

## AMPK as a metabolic switch in rat muscle, liver and adipose tissue after exercise

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### Abstract

An increasing body of evidence has revealed that activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK)-activated protein kinase increases fatty acid oxidation by lowering the concentration of malonyl coenzyme A (CoA), an inhibitor of carnitine palmitoyl transferase 1. Studies carried out primarily in skeletal muscle suggest that AMPK modulates the concentration of malonyl CoA by concurrently phosphorylating and inhibiting acetyl CoA carboxylase (ACC), the rate limiting enzyme in malonyl CoA synthesis, and phosphorylating and activating malonyl CoA decarboxylase (MCD), an enzyme involved in its degradation. We have recently observed that AMPK and MCD activities are increased and ACC activity diminished in skeletal muscle, liver and, surprisingly, in adipose tissue 30 min following exercise (treadmill run) in normal rats. In liver and adipose tissue these changes were associated with a decrease in the activity of glycerol-3-phosphate acyltransferase (GPAT), which catalyses the first committed reaction in glycerolipid synthesis and, which like ACC, is phosphorylated and inhibited by AMPK. Similar changes in ACC, MCD and GPAT were observed following the administration of 5-aminoimidazole 4-carboxamide-riboside (AICAR), further indicating that the exercise-induced alterations in these enzymes were AMPK-mediated.

**Conclusions:** (1) AMPK plays a major role in regulating lipid metabolism in multiple tissues following exercise. (2) The net effect of its activation is to increase fatty acid oxidation and diminish glycerolipid synthesis. (3) The relevance of these findings to the regulation of muscle glycogen repletion in the post-exercise state and to the demonstrated ability of AMPK activation to decrease adiposity and increase insulin sensitivity in rodents remains to be determined.

**Keywords** fatty acid oxidation, glycerolipid synthesis, malonyl coenzyme A, malonyl coenzyme A decarboxylase, glycerol 3-phosphate acyltransferase, glycogen synthesis, 5-aminoimidazole 4-carboxamide riboside, exercise.

A major factor regulating intracellular fatty acid oxidation is malonyl coenzyme A (CoA), an allosteric inhibitor of carnitine palmitoyltransferase (CPT1), the enzyme that controls the transfer of cytosolic long chain fatty acyl CoA (LCFA CoA) into mitochondria (McGarry & Brown 1997, McGarry, 2002). A large body of evidence has

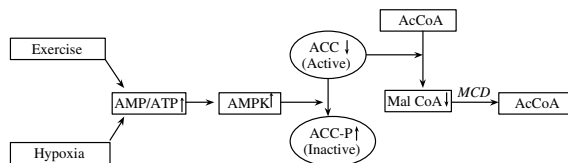
suggested that malonyl CoA levels diminish in exercising (contracting) rat and possibly human muscle as a result of the phosphorylation of acetyl CoA carboxylase (ACC), the rate limiting enzyme in its synthesis (Ruderman *et al.* 1999, Winder & Hardie 1999, Dean *et al.* 2000, Winder & Hardie, 1996). The evidence also suggests that this

phosphorylation reaction is catalysed by adenosine monophosphate (AMP)-activated protein kinase (AMPK), an enzyme activated in many cells by a change in their energy state, as reflected by an increase in the AMP/ATP ratio (see Fig. 1). In this brief review, we will examine three lines of investigation from our laboratory that impact on this classic view of AMPK regulation and its effects on fatty acid metabolism during exercise:

- (1) that AMPK also regulates malonyl CoA levels in muscle by activating malonyl CoA decarboxylase (MCD),
- (2) that AMPK activation is present well after the completion of exercise in muscle, and also in liver and adipose tissue,
- (3) these increases in AMPK post-exercise are associated with alterations in ACC and MCD activity in all three tissues and with a decrease in the activity of another AMPK-sensitive enzyme, glycerophosphate acyltransferase (GPAT), in liver and adipose tissue.

### Malonyl CoA decarboxylase

Malonyl CoA decarboxylase catalyses the decarboxylation of malonyl CoA to form acetyl CoA. In liver, the



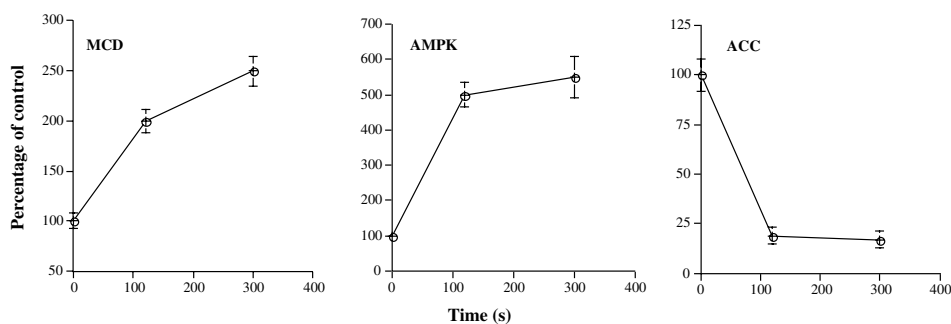
**Figure 1** Activation of AMP-activated protein kinase (AMPK) in muscle by exercise or hypoxia leads to phosphorylation and inhibition of acetyl CoA carboxylase (ACC), which in turn leads to a decrease in the concentration of malonyl CoA, which increases fatty acid entrance into the mitochondria where it is oxidized.

enzyme is to a considerable extent localized to mitochondria and peroxisomes by specific targeting sequences (Voilley *et al.* 1999); however, a modest but significant percentage of enzyme protein appears to be cytosolic (M. Bendayan, M. Prentki, A.K. Saha, N. Ruderman, unpublished data). Its distribution in skeletal muscle is not known. Presumably the role of this cytosolic MCD is to jointly regulate malonyl CoA levels with ACC, at least in selected situations. This notion had not been directly tested in skeletal muscle, however. For this reason, we assayed MCD activity in 500 g supernatant fractions of control and contracting rat muscle using both (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitation and immunoprecipitation with an antibody specific for the N-terminus of MCD to obtain purified enzyme (Saha *et al.* 2000). We found that contractions induced by electrical stimulation of the sciatic nerve caused a 2-fold increase in MCD activity within 2 min and that this was paralleled temporally by increases and decreases in the activity of AMPK and ACC, respectively (Fig. 2). Similar changes were also observed in a rat extensor digitorum longus muscle preparation incubated in a medium containing the AMPK activator, AICAR. In both situations, the alterations in MCD and ACC activity were reversed by treatment with protein phosphatase 2A, suggesting they were because of phosphorylation (Fig. 3).

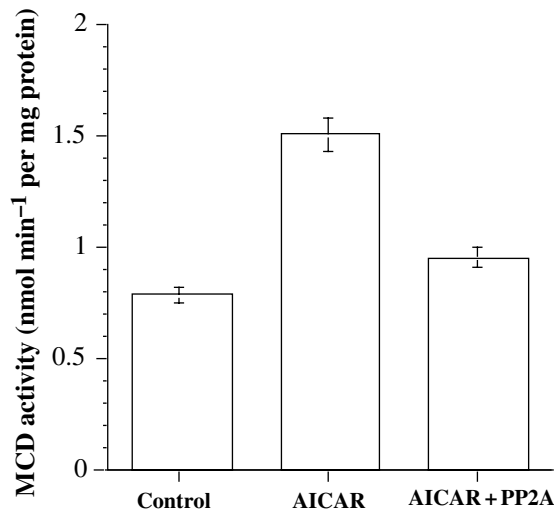
We concluded from these results that ACC and the cytosolic component of MCD are concurrently regulated by AMPK in contracting muscle and that observed changes in malonyl CoA are likely regulated by both enzymes.

### The post-exercise state: skeletal muscle

Following exercise, muscle oxidizes predominantly fatty acids to generate ATP, and glucose taken up by the muscle cell is used predominantly to replete glycogen stores (Ahlborg & Felig 1982, Bjorkman & Wahren



**Figure 2** Time course of changes in the activities of MCD, ACC and the  $\alpha_2$  isoform of AMPK during contractions. The sciatic nerve of one hindlimb was stimulated for periods ranging from 2 to 5 min and the other limb was used as the control. Immunoprecipitates were used to measure the activities of MCD, ACC and  $\alpha_2$  AMPK. Results are mean  $\pm$  SE of four to five sets of muscles (from Saha *et al.* 2000).



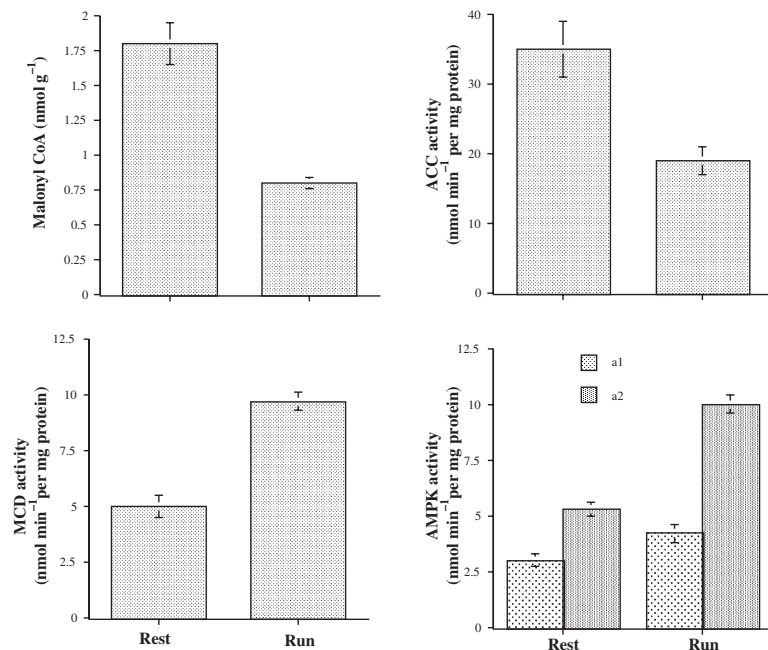
**Figure 3** Effect of AICAR on immunoprecipitable malonyl CoA decarboxylase activity. Muscles were incubated in the presence or absence of AICAR (2 mM) for 1 h. Some of the MCD immunopellets from the AICAR-incubated muscles were incubated at 37 °C for 2.5 h with 200 mU of protein phosphatase 2A (PP2A). Results are mean  $\pm$  SE of 10–12 experiments (from Saha *et al.* 2000).

1988, Wolfe *et al.* 1990, Wasserman *et al.* 2002). Changes in ACC and AMPK in skeletal muscle have been shown to persist for 15–90 min post-exercise (Rasmussen *et al.* 1998) or electrically-induced contractions (Vavvas *et al.* 1997, Hutber *et al.* 1997), and could play a role in modulating these events. To examine this question, rats weighing 320–360 g were

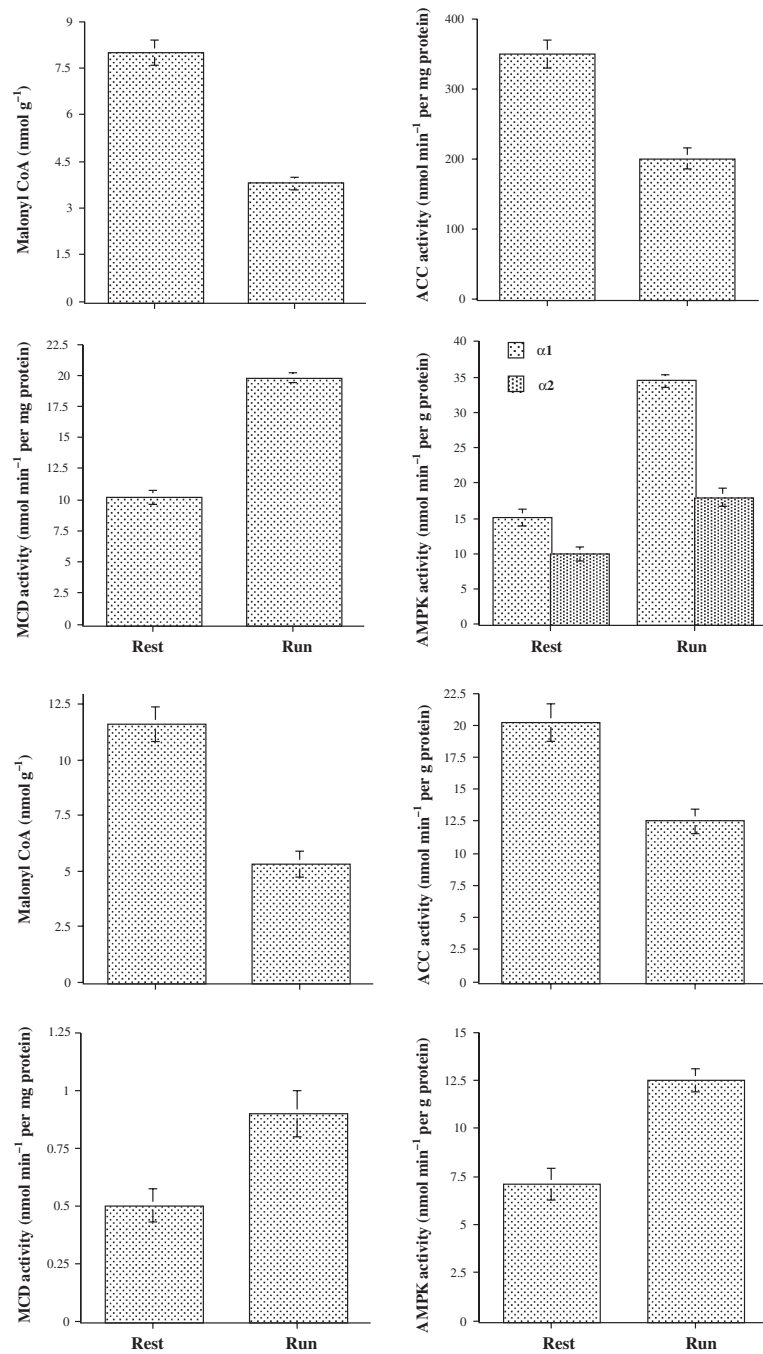
run for 30 min on a treadmill (21 m min<sup>-1</sup> on a 12% grade), anesthetized with pentobarbital, and muscle and other tissues analysed for AMPK, ACC, MCD and GPAT. As shown in Figure 4, 30 min after the cessation of the treadmill run, increases in  $\alpha$ 2-AMPK activity in muscle were present, and they were associated with significant increases in MCD activity and decreases in ACC activity and malonyl CoA concentration. No change was observed in the activity of the mitochondrial or microsomal form of GPAT (see below).

### The post-exercise state: liver and adipose tissue

Increases in AMPK activity accompanied by decreases in malonyl CoA content and ACC activity have been noted in rat liver following exercise in some circumstances, but not others (Carlson & Winder 1999). In addition, MCD and GPAT were not determined in these studies, nor was the effect of the exercise on adipose tissue examined. As shown in Figure 5, changes in AMPK, ACC, MCD and malonyl CoA very similar to those in muscle occurred in both liver and adipose tissue. In addition, the activity of mitochondrial GPAT, which was not altered in muscle, was diminished by approximately 50% in these tissues (Fig. 6). No change in microsomal GPAT was observed. Also of note, the exercise caused proportionate increases in the activities of the  $\alpha$ 1- and  $\alpha$ 2-AMPK isoforms in liver, whereas in muscle predominantly  $\alpha$ 2-AMPK activity was increased (Figs 4 and 5). Overall, the net effect of these changes should



**Figure 4** Effect of exercise on the concentration of malonyl CoA and the activities of AMPK ( $\alpha$ 1 and  $\alpha$ 2 isoforms), ACC and MCD in skeletal muscle. Rats were run on a treadmill for 30 min as described in the text. Values are mean  $\pm$  SE;  $n = 6$  rats in each group. \*Significantly different from resting control (adapted from Park *et al.* 2002).



**Figure 5** Effect of 30 min of treadmill running on malonyl CoA concentrations and the activities of AMPK, ACC and MCD in (top) liver and (bottom) adipose tissue. Only total AMPK activity was assayed in adipose tissue. See legend to Figure 4 for details.

be to increase fatty acid oxidation and decrease its esterification in liver and adipose tissue (Fig. 7).

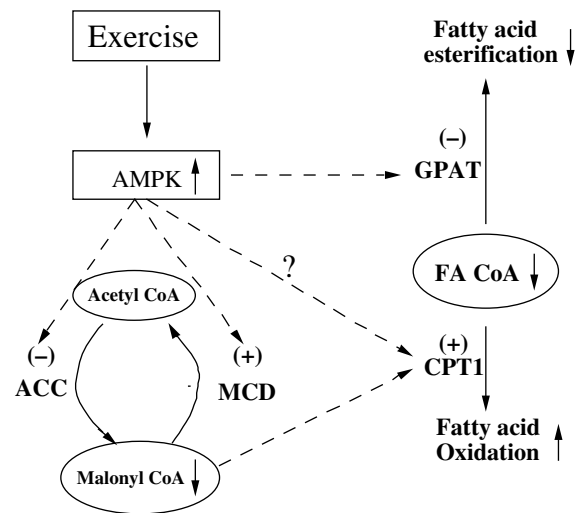
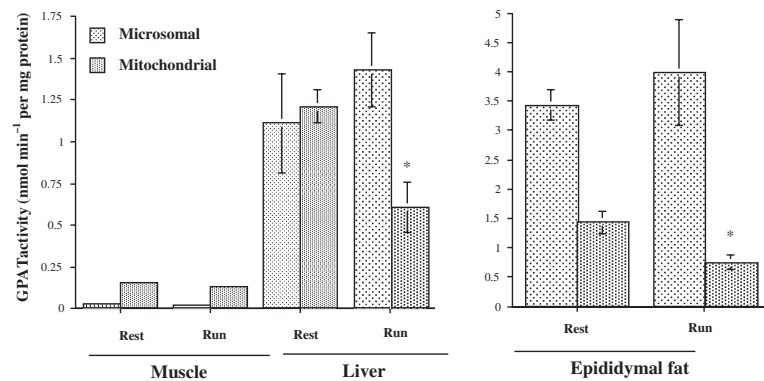
**Effect of AICAR administration *in vivo***

That the observed enzymic changes were attributable to AMPK activation was further suggested by the observation that nearly identical changes in ACC, MCD and GPAT were observed in the three tissues 2 h following the subcutaneous administration of AICAR (250 mg kg<sup>-1</sup> bw) (Park *et al.* 2002).

**Discussion**

As a metabolic switch during exercise, AMPK has been viewed primarily in light of its ability to inhibit ACC and stimulate fatty-acid oxidation and insulin-independent glucose transport in skeletal muscle (Winder & Hardie 1999, Musi *et al.* 2001). The data reviewed here suggest that this perception of AMPK is incomplete. Thus, they indicate that (1) changes in AMPK as a result of exercise occur in tissues other than muscle, (2) the activities of ACC, MCD and GPAT are concurrently

**Figure 6** Effect of exercise on GPAT activity in skeletal muscle, liver and adipose tissue. See legend to Figure 4 for details.



**Figure 7** Coordinated changes in enzymes of lipid metabolism after exercise because of activation of AMPK: a proposed model. AMPK activation causes changes in ACC and MCD activity that lower the concentration of malonyl CoA in liver, muscle and adipose tissue. In addition, AMPK activation causes inhibition of GPAT in liver and adipose tissue. It has also been suggested that AMPK phosphorylates a cytosolic protein that activates CPT1 in liver, although this remains to be confirmed. The net effect of these changes is to increase fatty acid oxidation and decrease its esterification (from Park *et al.* 2002).

altered, and (3) alterations in these parameters persist or, to be more precise, are present well after the completion of exercise, when the energy state of the tissues should have long as returned to control values.

The effect of prior exercise on AMPK activity in tissues other than skeletal muscle has received little attention. In the present study, rats were run at a rate of 21 m min<sup>-1</sup> for 30 min on a 12% grade and were evaluated 30 min after the cessation of exercise. In rats run at a similar intensity, but for a longer time (16 m min<sup>-1</sup> × 120 min on a 15% grade) Carlson & Winder (1999) failed to observe an increase in AMPK or a decrease in ACC activity in liver 0, 15 and 60 min

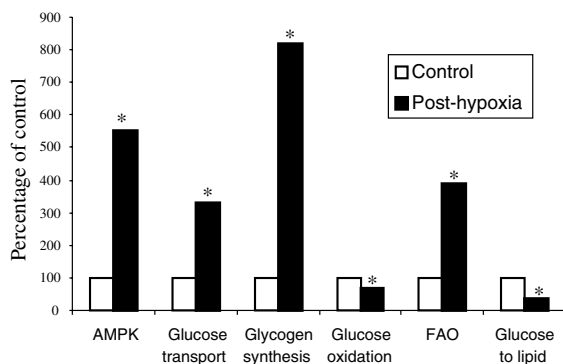
post-exercise. On the other hand, they did find changes in these enzymes after brief, intense exercise (32 m min<sup>-1</sup> × 10 min). The reason for these differing results remains to be determined.

The coordinate changes of MCD and ACC in muscle, liver and adipose tissue after exercise underscores the apparent importance to these tissues of closely regulating the concentration of malonyl CoA. Such dual regulation of malonyl CoA synthesis and degradation by AMPK would presumably allow for more rapid and larger decreases in its concentration, and increases in fatty acid oxidation, during and after exercise. In light of this, it is surprising that the decrease in malonyl CoA concentration in these situations is generally at most 2–3-fold. As discussed elsewhere, this could be explained if malonyl CoA is compartmented, and its whole-cell concentration does not accurately reflect its concentration in the immediate vicinity of CPT1 (McGarry & Brown 1997, Ruderman *et al.* 1999).

A novel finding was that decreases in the activity of GPAT, the first committed enzyme in the synthesis of triglycerides and other glycerolipids, were present in liver and adipose tissue after exercise and they closely paralleled changes in AMPK, ACC and MCD. Early studies suggested that AMPK activation might diminish the synthesis of glycerolipids by decreasing the concentration of malonyl CoA, and secondarily increasing fatty acid oxidation and diminishing the cytosolic concentration of LCFA CoA (Ruderman *et al.* 1999). Evidence that diminished glycerolipid synthesis could be related to AMPK-mediated inhibition of GPAT was first presented by Muoio *et al.* (1999). In studies with cultured hepatocytes, they found that AICAR-induced increases in fatty acid oxidation (40–50%) were associated with inhibition of both triacylglycerol synthesis (30–40%) and mitochondrial GPAT activity (20–30%). They also found that incubation of hepatic mitochondria with recombinant AMPK produced a similar decrease in GPAT activity, suggesting that the effect of AICAR in the intact cell was AMPK-mediated (Muoio *et al.* 1999). Interestingly, AICAR had similar effects on fatty acid oxidation and esterification in an

incubated rat soleus muscle preparation and it also decreases malonyl CoA (Alam & Saggerson 1998); however, they were not associated with a decrease in mitochondrial GPAT (Muoio *et al.* 1999). The investigators attributed this to the very low specific activity of GPAT in skeletal muscle. In keeping with the findings after exercise described here, they observed no change or a very delayed change in microsomal GPAT activity in hepatocytes incubated with AICAR.

The data are consistent with the notion that AMPK modulates many of the metabolic events that take place in skeletal muscle following, as well as during exercise. We have addressed this question in a model similar to that of prior exercise, muscle post-hypoxia. Thus, in EDL pre-incubated in a medium gassed with 95% N/5% CO<sub>2</sub> for 60 min and then reoxygenated for 30 min, we found sustained increases in AMPK activity together with increases in insulin-independent glucose transport (not shown) and fatty acid oxidation and decreases in glucose incorporation into lipid and to a lesser extent its oxidation to CO<sub>2</sub> (Fig. 8). Similar changes were observed after incubation with AICAR. These changes were associated with a greater than 8-fold increase in glycogen synthesis. AMPK activation probably contributes to glycogen repletion in muscle by increasing glucose transport and the concentration of glucose 6-phosphate, an activator of glycogen synthase. A direct effect of AMPK to activate glycogen synthase has been observed in some muscle fibre types by Goodyear and her co-workers (Aschenbach *et al.* 2002); however, others (Wojtaszewski *et al.* 2002) have shown that activation of AMPK by AICAR inhibits glycogen synthase in all three fibre types. Finally, two reports have suggested that activation of AMPK in muscle could be responsible for the enhanced sensitivity of the muscle cell to insulin that can persist for many hours following



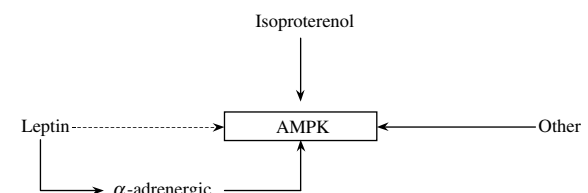
**Figure 8** Hypoxic muscles were pre-incubated in media gassed with 95% N/5% CO<sub>2</sub> and control muscles with 95% O<sub>2</sub>/5% CO<sub>2</sub>. Qualitatively similar results were obtained in muscles incubated with AICAR. FAO = fatty acid oxidation.

\**P* < 0.05 vs control (from Dean *et al.* 2001; D. Dean *et al.*, unpublished data).

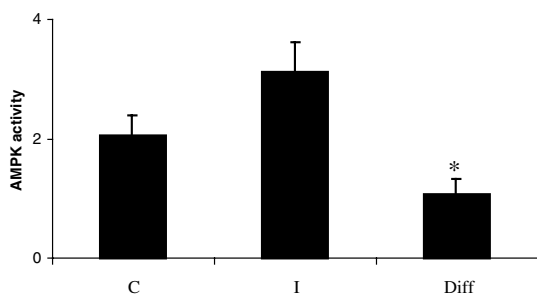
muscle contraction (Fisher *et al.* 2002, Iglesias *et al.* 2002). Earlier studies had suggested that this increase in insulin sensitivity is a major contributor to the rapid repletion of muscle glycogen stores in the post-exercise state (Richter *et al.* 1982, Ruderman *et al.* 1986).

The results also raise the possibility that AMPK-modulated changes in liver and adipose tissue contribute to the alterations in fuel homeostasis that characterize the post-exercise state. Thus, by increasing fatty acid oxidation in liver, AMPK activation could acutely stimulate hepatic glucose production (gluconeogenesis) (Rebrin *et al.* 1995), thereby providing glucose for muscle to replete its glycogen stores. Likewise, AMPK activation in the adipocyte, by inhibiting GPAT would diminish fatty acid re-esterification, thereby enabling it to release more fatty acid into the circulation for the fuel needs of muscle and liver. It should be noted, however, that AMPK activation in liver both by AICAR treatment *in vivo* (Lochhead *et al.* 2000) and the expression of a constitutively active AMPK in cultured hepatocytes (Becard *et al.* 2001) has been shown to diminish the activity of some key gluconeogenic enzymes by effects on their transcription. If so, prolonged, unopposed effects of AMPK activation on liver would, if anything, impair hepatic glucose production and muscle glycogen repletion. Likewise, AMPK activation by AICAR has been reported to inhibit lipolysis in isolated adipocytes (Sullivan *et al.* 1994, Winder & Hardie 1999), an effect which theoretically would decrease the availability of FFA for liver and muscle. The physiological relevance of both of these findings is unclear, as the rate of gluconeogenesis by liver during and after exercise is, if anything, increased (Ahlborg & Felig 1982, Bjorkman & Wahren 1988, Wasserman *et al.* 2002) as are plasma FFA levels (Wolfe *et al.* 1990, Wasserman *et al.* 2002).

An intriguing question raised by these studies is why AMPK activity is increased in liver and adipose tissue 30 min post-exercise. AMP and ATP levels cannot be expected to be altered at this time or even during the exercise. This raises the possibility that the increase in AMPK in these tissues, and possibly in muscle, was mediated by a systemic factor (Fig. 9). If so, one likely



**Figure 9** Possible factors accounting for the activation of AMPK observed in muscle, liver and adipose tissue post-exercise. The possibility that changes in cellular energy state during exercise initiated or contributed to the increases in AMPK activity has not been ruled out. See text for details.



**Figure 10** Activation of AMPK in rat soleus muscle incubated with isoproterenol for 60 min. Solei were incubated with 1 or 10  $\mu\text{M}$  isoproterenol with the muscle from the contralateral limb serving as control. Since the effect of isoproterenol at the two concentrations was the same, the data were pooled. Statistical analysis is based on a paired comparison of the control and treated muscles. Activity is expressed as  $\text{pmol min}^{-1} \text{mg}^{-1}$  supernatant protein (V. Kaushik *et al.*, unpublished data). \* $P < 0.004$ .

candidate would be catecholamines. It has long been known that plasma catecholamine levels increase during exercise (Christensen & Galbo 1983), and recent studies by Minokoshi *et al.* (2002) have demonstrated that an increase in AMPK activity in muscle caused by infusing leptin is, at least in part,  $\alpha$ -adrenergically mediated. In addition, Moule & Denton (1998) have shown that the  $\beta$ -adrenergic agonist isoproterenol increases AMPK activity in isolated adipocytes, and we have made a similar observation in incubated rat muscle (Fig. 10).

Finally, the data suggest AMPK concurrently increases fatty acid oxidation and diminishes its esterification in multiple tissues in the post-exercise state. The immediate implications of this dual effect on fuel homeostasis have already been discussed. Whether it has longer-term implications for gene expression in these tissues (Park *et al.* 2002) or for such problems as insulin resistance, obesity and lipotoxicity, all of which are associated with increases in tissue triglyceride deposition, remains to be determined (Ruderman *et al.* 1999, Unger & Orci 2001, Unger 2002).

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