Contraction-induced Changes in Acetyl-CoA Carboxylase and 5′-AMP-activated Kinase in Skeletal Muscle*

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The concentration of malonyl-CoA, a negative regulator of fatty acid oxidation, diminishes acutely in contracting skeletal muscle. To determine how this occurs, the activity and properties of acetyl-CoA carboxylase β (ACC-β), the skeletal muscle isoform that catalyzes malonyl-CoA formation, were examined in rat gastrocnemius-soleus muscles at rest and during contractions induced by electrical stimulation of the sciatic nerve. To avoid the problem of contamination of the muscle extract by mitochondrial carboxylases, an assay was developed in which ACC-β was first purified by immunoprecipitation with a monoclonal antibody. ACC-β was quantitatively recovered in the immunopellet and exhibited a high sensitivity to citrate (12-fold activation) and a Kₘ for acetyl-CoA (120 μM) similar to that reported for ACC-β purified by other means. After 5 min of contraction, ACC-β activity was decreased by 90% despite an apparent increase in the cytosolic concentration of citrate, a positive regulator of ACC. SDS-polyacrylamide gel electrophoresis of both homogenates and immunopellets from these muscles showed a decrease in the electrophoretic mobility of ACC, suggesting that phosphorylation could account for the decrease in ACC activity. In keeping with this notion, citrate activation of ACC purified from contracting muscle was markedly depressed. In addition, homogenization of the muscles in a buffer free of phosphatase inhibitors and containing the phosphatase activators glutamate and MgCl₂ or treatment of immunoprecipitated ACC-β with purified protein phosphatase 2A abolished the decreases in both ACC-β activity and electrophoretic mobility caused by contraction. The rapid decrease in ACC-β activity after the onset of contractions (50% by 20 s) and its slow restoration to initial values during recovery (60–90 min) were paralleled temporally by reciprocal changes in the activity of the α2 but not the α1 isofrom of 5′-AMP-activated protein kinase (AMPK). In conclusion, the results suggest that the decrease in ACC activity during muscle contraction is caused by an increase in its phosphorylation, most probably due, at least in part, to activation of the α2 isoform of AMPK. They also suggest a dual mechanism for ACC regulation in muscle in which inhibition by phosphorylation takes precedence over activation by citrate. These alterations in ACC and AMPK activity, by diminishing the concentration of malonyl-CoA, could be responsible for the increase in fatty acid oxidation observed in skeletal muscle during exercise.

In tissues such as liver (1) and heart (2, 3), malonyl-CoA regulates fatty acid oxidation by inhibiting carnitine palmitoyl transferase I, the enzyme that catalyzes the transfer of cytosolic long chain fatty acyl-CoA into mitochondria. Evidence has been presented that it plays a similar role in skeletal muscle (4–7), although definitive evidence is still lacking (see “Discussion”). Malonyl-CoA is synthesized from cytosolic acetyl-CoA by a reaction catalyzed by acetyl-CoA carboxylase (ACC).1 In liver, the principal ACC is the α isoform with a mass of 265 kDa (ACC-α); in skeletal muscle, it is a distinct 280-kDa β isoform (ACC-β) (8–14). Numerous studies have shown that the activity of ACC in liver is altered by starvation, refeeding, and incubation with insulin and glucose due to changes in its cellular content and phosphorylation state (15–22). In contrast, neither nutritional changes in vivo (23, 8) nor incubation with insulin and glucose (24) have been shown to affect the activity of ACC-β in skeletal muscle extracts, despite the fact that they produce substantial changes in malonyl-CoA content. Recent studies suggest that the rapid (min) increase in malonyl-CoA caused by insulin and glucose in incubated rat soleus muscle is caused by an increase in the cytosolic concentration of citrate, an allosteric activator of ACC and a substrate for its precursor, cytosolic acetyl-CoA (24).

Alterations in ACC activity due to phosphorylation may occur in skeletal muscle during voluntary exercise. Thus, Winder and Hardie (25) have demonstrated that after 5 min of swimming, malonyl-CoA levels and ACC activity in rat skeletal muscle are diminished and the activity of 5′-AMP-activated protein kinase is increased. Direct evidence that the decrease in ACC activity is due to phosphorylation is still lacking, however. In addition, it is unclear whether the observed decrease in ACC activity is a consequence of muscle contraction per se or a systemic effect of exercise. Finally, whether changes in the cytosolic concentration of citrate, such as those produced by insulin and glucose, continue to modulate ACC activity in muscle during exercise is not known.

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1 The abbreviations used are: ACC, acetyl-CoA carboxylase; PVDF, polyvinylidene difluoride; AMPK, 5′-AMP-activated protein kinase; PP2A, protein phosphatase 2A; PC, pyruvate carboxylase; PCC, propionyl-CoA carboxylase; PFK-I, phosphofructokinase-I.

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To examine these questions, ACC activity and tissue levels of citrate and malate, an antipporter for citrate efflux from the mitochondria, were assessed in control rat muscle and in muscle made to contract intensely by electrical stimulation. Because of difficulties in assaying ACC in frozen muscle due to the contamination of the extracts by other carboxylases (12), a novel method for studying ACC-β was developed, in which its properties and activity were assessed after immunopurification. The relationship between ACC activity and phosphorylation state was also addressed, as were the potential roles of the α1 and α2 5′-AMP-activated protein kinase isoforms in regulating ACC activity.

**EXPERIMENTAL PROCEDURES**

**Chemicals and Materials**—All radiochemicals were obtained from DuPont NEN. Protein phosphatase 2A was purchased from Upstate Biochemical Inc. (UBI), and okadaic acid was purchased from Calbiochem. Agarose-Protein A/agarose plus conjugate was obtained from Santa Cruz Biotechnology (Santa Cruz, CA), streptavidin-horseradish peroxidase from Amersham Corp., and PVDF membranes (0.45 μm) from Millipore. For enhanced chemiluminescence (ECL), luminol and p-chloromercuribenzoic acid were obtained from Sigma, and 30% H2O2 was obtained from Fisher. X-ray films were Fuji RX.

**Animal Care**—Male Sprague-Dawley rats weighing 55–67 g were obtained from Charles River Breeding Laboratories (Wilmington, MA). They were kept in the departmental animal house with a light-dark cycle of 6 p.m.–6 a.m. and were fed Purina rat chow ad libitum. In all studies, the rats were fasted for 18–20 h prior to an experiment.

**Muscle Stimulation**—Rats were anesthetized with sodium pentobarbital (55 mg/kg body weight intraperitoneally), and 45 min later the skin from both hindlimbs was removed, and the sciatic nerves were exposed. The right sciatic nerve was stimulated for 0–5 min with a bipolar electrode connected to a Grass stimulator (model S48) (5 pps, 100-ms trains of 2.5 V, 50 Hz, and 10-ms duration). The exposed muscle was kept moist with Ringer’s solution maintained at room temperature. At various times following stimulation, the gastrocnemius-soleus muscles of the stimulated and unstimulated contralateral limbs were rapidly excised and frozen in liquid nitrogen. All tissues were stored at −80 °C until analyzed.

**Buffers and Solutions**—Buffer A contained 30 mM NaHEPES, pH 7.4; 2.5 mM EGTA; 3 mM EDTA; 32% glycerol; 20 mM KCl; 40 mM β-glycerophosphate; 40 mM NaF; 4 mM NaPPi, 1 mM Na3VO4, 0.1% Nonidet P-40, 2 mM diisopropylphosphate, 2 mM phenylmethylsulfonyl fluoride; 5 mM α-protein, leupeptin, and pepstatin A; and 1 mM dithiothreitol. Buffer B contained 30 mM NaHEPES, pH 7.4; 2.5 mM EGTA; 3 mM EDTA; 70 mM KCl; 20 mM β-glycerophosphate; 20 mM NaF; 2 mM NaPPi; 1 mM Na3VO4; 0.1% Nonidet P-40; 2 mM diisopropylphosphate; 2 mM phenylmethylsulfonyl fluoride; 5 mM α-protein, leupeptin, and pepstatin A; and 1 mM dithiothreitol. Buffer C contained 30 mM NaHEPES, pH 7.4; 2.5 mM EGTA; 3 mM EDTA; 32% glycerol; 30 mM KCl; 85 mM NaCl; 0.1% Nonidet P-40; 2 mM diisopropylphosphate; 2 mM phenylmethylsulfonyl fluoride; 5 mM α-protein, leupeptin, and pepstatin A; and 1 mM dithiothreitol. Buffer D contained 100 mM NaCl; 20 mM NaHEPES, pH 7.4; 60 mM β-mecaptoethanol, and 1 mg/ml bovine serum albumin.

**Effect of Contraction on the Concentrations of Malonyl-CoA, Citrate, and Malate**—In agreement with previous results (4, 29), malonyl-CoA levels were diminished by 50% after 5 min of intense muscle contraction (1.9 ± 0.2 versus 0.9 ± 0.2 nmol/g of muscle, n = 4, p < 0.05). This was not attributable to a decrease in the cytosolic concentration of citrate, since the whole muscle cell concentration of citrate was increased (190 ± 16 versus 300 ± 34 nmol/g muscle, n = 4, p < 0.05). Furthermore, the concentration of malate, an antipporter for citrate efflux from the mitochondria, was also increased (280 ± 10 versus 390 ± 28 nmol/g of muscle, n = 4, p < 0.05), suggesting the possibility of citrate redistribution from mitochondria to cytosol (24).

**ACC-β Activity in Skeletal Muscle and Effect of Contraction**—Attempts to assay ACC activity directly in muscle supernatants were hindered by the presence of contaminating carboxylases, presumably pyruvate carboxylase (PC) and propionyl-CoA carboxylase (PCC) arising as a consequence of mitochondria breakage (Fig. 1A). Thus, when acetyl-CoA carboxylase activity was assayed by the 14CO2 fixation method in 100,000 × g supernatants obtained from frozen muscle extracts, a high but variable activity was noted in the absence of citrate (50–80% of total in different experiments), and activation by 10 mM citrate, an index of ACC activity, was modestly
Contraction-induced Changes in Muscle ACC and AMPK

The ACC-β-specific monoclonal antibody 7AD3 precipitates ACC-β that is free of contaminating carboxylases and that displays a high sensitivity to citrate activation. Cytosolic extracts of frozen gastrocnemii were incubated with the monoclonal antibody 7AD3 and Protein A/G-agarose beads as described under "Experimental Procedures." A, extracts before (PRE) and after (POST) immunoprecipitation and the immunopellets (IP) themselves were run on 7% SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes. They were then probed with streptavidin-horseradish peroxidase and developed with ECL. The identification of carboxylases on the gel is based on molecular weight. B, the different fractions from A were assayed for ACC activity by 14CO2 fixation in the absence (open bars) or presence (dark bars) of 10 mM citrate.

(382 ± 63 versus 957 ± 78 pmol/min/mg, n = 4, p < 0.05). Supernatants from freshly homogenized muscles contained the same amount of ACC as did frozen muscles, as judged by streptavidin blots (data not shown), but less of the other carboxylases. They also exhibited less citrate-independent 14CO2 fixation activity and a relatively greater activation by 10 mM citrate (86 ± 29 versus 610 ± 48 pmol/min/mg, n = 4, p < 0.05), suggesting that the contaminating carboxylases account principally for the citrate-independent activity.

To assay ACC in the absence of PC and PCC, a specific monoclonal antibody (7AD3) was used to immunoprecipitate ACC-β (see "Experimental Procedures"). As shown in Fig. 1A, the antibody immunoprecipitated the majority of the ACC and left behind PC and PCC. Equally important, carboxylase activity toward acetyl-CoA in the immunodepleted supernatants was not increased by 10 mM citrate, indicating that it was free of ACC activity (Fig. 1B). Nearly all (80–90%) of the citrate-dependent ACC activity present in the muscle supernatant was recovered in the immunoprecipitate, indicating that the antibody did not inhibit the enzyme. The immunopurified enzyme exhibited a Michaelis constant for acetyl-CoA ($K_m = 120 ± 4.2 \mu M$) similar to that reported by others for ACC-β purified by conventional methods (10). Thus, it appears that immunoprecipitation with the 7AD3 antibody can be a simple alternative to more conventional techniques for purifying and assaying ACC-β.

ACC activity in 7AD3 immunopellets, measured at close to physiological citrate levels (0.2 mM), was decreased by 90% after 5 min of intense muscle contractions ($78 ± 9$ versus $9.1 ± 4$ pmol/min/mg). Similar decreases in activity were observed at all assay citrate concentrations (Fig. 2A). In addition, the $K_{0.5}$ for citrate, which was $1.7 ± 0.12$ mM (mean ± S.E.) for the control muscles, was increased to $2.6 ± 0.3$ mM. Such decreases in the sensitivity and responsiveness of ACC to citrate are suggestive of increased enzyme phosphorylation (12, 15).

Decreased ACC Activity Induced by Contractions Is Attributable to Phosphorylation—Consistent with an increase in enzyme phosphorylation, the immunopurified enzyme from stimulated muscle showed a decrease in its electrophoretic mobility when subjected to SDS-polyacrylamide gel electrophoresis (Fig. 2, B and C). To obtain more direct evidence that phosphorylation decreased ACC activity, muscles were homogenized in lysis buffer with or without the phosphatase inhibitors NaF, Na2PPi, β-glycerophosphate, and Na3VO4, or without these phosphatase inhibitors but with okadaic acid present...
(another phosphatase inhibitor, see “Experimental Procedures”). As shown in Fig. 3A, homogenization in the absence of phosphatase inhibitors partially reversed the decrease in ACC activity caused by contractions, and it completely abolished it when the phosphatase activators glutamate and MgCl₂ were added to the muscle extract (see “Experimental Procedures”). Incubation of extracts from control muscles with glutamate and MgCl₂ in the absence of phosphatase inhibitors also enhanced ACC activity, although to a lesser extent. Under all conditions, these increases in ACC activity were prevented by 50 nM okadaic acid (Fig. 3A), confirming that they were due to the action of phosphatases.

Similar results were obtained when ACC isolated from stimulated muscles was treated with PP2A. In these studies, immunopellets from lysates containing phosphatase inhibitors were washed with phosphatase buffer (see “Experimental Procedures”) and then treated for 2.5 h with 500 milliunits of PP2A, 10 nM okadaic acid, and 100 mM glutamate/10 mM MgCl₂. Results are means ± S.E. of at least three separate sets of muscles.

**Fig. 3. Effect of phosphatases on ACC-β inhibition by contraction.** Muscles were made to contract by electrical stimulation of the sciatic nerve for 5 min. A, they were then homogenized in lysis buffer containing (+P.I.) or not containing (−P.I.) phosphatase inhibitors or in buffer free of phosphatase inhibitors to which 50 nM okadaic acid was added (−P.I./O.A.). Supernatants, obtained after low speed centrifugation, were incubated at 37 °C for 50 min with or without the addition of 100 mM glutamate, 10 mM MgCl₂ as indicated. ACC was immunoprecipitated (see “Experimental Procedures”), and its activity was measured at 0.2 mM citrate. B, effect of PP2A on the activity of immunoprecipitated ACC measured at 0.2 mM citrate. ACC-β immunopellets were incubated at 37 °C for 2.5 h with buffer D, either alone or containing, as indicated, 500 milliunits of PP2A, 10 nM okadaic acid, and 100 mM glutamate/10 mM MgCl₂. Results are means ± S.E. of at least three separate sets of muscles.

Time Course of Changes in the Activity of ACC-β, and of the α1 and α2 Isoforms of 5'-AMP-Activated Protein Kinase during and after Contractions—A logical candidate for regulating ACC phosphorylation during contraction is AMPK (25). To assess its role, we compared the time course of changes in the activities of ACC and the α1 and α2 isoforms of AMPK. The sciatic nerve of one limb was stimulated for periods ranging from 5 s to 5 min. Extracts (see “Experimental Procedures”) were used to immunoprecipitate and measure the activities of α1 AMPK (crosses), α2 AMPK (open diamonds), and ACC-β at 0.2 mM citrate (filled diamonds) during contractions (A) or at various intervals (0–90 min) after the cessation of 5 min of contractions (B). Results are means ± S.E. of at least three separate sets of muscles.

**Fig. 4.** The activity of ACC-β, and of the α1 and α2 isoforms of 5'-AMP-Activated Protein Kinase during and after contractions. The sciatic nerve of one limb was stimulated for periods ranging from 5 s to 5 min. Extracts (see “Experimental Procedures”) were used to immunoprecipitate and measure the activities of α1 AMPK (crosses), α2 AMPK (open diamonds), and ACC-β at 0.2 mM citrate (filled diamonds) during contractions (A) or at various intervals (0–90 min) after the cessation of 5 min of contractions (B). Results are means ± S.E. of at least three separate sets of muscles.

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ity to precontraction values followed a similar pattern. In contrast, no detectable activation of α1 AMPK was observed during the course of stimulation. A transient activation of its activity poststimulation did not achieve statistical significance ($p > 0.1$).

**DISCUSSION**

The results indicate that the activity of ACC and the concentration of malonyl-CoA in skeletal muscle decrease within seconds during intense contractions. They also strongly suggest that the decrease in ACC activity is secondary to phosphorylation, most probably due, at least in part, to activation of the α2 isoform of AMPK. In contrast, alterations in the cytosolic concentration of citrate, which acutely modulate ACC in muscle during changes in its fuel milieu (24), did not appear to play any role in the modulation of ACC activity during contraction.

The potential relevance of these findings relates to the regulation of fatty acid oxidation in muscle and the mechanism by which it is increased during and after exercise. Regulation of fatty acid oxidation by malonyl-CoA has been demonstrated in liver (1) and heart (2, 3), and it probably occurs in skeletal muscle (4–7). In the latter, malonyl-CoA levels are decreased by starvation (23) and exercise (25, 30, 31) and increased by refeeding after a fast, in keeping with the directional changes in fatty acid oxidation in these conditions. In addition, the decrease in fatty acid oxidation, observed in human muscle during a 5-h euglycemic-hypersulinemic clamp (7) is associated with a decrease in long chain acylcarnitine, suggesting inhibition of long chain fatty acyl-CoA transport into mitochondria. Although not measured, an increase in the concentration of malonyl-CoA is a likely cause for this finding. Unexplained is the observation that the inhibitory constants of malonyl-CoA for the carnitine palmitoyl transferase I isoforms that predominate in skeletal and cardiac muscle are 1–2 orders of magnitude lower than that for the carnitine palmitoyl transferase I isofrom of liver (32). Thus, at the measured concentration of malonyl-CoA in these muscles, carnitine palmitoyl transferase I should be totally inhibited. Presumably, much of the malonyl-CoA in heart and skeletal muscle is bound (33), and/or other factors, such as CoA or acetyl-CoA (34), alter its ability to inhibit carnitine palmitoyl transferase I in *vivo*.

Winder and Hardie (25), investigating the mechanism for the decline of malonyl-CoA levels in rat muscle during voluntary exercise, found that ACC activity, assayed in (NH4)2SO4 pellets, was diminished. The findings of the present study strongly suggest that this decrease in ACC activity is due to local changes induced by contraction rather than systemic factors altered by exercise. They also suggest that the decrease in ACC activity caused by contraction is due to phosphorylation of the enzyme. Evidence in support of this conclusion includes 1) the decreased electrophoretic mobility of ACC after contractions (Fig. 2B), 2) the concurrent decrease in responsiveness and sensitivity of ACC to citrate activation (Fig. 2A; Refs. 12 and 15), and 3) the restoration of the electrophoretic mobility and activity of ACC following dephosphorylation by endogenous or exogenous phosphatases, (Fig. 2C; Ref. 3). In addition, the reactivation by phosphatases was most complete when glutamate and MgCl2 were added to the medium. Gausin et al. (35) have described an ACC phosphatase in liver that is strongly activated (29-fold) by Mg2+/glutamate and inhibited by okadaic acid and that exhibits other properties of a type 2A phosphatase. In our studies, when both PP2A and MgCl2/glutamate were added to ACC-β immunopellets, ACC-β activity was increased to values equal to that of the untreated control (Fig. 3B). However, it did not completely reach the activity of ACC-β from control muscles treated with these agents, suggesting that either other phosphatases, in addition to PP2A, might be involved or that longer incubations are required to achieve maximum activation.

Several protein kinases have been shown to phosphorylate ACC obtained from liver and other tissues, with the greatest inhibition occurring when ACC is phosphorylated by the AMPK (36–45). Most studies indicate that AMPK is the major regulatory kinase acting on hepatic ACC and that ACC regulation by several effectors (glucagon, substrate depletion, inhibitors of oxidative phosphorylation, ischemia/hypoxia, arsenite, and heat shock) is due to the phosphorylation of specific serine residues (Ser-79, -1200, and -1215) on ACC (36–45). The regulation of AMPK activity occurs through alterations in cellular free 5'-AMP (36–45), such as occurs in skeletal muscle during contraction (46, 47). The mechanism by which 5'-AMP activates AMPK is complex, involving allosteric regulation of AMPK subunits and modulation of AMPK phosphorylation by AMPK kinase(s) and phosphatase(s) (48, 49).

Despite the fact that 5'-AMP is closely regulated in skeletal muscle, the effects of AMPK on muscle ACC have not been well studied. Winder and Hardie (25) have reported a modest activation of AMPK, accompanied by a decrease in ACC activity, in rat skeletal muscle following several minutes of swimming. However, the time courses of these changes in AMPK and ACC activity were not necessarily coincident (25). In the perfused rat heart, reciprocal changes in AMPK and ACC activity have been described during stop-flow ischemia/reperfusion, although again the time course of these changes leaves open the question of their cause-effect relationship (45). In both of these studies, AMPK activity was measured in crude extracts (concentrated by polyethylene glycol or (NH4)2SO4 precipitation) by SAMS peptide phosphorylation and without taking into account AMPK heterogeneity. AMPK is a heterotrimeric protein consisting of an α catalytic subunit associated with noncatalytic β and γ subunits, which are essential for optimal enzyme activity (27, 50–53). For each enzyme subunit, there is a recognized isoprotein family. In the case of the catalytic subunits, α1 and α2 polypeptides are known, each with wide tissue distribution (50). Rat skeletal muscle expresses both α1 and α2 subunits, as well as the β1 and γ1 noncatalytic subunits (50, 52). For this reason, we analyzed the effect of electrical stimulation on the two AMPK isoforms using specific antibodies to the α1 and α2 catalytic subunits. As shown in Fig. 4, the decrease in ACC activity during contractions and its restora-
tion during the recovery period were closely paralleled by reciprocal changes in α2-AMPK activity. In contrast, no significant change of α1 AMPK activity was observed. Why these AMPK isoforms are differentially regulated by contraction remains to be determined. Nevertheless, the data suggest that the α2-AMPK could be responsible, at least in part, for ACC phosphorylation under the conditions of these experiments. They do not rule out the possibility that activation of the α1 isoform by 5′-AMP also occurred but was not reflected in our assays, which were conducted at saturating concentrations of 5′-AMP (27, 28). For this reason, studies of the independent effects of the α1 and α2 isoforms on ACC-β activity in vitro need to be performed. The role of other factors, such as ACC phosphatase and/or other kinases, regulating the contraction-induced changes in ACC-β activity also warrants examination.

In contrast to the changes observed during contraction, the acute (minutes) increases in malonyl-CoA caused by incubating muscles with insulin and glucose are not due to increases in stable ACC activity (24). Rather, they correlate with increases in the cytosolic concentration of citrate, an allosteric activator of ACC, and a precursor of its substrate, cytosolic acetyl-CoA (24). In contracting muscle, however, decreases in malonyl-CoA were associated with increases in whole cell citrate and its mitochondrial antiontiser, malate, suggesting if anything an increase in cytosolic citrate. Thus, when the energy expenditure of muscle is substantially increased, phosphorylation appears to overcome the effects of citrate and becomes the dominant mechanism of ACC regulation (Fig. 5). Since free 5′-AMP levels in muscle increase markedly during contraction (46, 47), a logical conclusion is that these increases initiate ACC phosphorylation by activating AMPK. In that it is regulated by both citrate and by 5′-AMP via AMPK, ACC-β resembles another crucial metabolic enzyme, namely phosphofructokinase-I (PFK-I). PFK-I is inhibited by ATP, and this inhibition is potentiated by citrate and diminished by 5′-AMP, although without intermediation by AMPK (54, 55). An attractive hypothesis is that, by virtue of their dual effects on ACC-β and PFK-I, citrate and 5′-AMP complement each other in coordinating the regulation of these enzymes and secondarily the use of glucose and fatty acids as fuels for muscle (Fig. 5).

The standard assay for ACC activity measures the difference in acid-stable products generated by a CO2 fixation reaction in the presence and absence of exogenous acetyl-CoA. Such assays can be hindered by the presence of contaminating carboxylases (12). The most important of these appears to be propionyl-CoA carboxylase, which can catalyze the carboxylation of acetyl-CoA (to give malonyl-CoA) at 1/α10 the rate it carboxylates propionyl-CoA (56). In contrast to ACC, propionyl-CoA carboxylase is not activated by citrate (56). Thus, when present in large amounts, due to leakage from mitochondria, it could give an erroneously high value for ACC activity when assayed either in the absence of citrate or when citrate is present at low concentrations (0–0.3 mM). In keeping with this notion, extracts immunodepleted of ACC showed a large residual 13CO2 fixation activity that was not activated by citrate (Fig. 1). To avoid the problem of contaminating carboxylases, ACC was assayed after immunopurification with the monoclonal antibody 7AD3. The antibody recovered nearly all of the citrate-dependent ACC activity and protein present in the muscle supernatant, indicating that it did not inhibit ACC (Fig. 1). Finally, activation of ACC by citrate is classically portrayed as due to citrate-induced polymerization of protermic ACC subunits (57, 58). If so, when ACC is immobilized on beads during immunoprecipitation, one would not expect citrate to activate it 12-fold, as in the present study (Fig. 1). Thus, citrate activation of ACC may be dissociated from polymerization, as has been suggested by studies of chicken ACC (59–61).

In conclusion, we report here a simple assay that makes use of the monoclonal antibody 7AD3 to assay ACC-β activity in skeletal muscle, free of contaminating mitochondrial carboxylases. Using this assay, we were able to show that muscle contractions markedly diminish the activity of ACC-β within seconds. The data strongly suggest that such decreases in ACC activity are due to phosphorylation and that they are reversible by phosphatases, probably of type 2A. They also indicate that the α2 but not the α1 isoform of AMPK follows a pattern of activation/inactivation during and following contraction that closely mirrors that of ACC. Finally, the data suggest the existence of a dual mechanism for ACC regulation in muscle, in which inhibition by phosphorylation (e.g., during contraction) can take precedence over activation by citrate.

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