CoA-sensitive site of CPT 1. If, for example, a fraction of the total malonyl-CoA is located inside the mitochondria, the trained muscle, having more mitochondria than nontrained muscle, would be expected to have lower free cytosolic malonyl-CoA concentrations, given the same total muscle malonyl-CoA content.

Previous studies have provided information regarding the mechanism of the decline in muscle malonyl-CoA during exercise. Purified muscle ACC can be phosphorylated in vitro by AMP-activated protein kinase (AMPK) and by adenosine 3',5'-cyclic monophosphate-dependent protein kinase (PKA) (33, 34). Phosphorylation by PKA has no detectable effect on the activity of muscle ACC. Phosphorylation by AMPK results in an increase in $K_a$ for citrate and increases in the Michaelis constants for the substrates acetyl-CoA, ATP, and bicarbonate. The net effect of these changes in kinetic properties is a decrease in the activity of ACC (i.e., lower rate of malonyl-CoA synthesis), particularly at citrate concentrations found in the muscle. Within 5 min of the beginning of treadmill exercise, AMPK activity increases and ACC activity decreases concurrently with the decline in malonyl-CoA content of the working muscle of rats (33). In situ stimulation of rat gastrocnemius muscle at a frequency of 1/s causes an increase in estimated free AMP, a decrease in ACC, and a decrease in malonyl-CoA (14). The postulated sequence of events is as follows: 1) The increase in 5'-AMP associated with muscle contraction activates AMPK kinase. It is also conceivable that the rise in free calcium associated with contraction may activate a calcium/calmodulin-dependent kinase. 2) AMPK kinase phosphorylates and activates AMPK. The rise in 5'-AMP also allosterically activates AMPK. 3) AMPK phosphorylates and inactivates ACC. 4) The consequent decrease in malonyl-CoA relieves inhibition of CPT 1 and allows an increased rate of fatty acid oxidation as fatty acids become available (33).

Previous studies have demonstrated less increase in 5'-AMP during contraction in endurance-trained than in nontrained muscle in rats (7, 9). This may be the reason for the attenuation of the decline in muscle ACC activity during exercise in the endurance-trained rats of the present study. With a lower 5'-AMP concentration in the trained muscle, we would expect less activation of AMPK. With less phosphorylation of ACC by AMPK, the $K_a$ for citrate would be expected to be lower. At physiological citrate concentrations, this means that malonyl-CoA would be synthesized at a more rapid rate in the trained muscle. The results on ACC activity at 0.2 mM citrate and muscle malonyl-CoA appear to be consistent with this interpretation.

The $V_{max}$ for muscle ACC activity is also decreased during exercise. The fact that the exercise-induced increase in $K_a$ for citrate was attenuated in the endurance-trained rats but the exercise-induced decrease in $V_{max}$ was not implies that changes in these two kinetic characteristics of muscle ACC may be controlled by different kinases. It is clear from previous studies that $K_a$ is definitely increased by phosphorylation of muscle ACC by AMPK at one or more phosphorylation sites (33, 34). It is possible that the $V_{max}$ of ACC may be controlled by phosphorylation by a calcium/calmodulin kinase at another site. The rise in sarcoplasmic free calcium would be expected to be similar in trained and nontrained rats, but the increase in 5'-AMP would be predicted to be less in the trained rats. A second calcium/calmodulin-sensitive ACC kinase has not been identified.

As noted in MATERIALS AND METHODS, it was necessary to restrict food intake of the nontrained rats to maintain body weights near those of the training rats. During the final exercise test, it is essential that body weights be similar to have rats in each treatment group running at the same submaximal work rate. It is possible that this chronic food restriction resulted in adaptations that would accentuate the malonyl-CoA response to exercise in the nontrained rats. We have, however, observed similar decreases in malonyl-CoA in red quadriceps of nontrained rats that were not food restricted (33). These rats were larger but were run at the same speed and grade for up to 30 min.

In summary, endurance training led to an attenuation of the fall in muscle malonyl-CoA and to attenuation of the increase in $K_a$ for citrate of muscle ACC during submaximal bouts of treadmill exercise in rats. Liver and gastrocnemius muscle glycogen utilization during exercise was also less in trained than in nontrained rats during 1 h of treadmill exercise. We conclude that the decrease in carbohydrate utilization (with likely increased fat utilization) in trained rats during exercise is not due to a lower muscle malonyl-CoA and is more likely due to the previously reported increase in the capacity of muscle to oxidize fatty acids.

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