Postexercise recovery of skeletal muscle malonyl-CoA, acetyl-CoA carboxylase, and AMP-activated protein kinase

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Rasmussen, B. B., C. R. Hancock, and W. W. Winder. Postexercise recovery of skeletal muscle malonyl-CoA, acetyl-CoA carboxylase, and AMP-activated protein kinase. J. Appl. Physiol. 85(5): 1629–1634. 1998.—Previous studies have demonstrated that oxygen consumption and fat oxidation remain elevated in the postexercise period. The purpose of this study was to determine whether malonyl-CoA, an inhibitor of fatty acid oxidation, remains depressed in muscle after exercise. Rats were sprinted for 5 min (40 m/min, 5% grade) or run for 30 min (21 m/min, 15% grade). Red quadriceps malonyl-CoA returned to resting values by 90 min postexercise in the sprinting rats and remained significantly lower at least 90 min postexercise in the 30-min exercise group. AMP-activated protein kinase activity remained significantly elevated (P < 0.05) for 10 min after exercise in both groups. The most rapid rate of glycogen repletion was in the first 30 min postexercise. The respiratory exchange ratio decreased from a nonexercise value of 0.87 ± 0.01 to an average 0.82 ± 0.01 during the 90-min period after 30 min of exercise. Thus muscle malonyl-CoA remains depressed and fat oxidation is elevated for relatively prolonged periods after a single bout of exercise. This may allow fat oxidation to contribute more to muscle energy requirements, thus leaving more glucose for replenishment of muscle glycogen.

CARNITINE PALMITOYL-TRANSFERASE-1 (CPT-1) is the enzyme required to move long-chain fatty acyl-CoA molecules from the cytoplasmic to the intramitochondrial compartment where oxidation can occur (15). Malonyl-CoA, an inhibitor of CPT-1, has been demonstrated to decrease in muscle in response to exercise (21, 30–32) and in response to electrical stimulation (14, 20, 29). The decrease in malonyl-CoA is accompanied by increases in AMP-activated protein kinase (AMPK) activity and by decreases in acetyl-CoA carboxylase (ACC) activity (14, 29, 32). This mechanism has been postulated to couple muscle contraction with an increase in the rate of fatty acid oxidation by the muscle during exercise. The following sequence of events has been proposed (30, 32). 1) Muscle contraction increases 5'-AMP concentration in the muscle. 2) The increase in 5'-AMP activates AMPK allosterically and also activates AMPK kinase. 3) The AMPK kinase phosphorylates AMPK, resulting in further activation. 4) AMPK phosphorylates ACC, resulting in a decrease activity. 5) The decrease in ACC results in a decrease in malonyl-CoA, which decreases inhibition of CPT-1, allowing the rate of oxidation of long-chain fatty acids to increase. Credence to this postulated sequence was recently gained by using the rat perfused hindlimb preparation (17). When AMPK is activated by the 5'-AMP analog, 5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranosyl 5'-monophosphate (ZMP), the consequent decrease in ACC activity and malonyl-CoA is accompanied by an increase in the rate of palmitate oxidation. This is in agreement with previous studies on liver and heart that have shown an inverse correlation of malonyl-CoA content and fatty acid oxidation rate (1, 15, 23).

At submaximal work rates, the absolute rate of fatty acid oxidation increases during exercise (11, 22, 28). In human subjects and in rats exercised for prolonged time periods, fatty acid oxidation remains elevated above resting levels in the postexercise period (4, 33). We hypothesized that if muscle malonyl-CoA concentration does not recover rapidly after exercise, CPT-1 would remain active, allowing fatty acids to be utilized as an energy source during the time when muscle glycogen is being replenished. The purpose of this study was to determine the time course of malonyl-CoA, ACC activity, and AMPK activity in skeletal muscle after exercise.

MATERIALS AND METHODS

Animal care. Male Sprague-Dawley rats (Sasco, Omaha, NE) were housed in individual cages at a temperature of 19–21°C in a light-controlled room (12:12-h light-dark cycle) and were fed a normal diet (Harlan Teklad rodent diet, Madison, WI) and water ad libitum. Rats were run on a rodent treadmill (20–40 m/min) up a 5–15% grade (5–10 min/day) for 5–10 days to acclimatize them to running on the treadmill and to handling procedures. While rats were anesthetized with ether, jugular catheters were implanted 3–4 days before the final exercise test. The rats weighed 357 ± 3 g when they were killed at the end of the experiment.

Exercise test. Rats were anesthetized by injection of pentobarbital sodium via the catheter at rest or after running for 5 min on the treadmill at 40 m/min up a 5% grade (sprinting group) or running for 30 min at 21 m/min up a 15% grade (submaximal group). Prior studies have demonstrated that malonyl-CoA decreases significantly after sprinting for 5 min (21) and after submaximal endurance exercise of 30 min (31, 32). At 90–180 s after rats were anesthetized, the red region of the quadriceps was removed rapidly from both hindlimbs and was frozen between stainless steel block tongs at liquid nitrogen temperature. Blood was removed via the abdominal aorta. An aliquot was heparinized for collection of plasma to be used for determination of free fatty acids (FFA) (19). A perchloric acid extract of blood was made for measurement of glucose (3) and lactate (7).

Muscle assays. Muscle samples were kept in liquid nitrogen until they were analyzed. Red quadriceps muscles were ground to a powder while they were in liquid nitrogen. For ACC and AMPK assays, the frozen powder was weighed and then homogenized in a buffer containing (in mM) 100 mannitol,...
tol, 50 NaF, 10 Tris, 1 EDTA, 10 mercaptopoethanol, pH 7.5, and proteolytic enzyme inhibitors (5.0 ml/l aprotinin, 5 mg/l leupeptin, and 5 mg/l antitrypsin). The homogenate was immediately centrifuged at 48,000 g for 20 min. The ACC and AMPK were precipitated from the supernatant by addition of ammonium sulfate (144 mg/ml) and by stirring for 30 min on ice. The precipitate was collected by centrifugation at 48,000 g for 20 min. The pellet was dissolved in 10% of the original volume of the homogenate buffer and was centrifuged again to remove insoluble protein.

The supernatant was used for determination of ACC and AMPK activity. ACC activity was determined at citrate concentrations that varied between 0 and 20 mM by measuring the rate of incorporation of [14C]bicarbonate into malonyl-CoA, an acid-stable compound, at 37°C for 10 min as previously described (32). The data were fitted to a modified Hill equation by using the Grafit program (Sigma Chemical), which allows determination of maximal velocity (V_max) as a function of citrate concentration and determination of the activation constant for citrate (K_a). AMPK activity was determined by using the substrate SAMS peptide by the method described previously (5, 32).

Neutralized perchloric acid extracts of the red quadriceps muscles were used for the determination of malonyl-CoA (16). Glycogen content of the red quadriceps muscles was determined by using the anthrone method (9).

The respiratory exchange ratio (RER) was determined for three consecutive 30-min intervals for rats at rest and for the same rats (on a different day) after running on the treadmill for 30 min at 21 m/min up a 15% grade. The latter measurement was started 5 min postexercise. Rats were placed in a Plexiglas chamber. Air was passed through the chamber and into an airtight bag. After each 30-min interval, the volume of air in the bag was determined, along with the carbon dioxide and oxygen content (mass spectrometer). The RER was then calculated.

Results are expressed as means ± SE. Analysis of variance, followed by Fisher's least significant differences test, was used to determine statistical differences (P < 0.05) between treatment groups.

RESULTS

Figure 1 shows that malonyl-CoA in sprinting and endurance-run rats decreases significantly with exercise (P < 0.05). Malonyl-CoA remains significantly decreased for 60 min after exercise in the sprinting group and for at least 90 min in the 30-min exercise group.

ACC activity is reported in Fig. 2 and Table 1. ACC activity at the physiological citrate concentration (0.2 mM) remains significantly lower (P < 0.05) for 30 min after exercise in the sprinting group and for 90 min postexercise in the 30-min exercise group. Table 1 gives a more detailed presentation of the enzyme kinetics of ACC. V_max in the sprinting group remains significantly decreased (P < 0.05), and the K_a for citrate (calculated from the Hill equation) was significantly elevated for 30 min after the 5-min sprinting exercise. Similar results were observed for the rats that were run for 30 min.

AMPK activity is significantly increased (P < 0.05) for 10 min after a bout of sprinting or submaximal exercise (Fig. 3) and then rapidly returns to values near resting levels for both groups. This return to resting values after 10 min parallels the changes in K_a for citrate activation of ACC.

Figure 4 shows the time course of red quadriceps glycogen content from 0 to 90 min after bouts of sprinting or submaximal exercise. Glycogen content is significantly lower (P < 0.05) for 15 min postexercise after a 5-min bout of sprinting exercise and remains significantly decreased (P < 0.05) for 90 min after a 30-min bout of submaximal exercise. The glycogen content of the red quadriceps is higher at the 90-min postexercise time point vs. the 15-min time point (P < 0.05).

Plasma FFA concentration did not change with a 5-min bout of sprinting exercise but was significantly elevated (P < 0.05) for 5 min after the 30-min run.
Table 1. Time course of postexercise recovery of $V_{max}$ and $K_a$ of red quadriceps acetyl-CoA carboxylase

<table>
<thead>
<tr>
<th>Postexercise, min</th>
<th>ACC $V_{max}$, nmol·g⁻¹·min⁻¹</th>
<th>ACC $K_a$, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rats sprinted for 5 min</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>35.4 ± 1.7</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>0</td>
<td>22.4 ± 1.0*</td>
<td>12.2 ± 1.5*</td>
</tr>
<tr>
<td>5</td>
<td>21.9 ± 1.8*</td>
<td>9.9 ± 0.9*</td>
</tr>
<tr>
<td>10</td>
<td>25.4 ± 1.8*</td>
<td>9.6 ± 1.6*</td>
</tr>
<tr>
<td>15</td>
<td>27.0 ± 3.1*</td>
<td>8.9 ± 0.9*</td>
</tr>
<tr>
<td>30</td>
<td>30.9 ± 1.9</td>
<td>7.4 ± 1.3*</td>
</tr>
<tr>
<td>60</td>
<td>31.4 ± 1.1</td>
<td>5.0 ± 0.7</td>
</tr>
<tr>
<td>90</td>
<td>34.3 ± 2.4</td>
<td>4.9 ± 0.6</td>
</tr>
<tr>
<td><strong>Rats run for 30 min</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>40.3 ± 2.6</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>0</td>
<td>21.9 ± 1.6*</td>
<td>9.7 ± 0.7*</td>
</tr>
<tr>
<td>5</td>
<td>25.5 ± 2.7*</td>
<td>7.4 ± 0.5*</td>
</tr>
<tr>
<td>10</td>
<td>28.4 ± 2.4*</td>
<td>6.5 ± 0.4*</td>
</tr>
<tr>
<td>15</td>
<td>28.3 ± 1.0*</td>
<td>5.6 ± 0.4*</td>
</tr>
<tr>
<td>30</td>
<td>29.2 ± 2.5*</td>
<td>5.1 ± 0.6</td>
</tr>
<tr>
<td>60</td>
<td>33.3 ± 1.5*</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>90</td>
<td>33.8 ± 2.9*</td>
<td>4.1 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE from 5 to 7 rats. $V_{max}$, maximal velocity; ACC, acetyl-CoA carboxylase activity; $K_a$, activation constant. *Significantly different from resting group ($P < 0.05$).

Blood lactate was much higher ($P < 0.05$) after 5 min of sprinting than at rest (Table 2). Lactate also increased ($P < 0.05$) in the rats run for 30 min and remained elevated for 10 min postexercise ($P < 0.05$).

Table 3 shows that the RER is significantly decreased in the postexercise period in rats run for 30 min at 21 m/min. From these RER values, it was estimated that, with no prior exercise, the rats derived an average of 41% of calories from fat oxidation. After 30 min of treadmill exercise, the same rats derived 63, 60, and 53% of calories from fat oxidation during the periods 5–30, 30–60, and 60–90 min postexercise.

Table 2. Time course of postexercise recovery of plasma FFA, blood glucose, and blood lactate

<table>
<thead>
<tr>
<th>Postexercise, min</th>
<th>FFA, mM</th>
<th>Glucose, mM</th>
<th>Lactate, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rats sprinted for 5 min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>0.22 ± 0.03</td>
<td>6.3 ± 0.3</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>0</td>
<td>0.22 ± 0.03</td>
<td>8.6 ± 0.3*</td>
<td>7.2 ± 1.0*</td>
</tr>
<tr>
<td>5</td>
<td>0.21 ± 0.01</td>
<td>8.3 ± 0.4*</td>
<td>4.4 ± 0.8*</td>
</tr>
<tr>
<td>10</td>
<td>0.23 ± 0.05</td>
<td>7.8 ± 0.3*</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>15</td>
<td>0.27 ± 0.03</td>
<td>7.8 ± 0.2*</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>30</td>
<td>0.23 ± 0.03</td>
<td>7.4 ± 0.3*</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>60</td>
<td>0.21 ± 0.03</td>
<td>7.4 ± 0.4*</td>
<td>2.9 ± 0.8</td>
</tr>
<tr>
<td>90</td>
<td>0.22 ± 0.03</td>
<td>7.0 ± 0.3</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td><strong>Rats run for 30 min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>0.26 ± 0.03</td>
<td>6.9 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>0</td>
<td>0.39 ± 0.02*</td>
<td>8.1 ± 0.4*</td>
<td>4.2 ± 0.5*</td>
</tr>
<tr>
<td>5</td>
<td>0.35 ± 0.04*</td>
<td>8.6 ± 0.2*</td>
<td>3.5 ± 0.5*</td>
</tr>
<tr>
<td>10</td>
<td>0.34 ± 0.03</td>
<td>8.1 ± 0.6*</td>
<td>3.7 ± 0.5*</td>
</tr>
<tr>
<td>15</td>
<td>0.29 ± 0.03</td>
<td>7.9 ± 0.5</td>
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<tr>
<td>60</td>
<td>0.31 ± 0.03</td>
<td>8.1 ± 0.5*</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>90</td>
<td>0.32 ± 0.03</td>
<td>6.7 ± 0.2</td>
<td>1.3 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE from 5 to 7 rats. FFA, free fatty acids. *Significantly different from resting group ($P < 0.05$).

DISCUSSION

Wolfe et al. (33) have shown that the rate of fatty acid oxidation remains elevated above resting values after exercise. Their work in human subjects indicates that fatty acid oxidation is ~50% higher than at rest for at least 2 h after 4 h of treadmill exercise at 40% maximal oxygen consumption. Brooks and Gaesser (4) previously observed a significant decrease in RER in rats after prolonged exhausting exercise. The decrease in RER in the postexercise period in the current study is consistent with results of previous reports and provides further evidence of increased fat oxidation in the postexercise period. In addition, Treuth et al. (26) have
include: several factors regulating the substrate oxidation of nonexercising muscle fibers and other tissues. Body RER, the latter being diluted by absence of change in the red quadriceps is likely to be much larger than the change in whole body RER. The change in RER in the red quadriceps with changes observed in the red region of the quadriceps. In addition, the change in RER in the red region of the quadriceps muscle fibers involved in the exercise would exhibit a decrease in malonyl-CoA under these conditions. A previous study (31) indicates that malonyl-CoA does not decrease in the white region of the quadriceps muscle of rats until after at least 30 min of exercise and that the decrease is small compared with changes observed in the red region of the quadriceps. In addition, the change in RER in the red quadriceps is likely to be much larger than the change in whole body RER, the latter being diluted by absence of change in nonexercising muscle fibers and other tissues. Malonyl-CoA inhibition of CPT-1 is probably only one of several factors regulating the substrate oxidation mix in muscle. Other factors (11–13, 18, 25, 27, 28) may include: 1) plasma FFA concentration, 2) regulation of FFA uptake into the muscle cell via a plasma membrane fatty acid-binding protein, 3) control of fatty acid-binding proteins and fatty acyl-CoA-binding proteins in the transport and release of lipid to the mitochondria, 4) glucose availability, 5) carnitine availability, and 6) intramitochondrial acetyl-CoA concentration. The concentration of FFA in plasma was observed to increase during 30 min of exercise but was not markedly elevated in the postexercise period. However, increased rate of turnover of plasma FFA and/or supply of fatty acids from intramuscular lipolysis could also contribute to the increased rate of fat oxidation. The rate of oxidation of fatty acids, even in the muscle fibers involved in contraction, would be expected to be limited by the low plasma fatty acid concentration. In more prolonged bouts of exercise that continue until liver glycogen is depleted, insulin is depressed, and glucagon is elevated, plasma fatty acid concentration would be expected to be elevated for much longer after exercise (2).

Glycogen replenishment appeared to occur quite rapidly in the red region of the quadriceps muscle during the postexercise period in this study. Liver glycogen would not have been expected to be depleted by these relatively short bouts of exercise. Thus glucose would be readily available to the muscle fibers requiring replenishment. A recent study from this laboratory (17) demonstrated that 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), an analog of adenosine, can be taken up into muscle and phosphorylated to form ZMP, an analog of 5′-AMP. ZMP activates AMPK similarly to 5′-AMP. Perfusion of isolated rat hindlimb with AICAR thus can mimic the effect of exercise on AMPK, ACC, and malonyl-CoA. AICAR causes a decrease in malonyl-CoA, with a resultant increase in palmitate oxidation with no change in oxygen consumption, thus indicating a change in the substrate oxidation mix. That experiment also yielded the exciting observation of a twofold increase in glucose uptake in AICAR-infused muscle. This result led to the hypothesis that activation of AMPK not only increases availability of intramitochondrial long-chain fatty acids for oxidation but also increases availability of glucose (possibly by phosphorylation of undefined target proteins involved in translocation of GLUT-4 to the sarcolemma) to the working muscle. The contraction-induced increase in glucose transport (see Refs. 6, 10, and 11), which we postulate is due to AMPK activation, would be important for replenishment of glycogen stores after exercise. This fits well with the suggestion that AMPK serves as a fuel gauge of the mammalian cell (8), signaling the need for increased glucose uptake and increased fatty acid oxidation. The prolonged depression of malonyl-CoA in the postexercise period would allow these muscle fibers to rely more on fatty acid oxidation for energy needs, thus diverting more of the available glucose into glycogen.

Malonyl-CoA does not always mirror changes in ACC activity (24) because of allosteric control (inhibition of ACC by long-chain acyl-CoA and activation by citrate). Citrate dependence of ACC activity is measured simply to detect changes due to covalent modification (phosphorylation). The allosteric regulators are not retained in the final enzyme preparation. Saha et al. (24) reported malonyl-CoA to be elevated in isolated skeletal muscle in response to incubation with glucose, insulin, and acetoacetate, with no concurrent change in ACC activity. When ATP citrate lyase was inhibited with hydroxycitrate, the increase in malonyl-CoA was attenuated, implying that the increase in malonyl-CoA under these conditions was due to increases in cytosolic citrate.

The applicability of the results of the current study to human muscle is unclear at this time. Odland et al. (20) previously reported very low levels of malonyl-CoA in resting human muscle (compared with rat muscle) and no significant change during exercise. One of the challenges in studying biopsies from human muscle is the difficulty in obtaining a relatively homogenous sample of type IIa fibers. We have previously noted that...
malonyl-CoA content is much less in IIb fibers than in IIa fibers and that a decrease in IIb fibers is observed only after 30 min of exercise. Even at relatively high work rates, no change was seen in white quadriceps (composed of type IIb fibers) malonyl-CoA after a 5-min bout of exercise. Thus dilution of type IIa with IIb fibers in a biopsy would diminish the resting malonyl-CoA concentration measurement and would also reduce the magnitude of the decrease in malonyl-CoA. The data from the current study also suggest that care must be taken to avoid muscle contraction for an extended period before taking the biopsy of resting muscle. At the present time, however, it is not possible to draw any certain conclusion regarding the role of this control system in human muscle.

Vavvas et al. (29) electrically stimulated the gastrocnemius muscle group in rats and found that 60–90 min were required for ACC activity to return to resting values. The present study demonstrates that malonyl-CoA and ACC activity remain depressed in muscle for relatively prolonged periods after short bouts of high-intensity exercise and longer bouts of submaximal exercise in the rat. These results are consistent with the hypothesis that a reduction in muscle malonyl-CoA in the postexercise period allows increased fatty acid oxidation, thus diverting a greater proportion of the glucose taken up into muscle into the glycolysis synthetic pathway.

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