Effect of phosphorylation by AMP-activated protein kinase on palmitoyl-CoA inhibition of skeletal muscle acetyl-CoA carboxylase

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Rubink, D. S., and W. W. Winder. Effect of phosphorylation by AMP-activated protein kinase on palmitoyl-CoA inhibition of skeletal muscle acetyl-CoA carboxylase. J Appl Physiol 98: 1221–1227, 2005. —AMP-activated protein kinase (AMPK) has previously been demonstrated to phosphorylate and inactivate skeletal muscle acetyl-CoA carboxylase (ACC), the enzyme responsible for synthesis of malonyl-CoA, an inhibitor of carnitine palmitoyltransferase I and fatty acid oxidation. Contraction-induced activation of AMPK with subsequent phosphorylation/inactivation of ACC has been postulated to be responsible in part for the increase in fatty acid oxidation that occurs in muscle during exercise. These studies were designed to answer the question: Does phosphorylation of ACC by AMPK make palmitoyl-CoA a more effective inhibitor of ACC? Purified rat muscle ACC was subjected to phosphorylation by AMPK. Activity was determined on nonphosphorylated and phosphorylated ACC preparations at acetyl-CoA concentrations ranging from 2 to 500 μM and at palmitoyl-CoA concentrations ranging from 0 to 100 μM. Phosphorylation resulted in a significant decline in the substrate saturation curve at all palmitoyl-CoA concentrations. The inhibitor constant for palmitoyl-CoA inhibition of ACC was reduced from 1.7 ± 0.25 to 0.85 ± 0.13 μM as a consequence of phosphorylation. At 0.5 mM citrate, ACC activity was reduced to 13% of control values in response to the combination of phosphorylation and 10 μM palmitoyl-CoA. Skeletal muscle ACC is more potent ly inhibited by palmitoyl-CoA after having been phosphorylated by AMPK. This may contribute to low-muscle malonyl-CoA values and increasing fatty acid oxidation rates during long-term exercise when plasma fatty acid concentrations are elevated.

ACETYL-COA CARBOXYLASE (ACC) catalyzes synthesis of malonyl-CoA from acetyl-CoA. In lipogenic tissues such as liver, adipose tissue, and mammary glands, malonyl-CoA is the major source of two carbon units for synthesis of long-chain fatty acids (5, 6, 24). In both lipogenic and nonlipogenic tissues (such as heart and skeletal muscle), malonyl-CoA also serves as a regulator of fatty acid oxidation (5–7, 37, 39, 40, 47–50). Malonyl-CoA is an inhibitor of carnitine palmitoyltransferase I (CPT I), the key enzyme responsible for allowing transfer of long-chain fatty acyl-CoA into the mitochondrial matrix for β-oxidation to carbon dioxide (25).

At least two isoforms of ACC have been identified and characterized. ACC-1 or ACCα is found primarily in the lipogenic tissues (9, 22, 24, 28). ACC-2 or ACCβ is found in skeletal muscle and heart where lipogenesis is not a major pathway but where fatty acids serve as major substrates for ATP production (9, 16, 19, 22, 28, 43, 44). There is some question as to whether heart and skeletal muscle ACC-2 are identical in structure, as protein kinase A phosphorylation of the heart (10, 16, 28), but not muscle (33), ACC results in a decline in activity. Although some skepticism has been expressed regarding the importance of malonyl-CoA regulation of fatty acid oxidation in exercising muscle (particularly in human muscle), recent data on cellular localization of ACC-2 make this regulation more feasible (2). The skepticism stems from data on human subjects, in whom it has been difficult to demonstrate declines in muscle malonyl-CoA during exercise (23, 29–31, 42). When malonyl-CoA is elevated in muscle of human subjects due to hyperglycemia and hyperinsulinemia, long-chain fatty acid oxidation is markedly inhibited, however (35). The ACC-2 is a larger protein (272 vs. 265 kDa) than ACC-1 due primarily to an NH2-terminal extension that has a hydrophobic mitochondrial targeting sequence (1, 9, 19, 22, 43). ACC-2 has been demonstrated by immunohistochemical techniques and confocal microscopy to be closely associated with the mitochondria (2). Thus malonyl-CoA produced by ACC-2 is likely synthesized in close proximity to CPT I, thus allowing inhibition of the enzyme without marked changes in total cellular malonyl-CoA (2). When malonyl-CoA production is decreased as a result of phosphorylation of ACC, marked declines in malonyl-CoA concentration could occur in the region adjacent to CPT I without large changes in total tissue malonyl-CoA. In rat muscle, particularly in the fast-twitch red muscle fibers, the concentration of malonyl-CoA is considerably higher than in human muscle (8, 15, 29–31, 35, 48). Marked declines in malonyl-CoA occur in this tissue in response to exercise or electrically stimulated muscle contraction (21, 33, 34, 36, 48, 49). Hindlimb perfusion studies demonstrate a good correlation between the rate of fatty acid oxidation and the muscle malonyl-CoA content (26, 27, 51).

AMP-activated protein kinase (AMPK) has previously been shown to phosphorylate the muscle isoform of ACC (ACC-2) at the site equivalent to serine-79 in ACC-1 (12, 20, 33, 49, 52). AMPK is activated in response to muscle contraction (12, 18, 21, 33, 45, 49, 53). The downstream target, ACC-2, is phosphorylated and/or inactivated in exercising or electrically stimulated skeletal muscle (12, 15, 19, 21, 34, 36, 41, 45). Phosphorylation of ACC-2 by AMPK results in a shift of the citrate activation curve to the right, making the enzyme insensitive to allosteric activation at physiological citrate concentrations (33, 49, 52). This, coupled with removal of malonyl-CoA by malonyl-CoA decarboxylase, results in a marked decrease in malonyl-CoA concentration in the muscle (38, 40, 41).
41, 49). This is thought to remove inhibition of CPT I, allowing fatty acid oxidation to proceed as exercise continues.

In addition to activation by citrate, ACC-1 and ACC-2 have been shown to be inhibited by palmitoyl-CoA (32, 44). Muscle content of long-chain acyl-CoA increases in human muscle during the course of prolonged exercise bouts (46). No data are available regarding the effect of phosphorylation on palmitoyl-CoA inhibition of ACC. This study was designed to test the hypothesis that palmitoyl-CoA inhibition of ACC is enhanced by AMPK phosphorylation of ACC.

MATERIALS AND METHODS

ACC isolation. Rats (Sprague-Dawley, Sasco, Charles River) were given free access to food and water and were kept in a room with a 12:12-h light-dark cycle. All procedures using animals were approved by the Institutional Animal Care and Use Committee at Brigham Young University. Rats were anesthetized with pentobarbital sodium (52 mg/100 g body wt). Quadriceps, hamstrings, and gastrocnemius muscles from five to six rats (body weight 400–600 g) were removed and placed between stainless steel blocks at ice temperature for quick cooling. Visible fat tissue was removed. ACC was isolated as described previously (49). All muscles (including gastrocnemius, hamstrings, and quadriceps) were pooled and placed in ice cold buffer A (9 mM muscle wet wt) containing 225 mM mannitol, 75 mM sucrose, 10 mM Tris·HCl, 0.05 mM EDTA, 5 mM potassium citrate, and 2.5 mM MnCl2, pH 7.5. Leupeptin (10 mg/l), antitrypsin (10 mg/l), and aprotonin (10 μl/l) were added immediately before use. To optimize collection of nonphosphorylated ACC, phosphatase inhibitors were not utilized in the homogenizing medium. Muscles were minced with scissors and then homogenized with a Brinkmann Polytron PT 3000 homogenizer. After centrifugation at 17,000 g for 30 min at 4°C, the supernatant was combined with ammonium sulfate granules (200 g/l) and stirred at 4°C for 1 h. After centrifugation (17,000 g for 30 min at 4°C), the supernatant was poured off, and the precipitate was suspended in a minimal volume of buffer B (100 mM Tris·HCl, 0.5 M NaCl, 1 mM EDTA, 0.1 mM DTT, and 10% glycerol, pH 7.5). Proteolytic enzyme inhibitors were added at the same concentration as indicated for buffer A. A homogenizer was utilized to resuspend the pellet. This resuspended solution was dialyzed for 3 h in buffer C (100 mM Tris·HCl, 0.5 M NaCl, 1 mM EDTA, 0.1 mM DTT, 5 mM glycerol, pH 7.5). The dialyzed solution was centrifuged at 40,000 g for 15 min at 4°C to remove any insoluble material. The supernatant was applied to a freshly regenerated column of Promega Soft-Link, soft-release aprotinin (10 μg/l) and 0.05 mM EDTA, 0.68 mM EGTA, 0.68 mM DTT, 1.36 mM EGTA, 1.36 mM DTT, pH 7.4). Final concentrations in the incubation mixes from above were added to Laemmli’s solution, and proteins were separated on 5% SDS-PAGE gels (Tris·HCl Ready Gels, Bio-Rad, Hercules, CA) and then transferred to nitrocellulose membranes at 100 V for 50 min. Membranes were blocked in 5% dried milk (Bio-Rad) in PBST (139 mM NaCl, 2.7 mM KH2PO4, 9.9 mM Na2HPO4, and 0.1% Tween 20) for 1 h. The membrane designated for phospho-ACC determination was left overnight with phospho-ACC polyclonal antibody (Cell Signaling Technology, Beverly, MA) in 5% nonfat dried milk solution (1:5,000 dilution). Both membranes were washed twice in PBST and twice in PBS. The membrane designated for ACC determination was then exposed to streptavidin-horseradish peroxidase conjugate (Amerham Life Sciences, Arlington Heights, IL) in PBST (1:5,000 dilution) for 1 h at room temperature. The membrane designated for phospho-ACC determination was then exposed to horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amerham Life Sciences) in 3% nonfat dried milk in PBST (1:1,500 dilution) for 1 h at room temperature. After being washed twice with PBST and twice with PBS, the membranes were incubated in enhanced chemiluminescence-detection reagent and then visualized on enhanced chemiluminescence hyperfilm (Amerham Life Sciences). Relative amounts of ACC and phospho-ACC were then quantified using SigmaGel software (SPSS, Chicago, IL).

ACC activity measurements. The ACC activity assay was performed on phosphorylated ACC and nonphosphorylated ACC. Final concentrations in the assay mix were 50 mM HEPES, pH 7.5, 1.5 mM MgSO4, 2 mM DTT, 4 mM ATP, 12.5 mM KHCO3, 2 μCi [14C]bi carbonate, 20 mM citrate, 20 mM magnesium acetate, 0.75 mg/ml fatty acid free bovine serum albumin, and variable concentrations of acetyl-CoA (0, 0.0025, 0.005, 0.010, 0.025, 0.050, 0.100, 0.250, and 0.500 mM) and palmitoyl-CoA (0, 1, 10, and 100 μM) in a final volume of 0.19 ml. The reaction was started by addition of 0.01 ml phosphorylated or nonphosphorylated ACC. After 4-min incubation at 37°C, the reaction was stopped by addition of 50 μl of 5 M HCl. After mixing and centrifugation, 200 μl were added to a 7-ml scintillation vial and evaporated to dryness at 80°C. The residue (containing malonyl-CoA) was dissolved in 0.4 ml water and mixed with 5.5 ml Ecolite (ICN, Costa Mesa, CA) for determination of radioactivity. Five different preparations of muscle ACC were utilized to obtain these data. Data for all combinations of concentrations of acetyl-CoA and palmitoyl-CoA were obtained for each different ACC isolate. A second series of experiments were done using a fixed acetyl-CoA concentration (0.5 mM), variable citrate (0, 0.5, 1, 2.5, 5, 10, and 20 mM), and with palmitoyl-CoA at 0 and 10 μM. Four different runs, including all combinations of citrate and palmitoyl-CoA, were done on the phosphorylated and nonphosphorylated ACC from the same preparation for these data. A final series of reactions was performed on phosphorylated and nonphosphorylated ACC at an acetyl-CoA concentration of 0.5 mM, citrate concentration of 0.5 mM, with and without palmitoyl-CoA at 10 μM (n = 5 for each condition). Preliminary experiments indicated linearity with time and enzyme concentration in this range. Sigma Plot 8.0 Software for Enzyme Kinetics (Jandel Scientific) was utilized for analysis of the data and preparation of curves of best fit. Grafit (Erithacus Software, Horley, Surrey, UK) was utilized for curve fitting (Allosteric Kinetics, Hill equation) and calculations of activation constant (Ks) and Vmax for the citrate activation curves.

Statistical analysis. Number Cruncher Statistical Software (NCSS, Kaysville, UT) was also used to perform descriptive statistics, t-tests in the case of comparison of two means, and one-way analysis of variance followed by Fishers’ least significant difference post hoc test.
when more than two means were compared. Values are expressed as means ± SE. Differences between means were considered significant when $P < 0.05$.

RESULTS

Western blot technique was used to determine the appropriate time of incubation of ACC with AMPK to ensure maximum phosphorylation of ACC. Gels probed with the phospho-ACC antibody showed increases in the extent of phosphorylation of ACC as a function of time. The blot probed with streptavidin (for total ACC) showed no change in ACC as a function of time. Mean data from four determinations are summarized in Fig. 1. Representative blots are also shown. From these data it was determined that a 30-min incubation was sufficient for maximal phosphorylation of ACC by AMPK. This incubation time was utilized for all phosphorylations of ACC by AMPK.

Figure 2 shows the families of ACC activity curves generated when the purified muscle ACC was incubated with variable concentrations of acetyl-CoA at four palmitoyl-CoA concentrations. Each point on the curve represents five individual determinations using a separate ACC isolation for each determination. Using the Sigma Plot Enzyme Kinetics Software, the mixed partial inhibition model gave the best fit for these data ($R^2 = 0.98$). This implies both competitive and noncompetitive components of palmitoyl-CoA inhibition of ACC. It is apparent, when comparing the phosphorylated ACC with the nonphosphorylated ACC at each acetyl-CoA concentration, that palmitoyl-CoA is a more effective inhibitor after ACC is phosphorylated by AMPK.

Table 1 shows $V_{\text{max}}$ and $K_m$ for acetyl-CoA and inhibitor constant ($K_i$) for palmitoyl-CoA in phosphorylated and nonphosphorylated ACC. The marked reduction in $K_i$ in response to phosphorylation implies that the phospho-ACC is significantly inhibited at lower concentrations of palmitoyl-CoA than is the nonphosphorylated ACC.

Figure 3 shows ACC $V_{\text{max}}$ (as a function of acetyl-CoA concentration) at the four different palmitoyl-CoA concentrations. Note that the reduction in $V_{\text{max}}$ was proportionally greater in the phosphorylated ACC preparations compared with the nonphosphorylated preparations. Differences between phosphorylated and nonphosphorylated ACC activity were statistically significant at all palmitoyl-CoA concentrations ($P < 0.05$).

Figure 4 shows citrate dependence of nonphosphorylated and phosphorylated ACC at citrate concentrations ranging from 0 to 20 mM in the presence and absence of 10 μM

Table 1. Effect of phosphorylation of acetyl-CoA carboxylase with AMP-activated protein kinase

<table>
<thead>
<tr>
<th></th>
<th>Nonphosphorylated</th>
<th>Phosphorylated</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$, cpm/min</td>
<td>3,158±1</td>
<td>2,768±65</td>
</tr>
<tr>
<td>$K_m$ (acetyl-CoA), μM</td>
<td>45±3</td>
<td>54±4</td>
</tr>
<tr>
<td>Palmitoyl-CoA $K_i$, μM</td>
<td>1.7±0.25</td>
<td>0.85±0.13*</td>
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Values are means ± SE; $n = 5$. cpm. Counts/min; $K_i$, inhibitor constant.

*Significantly different from nonphosphorylated acetyl-CoA carboxylase, $P < 0.01$. 

![Fig. 1. Top: Western blots of phospho-acetyl-CoA carboxylase (ACC) (top blot) and ACC (bottom blot) at times following addition of AMP-activated protein kinase (AMPK) or buffer to the reaction mix. Bottom: graph shows summary of mean data from 4 determinations (n = 4 at each point). The 15-, 30-, and 45-min values for phospho-ACC are all significantly different (P < 0.001) from the nonphosphorylated ACC shown at the 0 time point. ACC values are not significantly different at any of the time points.](Fig. 1. Top: Western blots of phospho-acetyl-CoA carboxylase (ACC) (top blot) and ACC (bottom blot) at times following addition of AMP-activated protein kinase (AMPK) or buffer to the reaction mix. Bottom: graph shows summary of mean data from 4 determinations (n = 4 at each point). The 15-, 30-, and 45-min values for phospho-ACC are all significantly different (P < 0.001) from the nonphosphorylated ACC shown at the 0 time point. ACC values are not significantly different at any of the time points.)

![Fig. 2. Substrate saturation curves of nonphosphorylated ACC and of AMPK-phosphorylated ACC at different concentrations of palmitoyl-CoA. Values are means ± SE (n = 5 at each point). Palmitoyl-CoA inhibition was accentuated in ACC preparations phosphorylated by AMPK. See Table 1 and Fig. 3 for statistics. cpm, Counts per minute.](Fig. 2. Substrate saturation curves of nonphosphorylated ACC and of AMPK-phosphorylated ACC at different concentrations of palmitoyl-CoA. Values are means ± SE (n = 5 at each point). Palmitoyl-CoA inhibition was accentuated in ACC preparations phosphorylated by AMPK. See Table 1 and Fig. 3 for statistics. cpm, Counts per minute.)

![Fig. 3. ACC $V_{\text{max}}$ (as a function of acetyl-CoA concentration) at the four different palmitoyl-CoA concentrations.](Fig. 3. ACC $V_{\text{max}}$ (as a function of acetyl-CoA concentration) at the four different palmitoyl-CoA concentrations.)

![Fig. 4. Citrate dependence of nonphosphorylated and phosphorylated ACC at citrate concentrations ranging from 0 to 20 mM in the presence and absence of 10 μM](Fig. 4. Citrate dependence of nonphosphorylated and phosphorylated ACC at citrate concentrations ranging from 0 to 20 mM in the presence and absence of 10 μM)
palmitoyl-CoA. It is apparent that, with phosphorylated ACC, activity is much lower in the presence of palmitoyl-CoA at all concentrations of citrate. From these curves, the $K_a$ for citrate activation of ACC was 2.9 ± 0.3 for nonphosphorylated ACC, 5.8 ± 0.9 for phosphorylated, 3.9 ± 0.7 for nonphosphorylated ACC with addition of 10 μM palmitoyl-CoA, and 20.1 ± 3.0 for phosphorylated ACC with the addition of 10 μM palmitoyl-CoA. The combination of phosphorylation and addition of palmitoyl-CoA shifts the activation curve far to the right and makes citrate an ineffective activator of ACC at physiological citrate concentrations. The results of this treatment are significantly different from all others ($P < 0.05$).

Figure 5 illustrates the effect of phosphorylation and palmitoyl-CoA at 0.5 mM citrate, a concentration slightly above those expected in the muscle. An ACC preparation showing higher activity was utilized for these experiments to more accurately show the difference between treatments at near physiological concentrations of citrate. At this citrate concentration, activity of ACC was reduced to ~34% ($P < 0.001$) of the nonphosphorylated control value as a result of phosphorylation and to 13% of control with addition of 10 μM palmitoyl-CoA ($P < 0.001$). Again, palmitoyl-CoA appeared to be a more effective inhibitor of the phosphorylated ACC.

**DISCUSSION**

The importance of understanding regulation of ACC-2 has recently been emphasized by studies on transgenic mice deficient in ACC-2. Abu-Elheiga et al. (3) reported that mice lacking ACC-2 exhibited reduced fat storage and higher rates of fatty acid oxidation. The same group found that these ACC-2 mutant mice were resistant to obesity and diabetes induced by high-fat or high-carbohydrate diets (4). Some of these same beneficial effects might be observed if the enzyme were turned off by natural means. Muscle contraction has been demonstrated to activate AMPK, which then phosphorylates and inactivates ACC-2 (21, 33, 34, 45, 49). In rat studies, malonyl-CoA has been reported to decrease in muscle during exercise and in response to electrical stimulation of muscle (34, 36, 48, 49). This decline in malonyl-CoA has been postulated to be important in allowing fat oxidation to proceed in the working muscle (46, 47, 50). In hindlimb perfusion studies, fatty acid oxidation increases when AMPK is chemically inhibited.
activated using aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (26, 27, 51). A good correlation was observed between the rate of palmitate oxidation and the malonyl-CoA content of muscle (26, 27, 51).

Previous studies have demonstrated that palmitoyl-CoA is an inhibitor of liver ACC (32). In liver, ACC-1 is a lipogenic enzyme, which provides malonyl-CoA as the principal source of substrate for fatty acid synthesis. When carbohydrate is present in abundance, elevated concentrations of palmitoyl-CoA derived from high rates of fatty acid synthesis would be expected to have a negative feedback effect on ACC, thus preventing accumulation of long-chain fatty acyl-CoA to toxic, detergent levels. In carbohydrate-deficient states (such as fasting or prolonged exercise), elevated hepatic concentrations of palmitoyl-CoA resulting from lipolysis in adipose tissue would also be expected to inhibit ACC-1, resulting in a decline in malonyl-CoA and allowing an increased rate of fatty acid oxidation and ketogenesis.

Palmitoyl-CoA has also been reported to inhibit ACC isolated from skeletal muscle (ACC-2) (44). Skeletal muscle is not considered to be a lipogenic tissue, and the ACC-2 is thought to be involved primarily in regulation of fatty acid oxidation. During fasting, this mechanism may be important for causing a decline in malonyl-CoA, thus allowing the muscle to obtain a greater proportion of its energy from fatty acid oxidation. During prolonged exercise, it is also important that malonyl-CoA content of the muscle be reduced to relieve inhibition of CPT I and allow fatty acid oxidation to proceed to provide ATP for muscle contraction. This contraction-induced decrease in malonyl-CoA is thought to be mediated by AMPK phosphorylation/inactivation of ACC-2 and by AMPK-induced activation of malonyl-CoA decarboxylase (40, 41, 47, 50). Results of the present study suggest that ACC phosphorylation not only produces direct covalent modulation/inactivation but also that palmitoyl-CoA becomes more effective in inhibiting malonyl-CoA synthesis. The dual control ensures that ACC will be inactivated, allowing fatty acid oxidation to proceed.

Previous reports have shown a reduction in citrate activation of ACC in response to phosphorylation by AMPK, particularly at concentrations of citrate expected in muscle sarcoplasm (33, 49). Citrate activation curves in this study confirm those findings. In addition, it is clear that, at physiological citrate concentrations, the combination of phosphorylation by AMPK and 10 μM palmitoyl-CoA results in virtually complete inhibition of skeletal muscle ACC.

In 1987, Carling et al. (11) reported that palmitoyl-CoA at concentrations of 50–200 nM accelerated reactivation of crude preparations of dephosphorylated AMPK (then termed ACC kinase or ACK3). Data from a recent study by Clark et al. (13) demonstrate that elevated fatty acids (palmitate and oleate) can cause increased phosphorylation/activation of AMPK in perfused heart in the absence of changes in tissue content of AMP, ADP, ATP, and creatine phosphate. Although it is unclear if this mechanism exists in skeletal muscle, it is conceivable that elevated plasma fatty acids facilitate their own oxidation by enhancing phosphorylation of AMPK and ACC and by increasing palmitoyl-CoA, which inhibits ACC.

Skeletal muscle long-chain acyl-CoA concentrations have been reported to increase in response to fasting, to high-fat diets, and to exercise (17, 46). Values in the range of 1.6 to 8.3 μmol/kg have been reported for rat muscle (17). In human subjects exercising for 2 h, muscle long-chain acyl-CoA was reported to increase over sixfold from 18 to 82 μmol/kg dry mass (46). The sensitivity range of muscle ACC thus appears to be in the range of fluctuation of long-chain acyl-CoA concentrations in muscle. It is also apparent that a phosphorylation-induced decrease in the $K_i$ for palmitoyl-CoA inhibition of ACC would be expected to have physiologically relevant consequences in inhibiting malonyl-CoA synthesis.

The significance of palmitoyl-CoA inhibition of ACC in Type 2 diabetes is unclear. Rasmussen et al. (35) clearly demonstrated in healthy human subjects that physiological hyperglycemia with accompanying hyperinsulinemia produced elevated muscle malonyl-CoA, inhibition of CPT I, and inhibition of fatty acid oxidation. Although it is possible that palmitoyl-CoA concentration may be increased in muscle of patients with Type 2 diabetes, the elevated blood glucose may result in accelerated citrate production and hence malonyl-CoA synthesis (see Ref. 38), thus overwhelming this inhibitory effect of palmitoyl-CoA on ACC activity. One might anticipate that muscle contraction in these individuals would result in AMPK activation and thus increase the effectiveness of inhibition of ACC by palmitoyl-CoA.

In a previous study (44), malonyl-CoA exhibited product inhibition of muscle ACC with a $K_i$ of ~10 μM. Although we did not quantitate malonyl-CoA inhibition in this study, it is conceivable that the combination of palmitoyl-CoA and malonyl-CoA concentrations would more potently inhibit ACC activity in the intact muscle than would palmitoyl-CoA alone. Because the $K_i$ for palmitoyl-CoA is ~0.1 that of malonyl-CoA, however, it is likely that the long-chain acyl-CoA are far more important physiologically for ACC modulation.

It should be emphasized that regulation of malonyl-CoA concentration is only one mechanism controlling the rate of fatty acid oxidation in the muscle. The fatty acid concentration, the availability of carnitine, the concentration of acetyl-CoA (feedback inhibitor of fatty acid oxidation via the thiolase reaction), the availability of CoA in the mitochondrial matrix, and the overall energy charge all must be considered factors influencing total muscle fatty acid oxidation rate (23, 47).

In summary, palmitoyl-CoA was found to inhibit both non-phosphorylated and AMPK-phosphorylated ACC isolated from rat skeletal muscle. The potency of this inhibitor was increased in response to phosphorylation, evidenced by a decrease in the $K_i$. Phosphorylation thus not only has a direct inhibitory effect on ACC, but also alters the sensitivity of ACC to inhibition by this allosteric inhibitor. At physiological concentrations of citrate, the combination of phosphorylation and 10 μM palmitoyl-CoA essentially inhibits synthesis of malonyl-CoA by ACC. This may be important during long-term exercise when muscle fatty acyl-CoA concentrations are elevated and the muscle is highly dependent on fat oxidation for ATP production.

ACKNOWLEDGMENTS

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GRANTS

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