Activation of Malonyl-CoA Decarboxylase in Rat Skeletal Muscle by Contraction and the AMP-activated Protein Kinase Activator 5-Aminoimidazole-4-carboxamide-1-β-d-ribofuranoside*

Received for publication, May 1, 2000, and in revised form, June 2, 2000
Published, JBC Papers in Press, June 14, 2000, DOI 10.1074/jbc.C000291200

Asish K. Saha‡§, Alexandria J. Schwarsin‡, Raphael Roduit¶¶, Frédéric Massé¶, Virendar Kaushik‡, Keith Tornheim¶, Marc Prentki¶¶¶, and Neil B. Ruderman‡

From the ‡Diabetes Unit, Section of Endocrinology, and Departments of Medicine, Physiology, and Biochemistry, Boston Medical Center, Boston, Massachusetts 02118 and the §Molecular Nutrition Unit, Department of Nutrition and the ¶¶¶CR-CHUM, University of Montreal, Montreal, Québec H2L 4M1, Canada

Alterations in the concentration of malonyl-CoA, an inhibitor of carnitine palmitoyltransferase I, have been linked to the regulation of fatty acid oxidation in skeletal muscle. During contraction decreases in muscle malonyl-CoA concentration have been related to activation of AMP-activated protein kinase (AMPK), which phosphorylates and inhibits acetyl-CoA carboxylase (ACC), the rate-limiting enzyme in malonyl-CoA formation. We report here that the activity of malonyl-CoA decarboxylase (MCD) is increased in contracting muscle. Using either immunopurified enzyme or enzyme partially purified by (NH4)2SO4 precipitation, 2–3-fold increases in the V_{max} of MCD and a 40% decrease in its K_{m} for malonyl-CoA (190 versus 119 μM) were observed in rat gastrocnemius muscle after 5 min of contraction, induced by electrical stimulation of the sciatic nerve. The increase in MCD activity was markedly diminished when immunopurified enzyme was treated with protein phosphatase 2A or when phosphatase inhibitors were omitted from the homogenizing solution and assay mixture. Incubation of extensor digitorum longus muscle for 1 h with 2 mM 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside, a cell-permeable activator of AMPK, increased MCD activity 2-fold. Here, too, addition of protein phosphatase 2A to the immunopellets reversed the increase of MCD activity. The results strongly suggest that activation of AMPK during muscle contraction leads to phosphorylation of MCD and an increase in its activity. They also suggest a dual control of malonyl-CoA concentration by ACC and MCD, via AMPK, during exercise.

The role of malonyl-CoA, an inhibitor of carnitine palmitoyltransferase I, in regulating the oxidation of fatty acids in rat skeletal (1, 2) and cardiac (3, 4) muscle has been intensively investigated. Recent studies have demonstrated that its concentration in rat muscle is governed, at least in part, by changes in the activity of the muscle isoform of acetyl-CoA carboxylase (ACC_β) (5), the enzyme that catalyzes malonyl-CoA synthesis. Thus, in keeping with their observed effects on malonyl-CoA concentration and fatty acid oxidation, insulin and glucagon appear to activate ACC_β in muscle by increasing the cytosolic concentration of citrate, an allosteric activator of ACC_β and a precursor of its substrate, cytosolic acetyl-CoA. Conversely, decreases in malonyl-CoA concentration and increases in fatty acid oxidation in muscle during exercise (contraction) have been linked to decreases in ACC_β activity, attributable to its phosphorylation and inhibition by the α_2 isoform of AMP-activated protein kinase (AMPK) (5). AMPK can also be activated and the concentration of malonyl-CoA decreased by exposing resting muscle to 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), which is taken into the muscle and phosphorylated to form the 5’-AMP analogue ZMP (6).

Whether a change in malonyl-CoA turnover contributes to the alterations in its concentration in muscle during exercise and other conditions is not known. In a lipogenic tissue such as liver, the de novo synthesis of fatty acids is thought to be the major mechanism by which malonyl-CoA is utilized. In contrast, in skeletal muscle fatty acid synthesis occurs at a very low rate, if at all (7), and attention has been focused on malonyl-CoA decarboxylase (MCD) for removal of malonyl-CoA (1). Evidence has been presented that MCD is present in both cardiac (8, 9) and skeletal (1, 10, 11) muscle. In skeletal muscle, its activity is similar to that of ACC (1). In heart, in which MCD activity is substantially greater than in skeletal muscle, a decrease in the K_{m} of MCD for malonyl-CoA has been reported following an increase in its work load (9). On the other hand, no change in activity has been observed following ischemia-reperfusion of the heart, a situation in which AMPK is activated (8). The question of whether MCD is acutely regulated in skeletal muscle and, if so, how has not been studied previously.

In this study, we describe the characteristics of purified MCD from rat skeletal muscle and contraction-induced changes in its maximal activity and affinity for malonyl-CoA. In addition, the effects of the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), which is taken into the muscle and phosphorylated to form the 5’-AMP analogue ZMP (6), on the activity of MCD have been investigated. Recent studies have demonstrated that its concentration in rat muscle is governed, at least in part, by changes in the activity of the muscle isoform of acetyl-CoA carboxylase (ACC_β) (5), the enzyme that catalyzes malonyl-CoA synthesis. Thus, in keeping with their observed effects on malonyl-CoA concentration and fatty acid oxidation, insulin and glucagon appear to activate ACC_β in muscle by increasing the cytosolic concentration of citrate, an allosteric activator of ACC_β and a precursor of its substrate, cytosolic acetyl-CoA. Conversely, decreases in malonyl-CoA concentration and increases in fatty acid oxidation in muscle during exercise (contraction) have been linked to decreases in ACC_β activity, attributable to its phosphorylation and inhibition by the α_2 isoform of AMP-activated protein kinase (AMPK) (5). AMPK can also be activated and the concentration of malonyl-CoA decreased by exposing resting muscle to 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), which is taken into the muscle and phosphorylated to form the 5’-AMP analogue ZMP (6).

Whether a change in malonyl-CoA turnover contributes to the alterations in its concentration in muscle during exercise and other conditions is not known. In a lipogenic tissue such as liver, the de novo synthesis of fatty acids is thought to be the major mechanism by which malonyl-CoA is utilized. In contrast, in skeletal muscle fatty acid synthesis occurs at a very low rate, if at all (7), and attention has been focused on malonyl-CoA decarboxylase (MCD) for removal of malonyl-CoA (1). Evidence has been presented that MCD is present in both cardiac (8, 9) and skeletal (1, 10, 11) muscle. In skeletal muscle, its activity is similar to that of ACC (1). In heart, in which MCD activity is substantially greater than in skeletal muscle, a decrease in the K_{m} of MCD for malonyl-CoA has been reported following an increase in its work load (9). On the other hand, no change in activity has been observed following ischemia-reperfusion of the heart, a situation in which AMPK is activated (8). The question of whether MCD is acutely regulated in skeletal muscle and, if so, how has not been studied previously.

In addition, the effects of the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), which is taken into the muscle and phosphorylated to form the 5’-AMP analogue ZMP (6), on the activity of MCD have been investigated. Recent studies have demonstrated that its concentration in rat muscle is governed, at least in part, by changes in the activity of the muscle isoform of acetyl-CoA carboxylase (ACC_β) (5), the enzyme that catalyzes malonyl-CoA synthesis. Thus, in keeping with their observed effects on malonyl-CoA concentration and fatty acid oxidation, insulin and glucagon appear to activate ACC_β in muscle by increasing the cytosolic concentration of citrate, an allosteric activator of ACC_β and a precursor of its substrate, cytosolic acetyl-CoA. Conversely, decreases in malonyl-CoA concentration and increases in fatty acid oxidation in muscle during exercise (con-...
Regulation of Malonyl-CoA Decarboxylase in Skeletal Muscle

zole-4-carboxamide ribonucleoside (AICAR) and of treatment with phosphatases on MCD activity were examined.

EXPERIMENTAL PROCEDURES

Animals—Male Harlan Sprague-Dawley rats weighing approximately 55–70 g obtained from Charles River Laboratories (Wilmington, MA) were used except as indicated. They were housed in individual cages in a temperature-controlled room on a 12-h light cycle and fed standard purina rat chow and water ad libitum for 6 days prior to an experiment unless noted otherwise.

Rats—Rats were anesthetized with sodium pentobarbital (5.5 mg/100 g of body weight intraperitoneally), and 45 min later the skin from both hindlimbs was removed and the sciatic nerves exposed. The sciatic nerve of one limb was then stimulated for 2 and 5 min with a bipolar electrode connected to a Glass stimulator (model S48) (5 pulses/s, 100-ms trains of 2.5 V, 50 Hz, and 10-ms duration) to induce muscle contractions (5). The gastrocnemius muscles from this limb were removed and the unstimulated contralateral limb were then rapidly excised and frozen in liquid nitrogen. All tissues were stored at −80 °C until assay.

Incubation with AICAR—Rats were anesthetized with sodium pentobarbital and extensor digitorum longus (EDL) muscles were tied to stainless steel clips. Muscles were preincubated for 20 min at 37 °C in 12 × 75-mm test tubes containing 3.0 ml of Krebs-Henseleit solution containing: 5.5 mM glucose, 50 microunits/ml insulin, and 0.2% fatty acid-free bovine serum albumin as described previously (12). The media were gassed continuously with a 95% O2/5% CO2 mixture. Muscles were then transferred to different test tubes and incubated for 60 min with fresh medium with or without 2 mM AICAR. At the end of the incubation, muscles were removed, blotted on gauze pads, and frozen in liquid N2. Muscles were then homogenized in 0.1 M Tris-HCl (pH 8.0), 2 mM phenylmethylsulfonyl fluoride (PMSF), 5 μM aprotinin, 5 μM leupeptin, and 5 μM pepstatin A containing 40 mM β-glycerophosphate, 40 mM NaF, 4 mM Na3VO4, and 0.1 mM Na3VO4. To examine the effect of protein phosphatase 2A (PP2A), immunopurified MCD was washed twice with 0.1 M Tris-HCl (pH 8.0) containing 2 mM PMSF, 5 μM aprotinin, 5 μM leupeptin, and 5 μM pepstatin A and then incubated with 200 milliunits of PP2A (Upstate Biotechnology Inc., Lake Placid, NY; 1 unit of PP2A with or without 10 nM okadaic acid at 37 °C for 2.5 h. For the experiments of PP2A with or without 10 nM okadaic acid at 37 °C for 2.5 h. For the

MCD Assay—MCD activity was measured spectrophotometrically (13, 14) using a Hewlett-Packard model 8450A diode array spectrophotometer as described previously (10). In brief, the (NH4)2SO4-purified fraction from 150 μl of the muscle homogenate was added to a 700-μl reaction mixture composed of 0.1 M Tris-HCl (pH 8.0), 0.5 mM dithiothreitol, 10 mM l-malate, 0.5 mM NAD+, and 10 μg of malate dehydrogenase (1.0 unit) and preincubated for 10 min at room temperature, in the presence of phosphatase inhibitors (40 mM β-glycerophosphate, 40 mM NaF, 4 mM NaPPi, and 1 mM Na3VO4) except as noted. Citrate MCD was added, and the preincubuation was continued for an additional 2 min. Malonyl-CoA (0.3 mM) was then added to start the MCD reaction and the rate of NADH formation was measured over 7 min. Controls were run to correct for the small rate of NADH oxidation obtained when malonyl-CoA was not added. The optimum pH range for MCD was determined using the following buffers: 0.1 mM sodium acetate (pH 4.0–6.5), 0.1 mM sodium phosphate (pH 6.0–8.0), 0.1 mM Tris-HCl (pH 7.5–9.0), and 0.1 mM glycine NaOH (pH 8.0–10.0). Protein concentration was determined by the method of Bradford with bovine serum albumin as the standard (15). Immunopurified MCD was assayed in an identical manner except that the immunoprecipitate (~20 μl) was added to 100 μl of the 0.1 M Tris-HCl buffer before addition to the reaction mixture. Activity in all instances is expressed per mg protein in the 500 × g supernatant of the whole tissue extract.

Statistical Analysis—Results are expressed as means ± S.E. Statistical differences between multiple groups were determined by analysis of variance followed by the Student-Newman-Keuls multiple comparison test.

RESULTS

Studies of MCD Activity Using Enzyme Purified by (NH4)2SO4 Precipitation—After partial purification of skeletal muscle MCD with (NH4)2SO4, the maximal rate of malonyl-CoA decarboxylation was found between pH 7 and 8 (data not shown), a range similar to that previously reported for MCD in rat heart (13, 16), brain (14), and heart (8, 9). The rate of formation of acetyl-CoA increased with increasing concentrations of malonyl-CoA, and a typical Michaelis-Menten type substrate saturation pattern was observed (data not shown). From linear double-reciprocal plots, a Km for malonyl-CoA of 190 ± 13 μM (n = 5) was obtained for control muscle extracted in the presence of phosphatase inhibitors, a value similar to that found by Prentki and his co-workers for skeletal muscle MCD2 but 2–3-fold higher than that reported for MCD in rat heart (8, 9), liver (13), and brain (14). The effect of contraction on MCD activity was studied next. As shown in Fig. 1, MCD activity was increased more than 3-fold in gastrocnemius muscles after 5 min of electrically induced contractions. The Km for MCD for malonyl-CoA from these muscles was decreased to 119 ± 14 μM (n = 5) after stimulation of contractile activity. When the tissue was processed and the MCD assay performed in the absence of phosphatase inhibitors, the increase in activity was still observed; however, it was diminished by 50% (Fig. 1). Studies of MCD Purified by Immunoprecipitation—We next examined whether it was possible to assay MCD when it was immunoprecipitated by a N-terminal affinity-purified antibody. As shown in Fig. 2, the antibody immunoprecipitated nearly 75% of the MCD activity (Vmax) present in the muscle supernatant. In addition, the antibody immunoprecipitated over 90% of the MCD in the 500 × g supernatant as judged by Western blotting (data not shown). MCD activity following immunoprecipitation was very similar to that observed after (NH4)2SO4 precipitation (Fig. 1). For instance in control unstimulated muscles MCD activity was 3.5 ± 0.35 nmol/min/mg of initial supernatant protein when assayed after (NH4)2SO4 precipitation (Fig. 1) and 5.0 ± 0.9 and 3.8 ± 0.3 nmol/min/mg of protein (Figs. 2 and 3, respectively) when measured after immunoprecipitation.

2 M. Prentki, R. Roduit, and F. Masse, unpublished data.
Regulation of Malonyl-CoA Decarboxylase in Skeletal Muscle

**FIG. 1.** Effect of contractions induced by sciatic nerve stimulation on MCD activity in rat gastrocnemius muscle. Rats were anesthetized, and the sciatic nerve was either sham-operated (control) or electrically stimulated for 5 min (5 pulses/s, 100-ms trains of 2.5 V, 50 Hz frequency, and 10-ms duration). Gastrocnemius muscles were then excised and frozen in liquid N2. They were homogenized in 0.1 M Tris-HCl (pH 8.0) buffer in the absence (−PI) or presence (+PI) of phosphatase inhibitors (40 mM β-glycerophosphate, 40 mM NaF, 4 mM NaPPi, and 1 mM Na3VO4). (NH4)2SO4-purified enzyme from these muscles was assayed for MCD activity. Results are means ± S.E. of three separate studies. MCD activity was increased 2-fold from 3.8 to 8.0 nmol/min/mg of protein in gastrocnemius muscles after 5 min of electrically induced contractions. When immunoprecipitates from these muscles were treated with PP2A (200 milliunits), the observed nonprecipitable MCD activity 2-fold. Furthermore when PP2A was added to the immunopellets, it reversed the increase in MCD activity caused by contraction, which correlates well with previously published data from our laboratory (5) and that of Winder (6) showing activation of AMPK and inhibition of ACC at these times. To illustrate the comparison, we have included in Fig. 4 our previously published data (5) for AMPK activation and ACC inhibition after electrically induced contractions. Similar alterations in AMPK and ACC activity have also been observed when muscle is perfused (6) or incubated (17) with the cell-permeable AMPK activator AICAR. As shown in Fig. 5, incubation of the EDL with 2 mM AICAR for 1 h increased immunoprecipitable MCD activity 2-fold. Furthermore when PP2A was added to the immunopellets, it reversed the increase in MCD activity caused by AICAR (Fig. 5), much as it did the increase in MCD activity caused by contraction. Parenthetically, the lower activity of MCD in the EDL than in the gastrocnemius (Fig. 1) has been observed previously (1).

**FIG. 2.** An antibody to the N-terminal region of MCD quantitatively immunoprecipitates MCD activity in an extract of rat muscle. Extracts from frozen gastrocnemius (300–350 µg of protein) were incubated for 3 h with 4 µg of antibody and 20 µl of A/G-agarose beads. MCD activity was assayed in the extract prior to (PRE) and after (POST) immunoprecipitation or directly in the immunoprecipitate (I.P.). Results are means ± S.E. of three separate studies. MCD activity in all fractions is expressed per milligram of extract protein in the original 500 × g supernatant subjected to immunoprecipitation. One mg of protein in the supernatant corresponds to 6 mg of tissue wet weight.

As shown in Fig. 3, MCD activity in the immunoprecipitate was increased 2-fold from 3.8 ± 0.3 to 8.0 ± 0.8 nmol/min/mg of protein in gastrocnemius muscles after 5 min of electrically induced contractions. When immunoprecipitates from these muscles were treated with PP2A (200 milliunits), the observed increase in activity was markedly diminished (Fig. 3A), an effect prevented when the phosphatase inhibitor okadaic acid (10 nM) was added to the medium. As shown in Fig. 3B, treatment with higher concentrations of PP2A caused further decreases in MCD activity in both control and contracting muscle. The addition of phosphatase activators (sodium glutamate and MgCl2) to muscle extracts containing no phosphatase inhibitors decreased MCD activity both in control (4.0 ± 0.3 versus 3.0 ± 0.2 nmol/min/mg) and stimulated (8.0 ± 0.5 versus 4.5 ± 0.3 nmol/min/mg) muscles, further suggesting that the increase in MCD activity caused by contraction is related to phosphorylation.

Changes in MCD Activity in Relation to Activation of AMPK—A logical candidate for regulating MCD phosphorylation during contraction is AMPK (5). As shown in Fig. 4, MCD activity is increased even as early as after 2 min of muscle contraction, which correlates well with previously published data from our laboratory (5) and that of Winder (6) showing activation of AMPK and inhibition of ACC at these times. To illustrate the comparison, we have included in Fig. 4 our previously published data (5) for AMPK activation and ACC inhibition after electrically induced contractions. Similar alterations in AMPK and ACC activity have also been observed when muscle is perfused (6) or incubated (17) with the cell-permeable AMPK activator AICAR. As shown in Fig. 5, incubation of the EDL with 2 mM AICAR for 1 h increased immunoprecipitable MCD activity 2-fold. Furthermore when PP2A was added to the immunopellets, it reversed the increase in MCD activity caused by AICAR (Fig. 5), much as it did the increase in MCD activity caused by contraction. Parenthetically, the lower activity of MCD in the EDL than in the gastrocnemius (Fig. 1) has been observed previously (1).

**FIG. 3.** Effect of PP2A on the activity of MCD immunoprecipitated from contracting muscle. Muscles were stimulated and isolated as described in the legend to Fig. 1. MCD immunopellets were incubated at 37 °C for 2.5 h as described under “Experimental Procedures.” A, experiments with 200 milliunits of PP2A ± 10 nM okadaic acid (O.A.). B, dose dependence of PP2A action. Results are means ± S.E. of three separate sets of muscles.

**DISCUSSION**

The principal findings of this study are: 1) that MCD activity in skeletal muscle is acutely increased after contraction and 2) that this is likely due to its phosphorylation by AMPK. Acute decreases in the concentration of malonyl-CoA in rat skeletal muscle occurs primarily by suppression of MCD activity, most likely by phosphorylation of MCD by AMPK. The increase in AMPK activity observed after 2 min of contraction indicates that AMPK is activated by AMP, which is increased after contraction due to the release of AMP from ATP hydrolysis. The decrease in AMPK activity after 30 min of contraction may be due to the decrease in AMP caused by the decrease in ATP hydrolysis. The decrease in ACC activity after 2 min of contraction may be due to the decrease in AMPK activity caused by AMPK. The decrease in ACC activity after 30 min of contraction may be due to the decrease in ACC caused by the decrease in AMPK activity caused by AMPK.
skeletal muscle during exercise (18–20) and during electrically induced contractions in hindlimb muscle (5, 21) very likely contribute to the increase in fatty acid oxidation in these situations. Thus, studies from our laboratory (12, 21–23) and that of Winder (18, 19) indicate that such decreases in malonyl-CoA concentration are associated with reduced ACC activity. As shown by Vavvas et al. (5), ACC is phosphorylated and inhibited by the α2 isoform of AMP-activated protein kinase within seconds of the onset of muscle contraction, and its activity is diminished by 80% after as little as 2 min. The results of a present study suggest that another factor contributing to a decrease in malonyl-CoA and increase in fatty acid oxidation in muscle tissue could be an increase in MCD activity. Thus, MCD activity was increased 2–3-fold after 2 and 5 min of contraction. The increase in MCD activity observed during contraction was twice as great when phosphatase inhibitors were added to the homogenizing solution, suggesting it was attributable to phosphorylation (Fig. 1). In keeping with this conclusion, the increase in MCD activity was substantially reduced when immunoprecipitate enzyme was incubated with protein phosphatase 2A (Fig. 3, A and B) or when phosphatase inhibitors were omitted from the homogenizing solution (Fig. 1), an effect magnified when the phosphatase activators MgCl2 and glutamate were added. The data also strongly suggest that the activation and phosphorylation of MCD during contraction is mediated by AMPK. Several lines of evidence support this conclusion: 1) the time course of MCD activation during contraction parallels that of AMPK activation and ACC inhibition (Fig. 4) reported previously by us (5) and others (6), 2) MCD activity was increased in the EDL when it was incubated with the AMPK activator AICAR (Fig. 5), and 3) the increase in MCD activity was diminished by incubation of the immunopellet with PP2A. Consistent with possible regulation by AMPK, rat MCD possesses 34 serine residues (11), including several that could be in a recognition motif for AMPK (24). Definitive studies showing phosphorylation of specific sites on purified MCD by AMPK have not yet been reported, however. Interestingly, treatment of semi-purified MCD from rat heart with alkaline phosphatase has been shown to increase MCD activity (8). Thus, phosphorylation of MCD on sites other than that phosphorylated by AMPK probably inhibit the enzyme. The observation that immunoprecipitable MCD activity is still somewhat higher in contracting and AICAR-treated muscles than in control muscles following PP2A treatment suggest either that dephosphorylation was incomplete or that some other factors are involved in MCD regulation.

The finding that both contraction and AICAR activate MCD...
and inhibit ACCβ (5, 6) suggests that the two enzymes are jointly regulated by AMPK (Fig. 6). It also supports the view that both ACCα and MCD participate in regulating the concentration of malonyl-CoA in skeletal muscle. The latter could be difficult to prove, since molecular biological (11) and cell fractionation (25) studies suggest localization of MCD isoforms in mitochondrial, peroxisomal, and possibly cytosolic fractions in various cells. Thus, the activity of MCD in these three fractions, as well as the relative effect of AMPK activation on MCD activity in each of these locations, will need to be evaluated.

The relevance of the changes in MCD activity reported here to other tissues remains to be determined. Dyck et al. (8) did not observe an increase in MCD activity in rat heart during ischemia-reperfusion, a situation in which they had previously observed an increase in AMPK activity. In contrast, Goodwin and Taegtmeyer (9) found a 40% increase in MCD activity at subsaturating concentrations of malonyl-CoA in a perfused rat heart, when its work load was increased by the combination of 1 μM epinephrine and a 40% increase in afterload from 100 to 140 cm H2O. However, they did not observe an increase in the Vmax of the enzyme as we did here. Whether this reflects a difference in the tissue studied, the nature of the increase in muscle work, or the different MCD assays used in the two studies remains to be determined. Also requiring further study is the observation of Goodwin and Taegtmeyer (9) that the increase in fatty acid oxidation that occurs when heart work is increased correlates with an increase in MCD activity, but not with a decrease in assayed ACCβ activity. ACCβ activity in that study (9) was measured on the basis of the citrate activation of 14CO2 fixation, without first purifying the enzyme. As previously noted by Thampy (26), when this is done the ACCβ assay may be unreliable due to the presence of high but variable activities of propionyl-CoA carboxylase. Thus, the conclusion that MCD can regulate malonyl-CoA concentration and fatty acid oxidation independently of ACCβ remains open to question.

In conclusion, the results presented here indicate that MCD activity in muscle is increased by contraction and by incubation with AICAR. Previous work has shown that ACCβ is phosphorylated and inhibited under these conditions (5). Since a common factor in these situations is an increase in AMPK activity, this suggests that MCD and ACCβ may be jointly regulated by AMPK. The importance of this dual control of MCD and ACCβ activities to the regulation of the cytosolic concentration of malonyl-CoA and secondarily to fatty acid oxidation remains to be determined.

Acknowledgment—We gratefully acknowledge the expert technical assistance of Holly Couture.

REFERENCES
1. Alam, N., and Saggerson, E. D. (1998) Biochem. J. 334, 233–241
2. Ruderman, N. B., Saha, A. K., Vavvas, D., and Witters, L. A. (1999) Am. J. Physiol. 276, E1–E8
3. Awan, M. M., and Saggerson, E. D. (1993) Biochem. J. 295, 61–66
4. Kudo, N., Barr, A. J., Barr, B. L., Deasi, S., and Lopaschuk, G. D. (1995) J. Biol. Chem. 270, 17513–17520
5. Vavvas, D., Apazidis, A., Saha, A. K., Gamble, J., Patel, A., Kemp, B. E., Witters, L. A., and Ruderman, N. B. (1997) J. Biol. Chem. 272, 13255–13261
6. Merrill, G. F., Kurth, E. J., Hardie, D. G., and Winder, W. W. (1997) Am. J. Physiol. 273, E1107–E1112
7. Chen, K. S., Heydrick, S. J., Brown, M. L., and Ruderman, N. B. (1994) Am. J. Physiol. 266, E479–E485
8. Dyck, J. R. B., Barr, A. J., Barr, R. L., Kolattukudy, P. E., and Lopaschuk, G. D. (1998) Am. J. Physiol. 275, H2122–H2129
9. Goodwin, G. W., and Taegtmeyer, H. (1999) Am. J. Physiol. 277, E772–E777
10. Chien, D., Dean, D., Saha, A. K., Flatt, J. P., and Ruderman, N. B. (2000) Am. J. Physiol. 279, E259–E265
11. Volley, N., Roduit, R., Vicaretti, R., Bonny, C., Waecher, G., Dyck, J., Lopaschuk, G. D., and Prentki, M. (1999) Biochem. J. 340, 213–217
12. Saha, A. K., Kurowski, T. G., and Ruderman, N. B. (1995) Am. J. Physiol. 269, E283–E289
13. Kim, Y. S., and Kolattukudy, P. E. (1978) Arch. Biochem. Biophys. 190, 234–246
14. Kim, Y. S., Kolattukudy, P. E., and Boos, A. (1979) Int. J. Biochem. 10, 551–555
15. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
16. Jang, S. H., Cheesbrough, T. M., and Kolattukudy, P. E. (1989) J. Biol. Chem. 264, 3560–3565
17. Hayashi, T., Hirshman, M. F., Fujii, N., Hahnisovski, S. A., Witters, L. A., and Goodyear, L. J. (2000) Diabetes 49, 527–531
18. Rasmussen, B. B., and Winder, W. W. (1997) J. Appl. Physiol. 83, 1104–1109
19. Winder, W. W., and Hardie, D. G. (1996) Am. J. Physiol. 270, E299–E304
20. Huber, C. A., Hardie, D. G., and Winder, W. W. (1997) Am. J. Physiol. 272, E262–E266
21. Saha, A. K., Kurowski, T. G., Colea, J. R., and Ruderman, N. B. (1994) Am. J. Physiol. 267, E95–E101
22. Saha, A. K., Laybutt, D. S., Dean, D., Vavvas, D., Ellis, B., Kraegen, E. W., Shafrir, E., and Ruderman, N. B. (1999) Am. J. Physiol. 276, E1030–E1037
23. Saha, A. K., Vavvas, D., Kurowski, T. G., Apazidis, A., Witters, L. A., Shafrir, E., and Ruderman, N. B. (1997) Am. J. Physiol. 273, E941–E948
24. Hardie, D. G., Carling, D., and Carlson, M. (1998) Annu. Rev. Biochem. 67, 821–855
25. Sacksteder, K. A., Morrell, J. C., Wanders, R. J. A., Matalon, R., and Gould, S. J. (1999) J. Biol. Chem. 274, 24461–24468
26. Thampy, K. G. (1989) J. Biol. Chem. 264, 17631–17634