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

C. L. CARLSON AND W. W. WINDER
Department of Zoology, Brigham Young University, Provo, Utah 84602

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 AMPK 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 ± 1.1 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 (Kauc) 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 a decrease 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 anesthetic.

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...
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 supernatant was 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 using the Grafit program (Sigma Chemical, St. Louis, MO) which allows estimation of Vmax as a function of citrate concentration and of the Ks 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, respective.

### 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>0</th>
<th>15</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucagon, pg/ml</td>
<td>66 ± 3</td>
<td>170 ± 12*</td>
<td>153 ± 27*</td>
<td>87 ± 7</td>
</tr>
<tr>
<td>Plasma insulin, nmol/l</td>
<td>2.9 ± 0.6</td>
<td>3.3 ± 1.0</td>
<td>2.3 ± 0.6</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>Plasma FFA, mM</td>
<td>0.46 ± 0.09</td>
<td>0.84 ± 0.11*</td>
<td>0.65 ± 0.07</td>
<td>0.74 ± 0.12</td>
</tr>
<tr>
<td>Blood glucose, mM</td>
<td>5.9 ± 0.1</td>
<td>6.9 ± 0.4*</td>
<td>6.1 ± 0.5</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td>Blood hydroxybutyrate, mM</td>
<td>0.21 ± 0.05</td>
<td>0.81 ± 0.12*</td>
<td>1.14 ± 0.12*</td>
<td>0.91 ± 0.09*</td>
</tr>
<tr>
<td>Liver citrate, µmol/g</td>
<td>0.16 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>0.26 ± 0.03*</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>Liver glycogen, mg/g</td>
<td>61.8 ± 2.5</td>
<td>10.6 ± 2.4*</td>
<td>4.5 ± 1.5*</td>
<td>7.1 ± 1.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE (per g wet wt). FFA, free fatty acids.
*Significantly different from controls, P < 0.05.
Liver ACC activity (V_{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_{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, 6.0 ± 0.4</td>
<td>7.7 ± 0.6</td>
<td>8.5 ± 1.3*</td>
<td>6.1 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

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

Liver ACC activity (V_{max}) and K_a for citrate activation of ACC before and after exercise at 32 m/min up a 15% grade for 120 min.

<table>
<thead>
<tr>
<th>ACC</th>
<th>Control</th>
<th>Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_{max}, nmol·g^{-1}·min^{-1}</td>
<td>487 ± 27</td>
<td>280 ± 39*</td>
</tr>
<tr>
<td>K_a</td>
<td>5.9 ± 0.4</td>
<td>12.5 ± 1.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE (per g wet wt). *Significantly different from control liver, P < 0.05.

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_{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_{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_{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 modulation such as phosphorylation.
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 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 phosphofructo-kinase (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

---

Table 4. Hormone and metabolite concentrations before and after 10 min of exercise at 32 m/min up a 15% grade

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucagon, pg/ml</td>
<td>84 ± 6</td>
<td>337 ± 50*</td>
</tr>
<tr>
<td>Plasma insulin, ng/ml</td>
<td>3.8 ± 0.6</td>
<td>1.5 ± 0.2*</td>
</tr>
<tr>
<td>Plasma FFA, mM</td>
<td>0.17 ± 0.02</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>Blood glucose, mM</td>
<td>6.2 ± 0.1</td>
<td>6.6 ± 0.2</td>
</tr>
<tr>
<td>Blood 3-hydroxybutyrate, mM</td>
<td>0.15 ± 0.01</td>
<td>0.21 ± 0.02*</td>
</tr>
<tr>
<td>Liver citrate, µmol/g</td>
<td>0.14 ± 0.01</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>Liver glycogen, mg/g</td>
<td>64.2 ± 7.5</td>
<td>53.6 ± 2.2</td>
</tr>
</tbody>
</table>

Values are means ± SE (per g wet wt). *Significantly different from control values, $P < 0.05$. 

---

Fig. 3. Effect of running at 32 m/min up a 15% grade for 10 min on AMPK activity (A), activity of ACC at physiological citrate (0.2 mM, B), and malonyl-CoA content (C) of liver. Values are means ± SE; $n = 7$ and 9 rats for control and exercised, respectively. *Significantly different from resting control, $P < 0.05$.

Fig. 4. Effect of increasing citrate concentrations on activity of ACC in rats at rest and after running at 32 m/min up a 15% grade for 10 min. Values are means for 7 control rats and 9 exercised rats. SE were determined but are not shown. See Table 4 for statistics.
10-min bout of high 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 PKA that produces inhibition, is the one phosphorylated in response to incubation of hepatocytes with glucagon (22). It is possible that the reduction in glycolytic flux induced by PFK-2 phosphorylation by PKA is accompanied by an increase in the AMP-to-ATP ratio. This change by itself would induce an increase in AMPK activity (9).

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 \( K_a \) and an increase in \( K_s \) 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 decline 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 glycoegen synthase (9). It has been proposed that AMPK protects cells against environmental stresses that deplete ATP by inhibiting energy that requires biosynthetic pathways (9). The net effect would be diversion of glucose carbons away from biosynthetic pathways, leaving more for the exercising muscle. Lactate and pyruvate derived from the working muscle and entering the liver could also be utilized for gluconeogenesis rather than for fatty acid and cholesterol synthesis. In this case, the hormonal control system appears to be designed for provision of energy for the entire organism and not just for the liver cells themselves. The effects mediated by liver AMPK activation, and consequent decreases in glycogen, cholesterol, and fatty acid synthesis, would appear to be beneficial to the working muscle and other extrahepatic tissues that require glucose.

The small increase in 3-hydroxybutyrate in the face of the reduction in malonyl-CoA is likely caused by the lack of increase in plasma FFA over the 10-min exercise period. Obviously, a large increase in 3-hydroxybutyrate would not be necessary as an alternative energy substrate for nervous and other tissues during intense short-term exercise when blood glucose levels are increased. The slight increase observed in blood 3-hydroxybutyrate concentration is probably caused by increased oxidation of available long-chain fatty acids, decreased malonyl-CoA, and consequent release of CPT-1 inhibition.

It is possible that methodological problems inherent with small animal studies may have influenced these results. The liver was removed as quickly as possible (usually within 1 min of cessation of running and after injection of anesthetic via the jugular catheter), but it is conceivable that changes occurring in the liver and blood during exercise may have diminished to some extent during this interval. It is also possible that the stress associated with jugular catheterization 3 days before the exercise test could have produced nonphysiological responses. Tissues and blood were collected 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 \( K_s \) 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 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.

We appreciate the assistance of Dan Hibbert, Amy Sorenson, and Blake Rasmussen with preliminary experiments.

This research was supported by the National Institute of Arthritis and Musculoskeletal and Skin Disease Grant AR-41438 and by Professional Development Funds from Brigham Young University.

Address for reprint requests: W. W. Winder, 545 WDB, Dept. of Zoology, Brigham Young Univ., Provo, UT 84602 (E-mail: william_winder@byu.edu).

Received 29 June 1998; accepted in final form 20 October 1998.

REFERENCES


