Liver AMP-activated protein kinase and acetyl-CoA carboxylase during and after exercise

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Carlson, C. L., and W. W. Winder. Liver AMP-activated protein kinase and acetyl-CoA carboxylase during and after exercise. J. Appl. Physiol. 86(2): 669–674, 1999.—Exercise induces a decline in liver malonyl-CoA, an inhibitor of carnitine palmitoyltransferase-1. The purpose of these experiments was to determine whether this decrease in malonyl-CoA is accompanied by an activation of AMP-activated protein kinase (AMPK) and inactivation of acetyl-CoA carboxylase (ACC). Rats were killed at rest, after 10 min of running at 32 m/min up a 15% grade or at 0, 15, or 60 min postexercise after 120 min of running at 16 m/min. There was no significant difference in AMPK and ACC activities after 120 min of exercise, although a trend toward a decrease in ACC and an increase in ACC was noted 15 min postexercise. After 10 min at 32 m/min, however, maximal ACC activity decreased from 487 ± 27 to 280 ± 39 nmol·g⁻¹·min⁻¹, and the activation constant for citrate activation of ACC increased from 5.9 to 12.5 mM. AMPK activity increased from a resting value of 4.7 ± 0.4 to 9.8 ± 2.0 pmol·mg⁻¹·min⁻¹ after exercise. These data provide indirect evidence of phosphorylation and inactivation of liver ACC during heavy exercise. In contrast, the decrease in malonyl-CoA during long-term, low-intensity exercise may occur by mechanisms other than phosphorylation of ACC.

Postexercise ketosis occurs after prolonged bouts of endurance exercise or after shorter bouts in fasted or carbohydrate-deficient states (2, 3, 7, 13, 14). In rats, the elevated 3-hydroxybutyrate levels seen during and after prolonged bouts of submaximal exercise are accompanied by a decline in liver malonyl-CoA (2, 3, 7). Malonyl-CoA is the first committed intermediate in the lipogenic pathway and is also an inhibitor of carnitine palmitoyltransferase-1 (CPT-1) (17, 18). CPT-1 activity can be limiting for fatty acid oxidation and ketogenesis in the liver (17, 18). The decline of liver malonyl-CoA has been postulated to be responsible for the increase in blood ketone body production during and after exercise (2, 3, 7, 13).

Malonyl-CoA is synthesized by acetyl-CoA carboxylase (ACC). ACC is subject to both allosteric and covalent regulation. Allosterically, citrate is an activator of ACC, whereas palmityl-CoA is an inhibitor (8, 11, 12). The principal liver isoform of ACC can be phosphorylated and inactivated by cAMP-dependent protein kinase and by 5′-AMP-activated protein kinase (AMPK) (9, 12). Liver ACC is hormonally activated by insulin and inhibited by glucagon and epinephrine (9, 12, 16). In isolated liver cells, insulin has been shown to increase ACC activity by inducing dephosphorylation of the enzyme (9). Glucagon and epinephrine both decrease hepatic ACC activity (11, 12, 22). The mechanism of the inactivation of liver ACC by glucagon is not clearly defined.

In skeletal muscle, the exercise-induced decline in malonyl-CoA is accompanied by increased activity of AMPK, a decrease in the maximal velocity (Vmax) of ACC, and an increase in the activation constant (Kc) for citrate activation of ACC (28). This change in kinetic properties of ACC mimics those caused by in vitro phosphorylation of ACC by AMPK (9, 28). High rates of fatty acid oxidation during reperfusion of ischemic hearts are also associated with decreases in malonyl-CoA levels. This has been shown to result from an activation of AMPK with consequent phosphorylation and inactivation of ACC (9).

The relationship between AMPK, ACC, and malonyl-CoA to ketogenesis has not been extensively examined in the liver during and after exercise. Although it has been previously demonstrated that malonyl-CoA decreases in liver and skeletal muscle during exercise, the mechanism of the decrease in liver malonyl-CoA is not well understood. This study was designed to determine whether the decrease in liver malonyl-CoA levels caused by exercise is accompanied by an increase in the activity of liver AMPK and a concomitant decrease in liver ACC activity.

Materials and Methods

Animal care and surgical preparation. Sprague-Dawley rats (Sasco, Wilmington, MA) were housed in individual cages at a temperature of 19–21°C in a light-controlled (12:12-h light-dark cycle) room and fed a normal diet (Harlan Teklad rodent diet, Madison, WI) and water ad libitum until the day preceding the exercise test, at which time they were fed 5 g of food/100 g body weight or ad libitum, depending on the conditions required for the specific experiment.

Rats were taught to run on a motor-driven treadmill for at least 5 days before the test, for 5–10 min/day at either 16 or 32 m/min, as dictated by the subsequent test. Poor runners were excluded initially from the study. Three days before the exercise test, jugular catheters were implanted, with the use of aseptic techniques to allow rapid administration of anesthesia.

Exercise tests. Tests were conducted between 10:30 AM and 1:30 PM to avoid large diurnal variations. In experiment 1, rats (body wt, 423 ± 10 g) were run on a treadmill at 16 m/min up a 15% grade. Oxygen consumption was not measured, but, on the basis of previous studies (1), this work rate was estimated to elicit 60–70% of the maximal oxygen consumption. Rats were anesthetized by injection of pentobarbital sodium via jugular catheter at rest, or immediately after 120 min of exercise, or at 15 or 60 min postexercise. In experiment...

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2, rats (body wt, 460 ± 15 g) were anesthetized at rest or after running for 10 min at 32 m/min up a 15% grade. This work rate was estimated to represent ~80–90% of maximal oxygen consumption. Livers were removed rapidly (60–90 s after administration of anesthesia) and then frozen, by using stainless steel block tongs at liquid nitrogen temperature, for analysis of ACC and AMPK activity along with malonyl-CoA, glycogen, and citrate levels. Blood was removed via the abdominal aorta. For analysis of glucagon and insulin, an aliquot (2 ml) was added to tubes that contained 0.03 ml (10.0 trypsin inhibitor units/ml) of aprotinin and 0.1 ml of a solution that contained 24 mg EDTA + 0.5 mg leupeptin/ml, pH 7.4. An aliquot was heparinized for collection of plasma for measurement of free fatty acids (FFA) (20). A perchloric acid (PCA) extract of blood (1 ml blood/2 ml 10% PCA) was made for measurement of 3-hydroxybutyrate (24) and glucose (4). Blood samples were centrifuged at 4°C, and the supernatants were stored in glass test tubes at −20°C.

Liver assays. Liver samples were kept under liquid nitrogen until analyzed. Tissue was ground to a powder under liquid nitrogen. For ACC and AMPK assays, the frozen powder was weighed (0.7 g) and then homogenized in a buffer containing (in mM) 200 mannitol, 50 NaF, 10 Tris, 1 EDTA, 10 β-mercaptoethanol, pH 7.5, and proteolytic enzyme inhibitors (10 ml/l aprotinin, 10 mg/l leupeptin, and 10 mg/l antitrypsin). The homogenate was immediately centrifuged at 48,000 g for 30 min. The ACC and AMPK were precipitated from the supernatant by the addition of 144 mg ammonium sulfate/ml and stirring on ice for 30 min. The precipitate was then collected by centrifugation at 48,000 g for 30 min. The pellet was dissolved in 10% of the original volume of homogenate buffer and centrifuged again to remove insoluble protein.

The supernatant was frozen at −70°C and used for determination of ACC and AMPK activity. ACC activity was determined at citrate concentrations that ranged from 0 to 20 mM by measurement of the rate of incorporation of [14C]bicarbonate into malonyl-CoA (acid stable) at 37°C for 2 min as previously described (28). The data were fitted to the Hill equation by nonlinear regression by using the Grafit program (Sigma Chemical, St. Louis, MO) which allows estimation of Vmax as a function of citrate concentration and of the Kc for citrate activation of ACC. AMPK activity was determined by using the SAMS peptide (15-amino acid peptide substrate) by the method described previously (5, 28).

Neutralized PCA extracts of the liver were used for determination of malonyl-CoA (19) and citrate (21). Fatty acid synthetase was isolated from livers of fasted-refed rats by the procedure described by Linn (15) and was used for malonyl-CoA assays. Liver glycogen content was determined by the anthrone method (10).

Hormone assays. Glucagon and insulin were assayed by double-antibody radioimmunoassay by using kits from Linco Research (St. Louis, MO).

Results are expressed as means ± SE. Analysis of variance and Fisher’s least significant difference (as a post hoc test) were used to determine statistical differences (P < 0.05) among four treatment groups, whereas the Student’s t-test was used to determine statistical differences (P < 0.05) between two treatment groups.

RESULTS

Experiment 1. Rats subjected to light exercise (120 min at 16 m/min up a 15% grade) and allowed to recover for 0, 15, or 60 min showed significantly higher plasma 3-hydroxybutyrate levels than did the nonexercised rats (Table 1). Liver malonyl-CoA levels were significantly lower after exercise than control values and remained low for the duration of the recovery period (Fig. 1C). Liver AMPK and ACC activity at 0.2 mM citrate was not significantly different after exercise compared with control values (Fig. 1, A and B, respect-

Table 1. Hormone and metabolite concentrations before and after exercise at 16 m/min up a 15% grade for 120 min

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Postexercise, min</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Plasma glucagon, pg/ml</td>
<td>66 ± 3</td>
<td>170 ± 12*</td>
</tr>
<tr>
<td>Plasma insulin, ng/ml</td>
<td>2.9 ± 0.6</td>
<td>3.3 ± 1.0</td>
</tr>
<tr>
<td>Plasma FFA, mM</td>
<td>0.46 ± 0.09</td>
<td>0.84 ± 0.11*</td>
</tr>
<tr>
<td>Blood glucose, mM</td>
<td>5.9 ± 0.1</td>
<td>6.9 ± 0.4*</td>
</tr>
<tr>
<td>Blood hydroxybutyrate, mM</td>
<td>0.21 ± 0.05</td>
<td>0.81 ± 0.12*</td>
</tr>
<tr>
<td>Liver citrate, µmol/g</td>
<td>0.16 ± 0.02</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>Liver glycogen, mg/g</td>
<td>61.8 ± 2.5</td>
<td>10.6 ± 2.4*</td>
</tr>
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</table>

Values are means ± SE (per g wet wt). FFA, free fatty acids. *Significantly different from controls, P < 0.05.
preexercise control values, but the concentration decreased to control levels during the recovery period (Table 1).

Experiment 2. Rats were fed ad libitum before this exercise test (10 min of running at 32 m/min up a 15% grade). Although the rats only ran for 10 min, a small, but statistically significant, increase in blood 3-hydroxybutyrate concentration was observed (Table 3).

Liver malonyl-CoA levels declined significantly from control values, whereas liver AMPK activity was significantly increased (Fig. 3). The increase in AMPK activity was accompanied by a decrease in activity of ACC at 0.2 mM citrate. Figure 4 shows a marked change in the shape of the citrate-activation curve for ACC. The $V_{\text{max}}$ was significantly lower and the citrate $K_{a}$ for ACC was significantly higher in the exercised rats than in resting controls (Table 3).

Plasma glucagon was significantly increased in exercised rats compared with controls, whereas insulin was significantly decreased compared with control values (Table 3). The glucagon-to-insulin ratio was elevated after exercise to a much greater degree in the 10-min exercise bout at 32 m/min than it was during and after prolonged exercise of low intensity. Levels of all metabolites measured showed no significant difference between control and exercised values (Table 4). Although not a significant decrease, liver glycogen decreased $\sim 1.0 \text{ mg g}^{-1}\text{min}^{-1}$. Citrate levels after exercise were not different from control values, and there was no significant increase in FFA.

**DISCUSSION**

Studies with the liver isoforms of ACC have demonstrated that in vitro phosphorylation by AMPK and protein kinase A (PKA) causes a decrease in $V_{\text{max}}$ and an increase in $K_{a}$ for citrate activation (9, 12). The skeletal muscle ACC is phosphorylated by both AMPK and PKA, but only the AMPK phosphorylation causes an increase in $K_{a}$ and decrease in $V_{\text{max}}$ (28, 29). Changes in kinetic properties of the ACC partially purified in the presence of phosphatase inhibitors may be used to estimate changes in the phosphorylation state in animals exposed to various perturbations. That is, a significant increase in $K_{a}$ and a significant decrease in $V_{\text{max}}$ of ACC provide indirect evidence that the liver ACC has been phosphorylated. In the ammonium sulfate precipitation step, allosteric modulators of ACC activity would be discarded in the supernatant, and any activity changes (compared with control) are likely caused by covalent modification such as phosphorylation.

**Table 2.** Liver ACC activity ($V_{\text{max}}$) and $K_{a}$ for citrate activation of ACC before and after exercise at 16 m/min up a 15% grade for 120 min.

<table>
<thead>
<tr>
<th>Postexercise, min</th>
<th>Control</th>
<th>0</th>
<th>15</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$, nmol·g$^{-1}$·min$^{-1}$</td>
<td>514±37</td>
<td>532±46</td>
<td>453±70</td>
<td>533±49</td>
</tr>
<tr>
<td>$K_{a}$, mM</td>
<td>6.0±0.4</td>
<td>7.7±0.6</td>
<td>8.5±1.3*</td>
<td>6.1±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE (per g wet wt). ACC, acetyl-CoA carboxylase; $V_{\text{max}}$, maximal velocity; $K_{a}$, activation constant. *Significantly different from control liver, $P < 0.05$.

**Table 3.** Liver ACC activity ($V_{\text{max}}$) and $K_{a}$ for citrate activation of ACC before and after 10 min of exercise at 32 m/min up a 15% grade.

<table>
<thead>
<tr>
<th>ACC</th>
<th>Control</th>
<th>Exercised</th>
</tr>
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<tbody>
<tr>
<td>$V_{\text{max}}$, nmol·g$^{-1}$·min$^{-1}$</td>
<td>487±27</td>
<td>280±39*</td>
</tr>
<tr>
<td>$K_{a}$, mM</td>
<td>5.9±0.4</td>
<td>12.5±1.8*</td>
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Values are means ± SE (per g wet wt). *Significantly different from control liver, $P < 0.05$. 

![Fig. 2. Effect of increasing citrate concentration on ACC activity in rats at rest and at 0, 15, and 60 min postexercise (16 m/min up a 15% grade for 120 min). Each point is the mean of determinations from 8 rats. SE were determined but are not shown. See Table 2 for statistics.](image-url)
In the present study, when rats exercised at a low rate (16 m/min) for 2 h, no changes in $K_a$ or $V_{\text{max}}$ for citrate activation of ACC were observed at the end of exercise. At this point, malonyl-CoA was significantly decreased and blood 3-hydroxybutyrate concentration was elevated compared with the preexercise values. It appears unlikely that the decrease in malonyl-CoA under these conditions was caused by phosphorylation and inactivation of ACC by either PKA or AMPK. At the 15-min-postexercise time point, however, it appeared that a small change in phosphorylation state had occurred in ACC. At this time point, a significant increase in $K_a$ was observed. The glucagon-to-insulin ratio was higher at this time point than at any other. Trends toward an increase in AMPK and a decrease in ACC $V_{\text{max}}$ were noted at the end of exercise and in the postexercise period, but there was no statistical significance.

Other mechanisms exist for decreasing malonyl-CoA in the liver. With the increase in plasma FFA that occurred during the prolonged exercise bout, it is likely that palmitoyl-CoA would have been elevated in the hepatocytes. Palmitoyl-CoA is an allosteric inhibitor of ACC and, hence, of malonyl-CoA synthesis (8, 9, 11). In addition, glycolytic flux would have declined in liver during the course of the exercise bout. Previous studies have demonstrated that cAMP increases in liver during prolonged exercise (26). The consequent activation of PKA would result in phosphorylation of phosphofructokinase (PFK)-2 and of pyruvate kinase (23, 25). Phosphorylation of PFK-2 converts this bifunctional enzyme to fructose-2,6-bisphosphatase, which in turn would decrease the concentration of the potent PFK-1 activator fructose-2,6-bisphosphate. Fructose-2,6-bisphosphate concentrations have also been demonstrated to decrease in the liver during prolonged submaximal exercise (6, 27, 30). Phosphorylation of pyruvate kinase by PKA also results in inactivation (25). The net effect of these phosphorylations would be decreased conversion of glucose carbons to malonyl-CoA and diversion of glucose derived from liver glycogenolysis to working muscle. The decrease in malonyl-CoA in this case would be caused by a diminished substrate supply rather than by allosteric or covalent regulation of ACC. It is likely that both the higher palmitoyl-CoA and the diminished substrate supply play a role during prolonged mild exercise bouts. Whatever the mechanism, the decline in liver malonyl-CoA would have the effect of allowing increased CPT-1 activity and increased rate of fatty acid oxidation and ketogenesis.

It is apparent that the significant decrease in malonyl-CoA induced by intense short-term exercise was accompanied by a significant increase in AMPK activity and a concurrent increase in $K_a$ and decrease in $V_{\text{max}}$ of ACC. These changes in kinetic properties provide indirect evidence that the ACC was phosphorylated during this process.
10-min bout of higher intensity exercise. The increase in \( K_a \) would have the functional effect of making the ACC considerably less sensitive to citrate activation at the prevailing citrate concentrations. These changes could explain the decline in malonyl-CoA that occurs with higher intensity exercise. A diminished substrate supply to ACC could also contribute. The liver content of fructose-2,6-bisphosphate decreases very rapidly during exercise bouts of high intensity (30). This would have the effect of decreasing glycolysis and increasing gluconeogenesis, thus decreasing availability of substrate for malonyl-CoA synthesis. Because plasma FFA did not increase during the high-intensity bout, it is unlikely that inhibition of ACC by long-chain acyl-CoA played a role in causing the decrease in malonyl-CoA.

The 260-kDa isoform of liver ACC (ACC-\( \alpha \)) has eight different phosphorylation sites. Although several kinases can phosphorylate ACC-\( \alpha \), only phosphorylation by AMPK and PKA causes inactivation. AMPK is thought to be the mediator of the effect of glucagon on ACC inactivation in liver cells, but the mechanism of the effect is not known. AMPK appears not to have a phosphorylation target site for PKA, yet serine 79, the target site on ACC for AMPK that produces inhibition, is the only phosphorylated in response to incubation of hepatocytes with glucagon (22). It is possible that ACC activity that was seen in response to intense exercise is AMPK phosphorylated and inactivated in liver cells, but the mechanism of ACC inactivation in liver cells, but the mechanism of ACC inactivation is not known. AMPK appears not to have a phosphorylation target site for PKA, yet serine 79, the target site on ACC for AMPK that produces inhibition, is the only phosphorylated in response to incubation of hepatocytes with glucagon (22). It is possible that ACC was allosterically inhibited by long-chain acyl-CoA.

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Previous studies in rats during exercise have demonstrated that hepatic cAMP increases during short, high-intensity bouts of treadmill running as well as during prolonged, moderate-intensity bouts (3, 26, 30). Therefore, it is possible that the decrease in liver malonyl-CoA in the high-intensity exercise was caused by PKA phosphorylation of ACC rather than by AMPK phosphorylation of ACC. In vitro studies have demonstrated that PKA phosphorylation of purified liver ACC results in an increase in \( V_{\text{max}} \) for citrate activation but only a small decrease in \( K_a \) for citrate activation (12). On the other hand, phosphorylation of purified liver ACC by AMPK results in an increase in \( K_a \) and a marked decrease in \( V_{\text{max}} \). The kinetic changes observed in liver ACC in response to running the rats at 32 m/min for 10 min resemble more closely the changes seen with AMPK phosphorylation than with PKA phosphorylation. That is, a marked decrease in \( V_{\text{max}} \) was observed along with the increase in \( K_a \) for citrate activation. This line of reasoning does not rule out the possibility of concurrent phosphorylation by PKA. Both may be important under these conditions. It is not possible with this experimental design to conclude with certainty a cause-effect relationship between the increase in AMPK and the decrease in ACC activity.

A possible function of the increased liver AMPK activity that was seen in response to intense exercise may be inhibition of the energy requiring biosynthetic pathways of fatty acid, cholesterol, and glycogen synthesis (9). AMPK phosphorylates and inactivates liver ACC, 3-hydroxymethylglutaryl-CoA reductase, and glucose to fatty acids, thus diverting glucose derived from nonexercised rats by the same method as from exercised rats and should, therefore, control for such factors. These precautions, however, must be considered before extrapolation of the results of the studies to human subjects during exercise.

In summary, the decreased liver malonyl-CoA content after light, long-term exercise in untrained rats is possibly caused by mechanisms other than phosphorylation of ACC, although trends are seen toward a decrease in ACC activity in the postexercise period. A possible explanation for the decreased malonyl-CoA is that ACC was allosterically inhibited by long-chain fatty acyl-CoA. Decreased substrate supply caused by inhibition of hepatic glycolysis could also contribute. During high-intensity exercise, the increase in \( K_a \) and decrease in \( V_{\text{max}} \) of ACC, together with the elevated AMPK activity, provide indirect evidence of phosphorylation and inactivation of ACC, thus decreasing malonyl-CoA synthesis. Phosphorylation of ACC by PKA may also play a role. In both cases, the decrease in malonyl-CoA would have the effect of reducing conversion of glucose to fatty acids, thus diverting glucose derived from nonexercised rats by the same method as from exercised rats and should, therefore, control for such factors. These precautions, however, must be considered before extrapolation of the results of the studies to human subjects during exercise.

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from liver glycogenolysis to the working muscles and other tissues that require glucose. When plasma fatty acids are elevated in long-term exercise, the decline in liver malonyl-CoA would have the effect of increasing fatty acid oxidation and ketogenesis, thus providing an alternate substrate to glucose when carbohydrate stores are limited.

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