

Malonyl-CoA and AMP-activated protein kinase: An expanding partnership

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Abstract

Insulin resistance in skeletal muscle is present in humans with type 2 diabetes (noninsulin-dependent diabetes mellitus) and obesity and in rodents with these disorders. Malonyl CoA is a regulator of carnitine palmitoyl transferase I (CPT I), the enzyme that controls the transfer of long chain fatty acyl CoA into mitochondria where it is oxidized. In rat skeletal muscle, the formation of malonyl CoA is regulated acutely (in minutes) by changes in the activity of acetyl CoA carboxylase (ACC), the enzyme that catalyzes malonyl CoA synthesis. ACC activity can be regulated by changes in the concentration of citrate which is both an allosteric activator of ACC and a source of its precursor, cytosolic acetyl CoA. Increases in cytosolic citrate leading to an increase in the concentration of malonyl CoA occur when muscle is presented with insulin and glucose, or when it is made inactive by denervation. In contrast, exercise lowers the concentration of malonyl CoA, by activating an AMP activated protein kinase (AMPK), which phosphorylates and inhibits ACC. Recently we have shown that the activity of malonyl CoA decarboxylase (MCD), an enzyme that degrades malonyl CoA, is also regulated by phosphorylation. The concentration of malonyl CoA in liver and muscle in certain circumstances correlates inversely with changes in MCD activity. This review will describe the current literature on the regulation of malonyl CoA/AMPK mechanism and its physiological function. (*Mol Cell Biochem* **253**: 65–70, 2003)

Key words: insulin resistant, malonyl CoA, acetyl CoA carboxylase, malonyl CoA decarboxylase, AMP-activated protein kinase

Abbreviations: ACC – acetyl CoA carboxylase; MCD – malonyl CoA decarboxylase; AMPK – AMP-activated protein kinase; AICAR – 5-amino 4-imidazolecarboxamide riboside; CPT I – carnitine palmitoyl transferase; PP2A – protein phosphatase 2A.

Introduction

Malonyl CoA is an allosteric inhibitor of carnitine palmitoyl-transferase (CPT) I, the enzyme that controls the transfer of long chain fatty acyl (LCFA) CoAs into the mitochondria where they are oxidized [1]. A large body of evidence has suggested that malonyl CoA levels diminish in exercising (contracting) rat and possibly in human muscle as a result of the phosphorylation of acetyl CoA carboxylase (ACC), the rate limiting enzyme in its synthesis [2–5]. There is also evidence that phosphorylation of ACC is catalyzed by 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 [6]. The net effect of AMPK

activation is stimulation of skeletal muscle glucose uptake and fatty acid oxidation [7]. The latter occurs because AMPK phosphorylates and inhibits ACC and phosphorylates and activates malonyl CoA decarboxylase (MCD), leading to a decrease in the concentration of malonyl CoA [8]. This review will focus on the physiological functions of malonyl CoA and AMPK and their regulation.

The malonyl CoA fuel sensing and signaling mechanism

Malonyl CoA is both an intermediate in the synthesis of fatty acids and an inhibitor of carnitine palmitoyl transferase I

(CPT I), the enzyme that regulates the transfer of long chain fatty acyl CoA molecules into the mitochondria where they are oxidized. In tissues, such as skeletal and cardiac muscle, in which the synthesis of fatty acids *de novo* is minimal, the latter is presumably its primary role. The early studies of McGarry *et al.* [1] demonstrated that the concentration of malonyl CoA in muscle is diminished by as much as 80% after 48 h of starvation and is restored to initial values after 24 h of refeeding. It has become evident that malonyl CoA levels in muscle can also be acutely (minutes) regulated. We have shown that the concentration of malonyl CoA in rat soleus muscle is increased by 2–6 fold when it is incubated for 20 min with insulin and glucose, in keeping with the decreased need for fatty acid oxidation in these muscle [2], whereas during exercise [3] or electrically-induced contractions [4, 5] or when a muscle is incubated in a medium devoid of glucose [2], malonyl CoA levels are diminished within seconds to minutes. This rapid response of malonyl CoA to change in the fuel supply or energy expenditure of the muscle cell has been referred to as the malonyl CoA fuel sensing and signaling mechanism (Fig. 1). Over the past several years many laboratories including ours has examined how this mechanism operates in the muscle cell, and its interaction with other fuel sensing mechanisms, including that mediated by AMP-activated protein kinase (AMPK) and the glucose-fatty acid cycle. In addition, evidence has been presented that malonyl CoA can serve as a link between fuel metabolism and signal transduction in a variety of tissues and that disturbances in this linkage could contribute to such pathophysiological events as insulin resistance in muscle [9], impaired insulin secretion in the pancreatic β -cell [10] and the development of obesity [11].

Regulation of acetyl CoA carboxylase

Studies of malonyl CoA regulation in skeletal muscle have focused on acetyl CoA carboxylase (ACC), the rate limiting

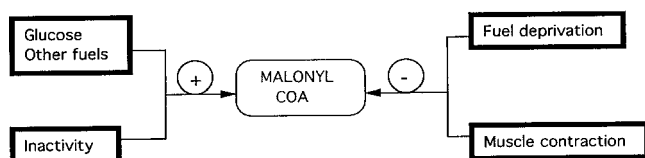


Fig. 1. The malonyl CoA fuel-sensing and signaling mechanism in skeletal muscle. According to this scheme, malonyl CoA is a component of a fuel-sensing and signaling mechanism that responds to changes in glucose availability and energy expenditure. Thus, when the muscle is provided with a surplus of glucose and fuels other than fatty acids, or it does not use available glucose because of inactivity, malonyl-CoA levels increase. Conversely, malonyl-CoA levels decrease when the muscle is glucose-deprived or energy use is increased by contraction. (Reproduced from [21]).

enzyme in malonyl CoA formation (Fig. 2). Two principal isoforms of ACC have been identified, a 265 KDa isoform commonly referred to as ACC $_{\alpha}$, which predominates in lipogenic tissues such as liver and adipose tissues, and a 280 KDa protein (ACC $_{\beta}$), which is the major isoform expressed in skeletal muscle. Heart possess an ACC similar in molecular weight and many of its regulatory characteristics to ACC $_{\beta}$; A large body of work indicated that hepatic ACC activity is altered acutely (min-hours) by changes in its phosphorylation state possibly mediated by an AMPK, and chronically (hours-days) by changes in its abundance, due to alterations in gene expression at the levels of transcription and mRNA stability [12–14]. Thus, hepatic ACC activity has been shown to decrease during starvation (as do levels of malonyl CoA) and increase with refeeding, initially due to changes in phosphorylation state and later due to changes in abundance. In general, insulin and glucose have been shown to diminish hepatic ACC phosphorylation and induce its synthesis, whereas glucagon and catecholamines have the opposite effects [12, 13, 15]. ACC $_{\beta}$ from skeletal muscle has kinetic properties similar to those of hepatic ACC $_{\alpha}$ [16]; however, unlike the liver enzyme it shows little change in assayable activity or abundance in response to alterations in nutritional status [17, 18]. Likewise, we have found no change in ACC $_{\beta}$ activity in response to insulin and glucose either in incubated muscle [2] or *in vivo* [19]. Two types of regulation of ACC $_{\beta}$ have so far been found: (1) regulation by insulin and glucose, which appears to be mediated acutely by changes in the cytosolic concentration of citrate, an allosteric activator of ACC and a precursor of its substrate, cytosolic acetyl CoA (LC CoA) [20] and (2) regulation by phosphorylation, which occurs during exercise and is attributed to activation of an AMPK isoform.

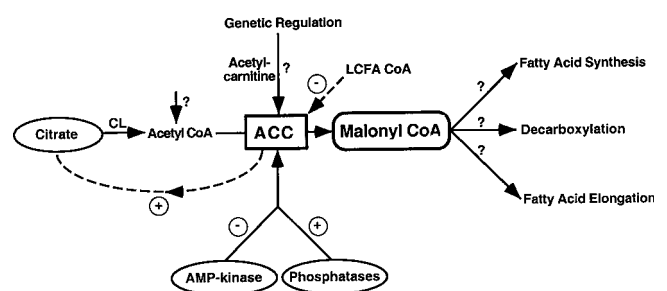


Fig. 2. Regulation of ACC $_{\beta}$ activity and malonyl CoA concentration in skeletal muscle. ACC $_{\beta}$ is regulated by phosphorylation-dephosphorylation (AMP-kinase and phosphatases) and by changes in the cytosolic concentration of citrate. It has been suggested that ACC $_{\beta}$ activity is also governed by the supply of its substrate, cytosolic acetyl CoA, and by the cytosolic concentration of long chain fatty acyl CoA (LCFA CoA), an allosteric inhibitor. Whether ACC $_{\beta}$ abundance is regulated at a genetic level is uncertain. The quantitative importance of each of the pathways depicted for malonyl CoA utilization is not known. ACC – acetyl CoA carboxylase $_{\beta}$; CL – ATP citrate lyase (see text for details). (Reproduced from [21]).

Whether cytosolic citrate is a physiological regulator of ACC_α in liver has been questioned, because changes in its whole-tissue concentration do not appear to parallel changes in malonyl CoA concentration induced by nutritional variation [21]. In addition, alterations in ACC activity due to phosphorylation and dephosphorylation seemed more than adequate to explain observed changes in malonyl CoA concentration in liver. In contrast, changes in assayable ACC activity, indicative of phosphorylation and dephosphorylation, have not been found in rat skeletal muscle in response to starvation or refeeding [22] or exposure to high concentrations of insulin and glucose, despite marked changes in the concentration of malonyl CoA [23]. Instead, in muscle exposed to glucose and insulin, we found that an increase in the concentration of malonyl CoA correlated closely with increases in the whole cell concentration of citrate and to an even greater extent, the sum of the concentration of citrate and malate [19]. Since malate is a counter ion for citrate efflux from the mitochondria, we reasoned that changes in its concentration could reflect a redistribution of citrate from the mitochondria to the cytosol.

Operation of malonyl CoA fuel sensing mechanism in muscle *in vivo*

The activity of an AMPK is increased when ACC_β activity is decreased during voluntary exercise [24], indicating that this component of the malonyl CoA fuel sensing and signaling mechanism operates in rat muscle *in vivo*. Thus we have found high levels of malonyl CoA in muscle of a wide variety of hyperglycemic and/or hyperinsulinemic rodents, including the *fa/fa* rat, the KKAY mouse, rats infused with glucose for 1–4 days and the Goto-Kakizaki (GK) rat, as well as in muscle of normoinsulinemic, normoglycemic rats made insulin resistant by denervation [21]. In none of these situations was the assayable activity of ACC increased, suggesting that the observed change in cytosolic citrate was the principal determinant of the rate of malonyl CoA formation (Table 1).

One condition in which changes in the concentration of malonyl CoA in muscle are unexplained is the fasted-refed state. In rats fasted for 48 h, we found a 2 fold increase in the malonyl CoA content in various muscles after 3–24 h of refeeding, with modest increase in the citrate and malate concentration without any consistent changes in ACC or MCD activity. Possibly, during refeeding the activity of ACC is increased allosterically by a decrease in the concentration of LC-CoA. This remains to be determined.

Malonyl CoA hypothesis and insulin resistance in humans

The existence of malonyl CoA fuel-sensing and signaling mechanism in humans is suggested by Bavenholm *et al.* [25]. They found that the concentrations of malonyl CoA, citrate and malate increased concurrently in human leg muscle during a euglycemic-hyperinsulinemic clamp. In addition, whole body fatty acid oxidation and presumably muscle fatty acid oxidation were markedly diminished in the subjects. In an earlier study, Sidossis *et al.* [26] reported that decreases in oleate oxidation in humans undergoing a euglycemic-hyperinsulinemic clamp are accompanied by decreases in the concentration of long chain acyl carnitine in muscle, suggesting inhibition of CPT 1. This report also suggest that malonyl CoA levels are regulated in human muscle and that, as in rat, they play a role in the regulation of fatty acid oxidation.

Role of AMP-activated protein kinase

Decreases in assayable ACC_β activity in skeletal muscle due to phosphorylation have been described in response to a variety of stressful stimuli including ischemia/hypoxia, inhibition of oxidative phosphorylation and exercise [6, 27–29]. In these circumstances phosphorylation of ACC_β appears due to activation of AMPK [12–14]. This heterotrimeric enzyme, which contains distinct α, β and γ subunits, is regulated by

Table 1. Lipid metabolites and protein kinase C (PKC) in muscle of insulin-resistant rodents

	TG	DAG	LCFA CoA	Malonyl CoA	Altered PKCs
KKAY	+	+	ND	+	ND
Zucker rat	+	+	ND	+	Yes (ε,)
Glucose-infused rat	+	ND	+	+	Yes (ε,)
Fat-fed rat	+	+	+	+	Yes (ε, θ)
GK rat	+	+	ND	+	Yes (ε,)
Denervated	ND	+	ND	+	Yes (ε, θ)

Compiled data from our laboratory [2, 9, 21–23] and laboratories of Farese [48], and Kraegen [45, 46] and Biden [47]. TG – muscle triglycerides; DAG – muscle diacylglycerol; LCFA CoA – long chain fatty acyl CoA. An increase in concentration is indicated by (+). ND – not done. Recent studies suggest that increases in protein kinase C (PKC) activity and distribution (and especially that of PKCε or θ) occur in many of these rodents.

changes in a cell's energy state. Increases in the AMP/ATP ratio of a cell, such as occur in response to ischemia and other stresses, can activate AMPK by a several mechanisms. The activation of AMPK, in turn, sets in motion a number of events that both increase ATP generation (e.g. increased fatty acid oxidation) and decrease its utilization for processes not required for a cells immediate viability (Fig. 3). As shown by several groups, phosphorylation of specific serine residues on ACC by AMPK results both in inhibition of basal ACC activity and its activation by citrate [5, 6, 12]. Interestingly, only the $\alpha 2$ isoform of AMPK is activated in skeletal muscle during contraction [5], whereas in response to ischemia/hypoxia both the $\alpha 1$ and $\alpha 2$ isoforms appear to be activated [28–30]. As reviewed by Hardie and Carling [6], activation of AMPK during these situations is secondary to an increase in the AMP/ATP ratio that can increase AMPK activity by several mechanisms. They include: activation of an AMPK-kinase that phosphorylates and activates AMPK. It is generally held that AMPK is a component of a fuel sensing mechanism that alters the cell to a change in its energy charge [7, 31]. Winder *et al.* [32, 33] demonstrated a specific role for AMPK in the regulation of glucose uptake. In an isolated rat hindquarter preparation they demonstrated that perfusion with the AMPK activator AICAR increased glucose uptake by 2 fold. Subsequent studies carried out by Goodyear *et al.*

[34, 35] established that this effect of AICAR was due to increased in glucose transport, and that it involved GLUT4 translocation. The precise mechanism by which AMPK activation stimulates glucose transport is still unknown.

Regulation of malonyl CoA decarboxylase

The observations that the concentration of malonyl CoA diminishes by 50% within 20 min when an isolated soleus is deprived of glucose [2] and even more rapidly during electrically-induced contractions [5] suggests that malonyl CoA utilization as well as synthesis may be regulated. Since muscle does not use malonyl CoA for fatty acid synthesis, as does liver, a principal focus has been on malonyl CoA decarboxylase (MCD). Evidence has been presented that MCD is present in both cardiac [36–40] and skeletal muscle [8, 41–43]. In skeletal muscle, its activity is similar to that of ACC and is 10–40 times greater than that of fatty acid synthase. In heart, where MCD activity is substantially greater than in skeletal muscle, a 10-fold increase in fatty acid oxidation observed between days one and seven after birth is accompanied by 80–95% decreases in ACC activity and malonyl CoA levels and a significant, although more modest (40%), increase in MCD activity [38]. The question of whether and how MCD is acutely regulated has received little attention. Dyck *et al.* [39] failed to observe an increase in MCD activity in cardiac muscle during ischemia-reperfusion, a situation in which they had previously observed a decrease in ACC activity at a sub-saturating concentration of acetyl CoA. Results from our laboratory suggested that the concentration of malonyl-CoA in liver and muscle in certain circumstances correlates inversely with changes in MCD activity. Thus, increases in MCD activity have been observed in rat liver during starvation [39] and in skeletal muscle [8] in response to electrically induced contractions. In the latter situation, the increase in activity was attributable to activation of AMP-activated protein kinase (AMPK) an enzyme that also phosphorylates and inhibits ACC [8]. Despite this, the physiological role of MCD in regulating the concentration of malonyl-CoA remains open to question as are the mechanisms by which its activity is regulated [42]. We have shown recently that voluntary exercise also increases the activity of MCD and that this too is secondary to a change in AMPK activity [43]. The increase in MCD activity is accompanied by decrease in ACC activity and malonyl-CoA concentration. Such a coordinate regulation of ACC and MCD attributable to AMPK has also been observed in rat muscle made to contract by electrical stimulation of the sciatic nerve *in vivo* and following incubation of the rat extension digitorum longus muscle with the AMPK activator AICAR. Based on this study we suggested that

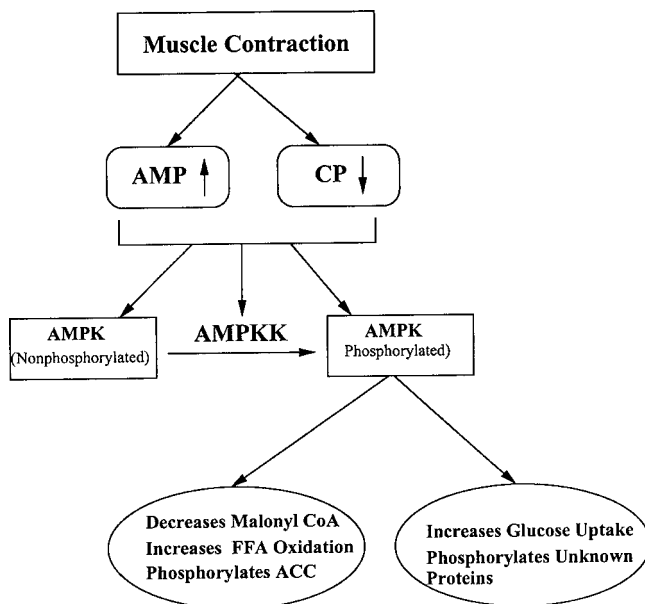


Fig. 3. Mechanisms of muscle AMPK activation in response to contraction. AMPK is activated allosterically by an increase in AMP concentration. The increase in AMP and the decrease in CP as muscle contracts result in allosteric activation of AMPK. AMP binds to AMPK, making it a better substrate for the upstream kinase, AMPK kinase. CP – Creatine phosphate; AMPKK – AMP-activated protein kinase kinase; ACC – acetyl CoA carboxylase; FFA – free fatty acid.

MCD and ACC_β might be jointly regulated by AMPK (Fig. 4). The importance of this dual control of MCD and ACC_β activities to the regulation of the cytosolic concentration of malonyl CoA and secondarily to fatty acid oxidation remains to be determined.

Conclusion

The malonyl CoA glucose sensing mechanism has been implicated in the regulation of insulin secretion by glucose in the pancreatic β-cell [10, 44], as well as the damage to the β-cell caused by sustained high blood glucose levels. In addition, glucose-induced changes in the enzyme protein kinase C, which could be explained by dysregulation of the malonyl CoA/LC CoA mechanism, have been implicated in causing cardiovascular, eye and kidney disease in patients with diabetes (reviewed by [11]). An increasing body of evidence also suggests that dysregulation of the malonyl CoA fuel sensing and signaling mechanism could play a role in the pathogenesis of obesity and the insulin resistance syndrome. Finally, it has been suggested that the malonyl CoA/LCFA

CoA mechanism mediates the response of glucose-sensitive cells in the brain to acute changes in blood glucose concentration [21]. If so, it might play a pivotal role in mediating the response of the central nervous system to hypoglycemia in diabetic patients treated with insulin. Thus, an understanding of how this mechanism works could have important ramifications for many aspects of diabetes. How changes in malonyl CoA level relate to the formation and action of leptin, uncoupling proteins, TNF-α and other molecules whose role in the pathophysiology of obesity and insulin resistance is now being intensively studied is a potentially fruitful area for research. Development of pharmacological agents and other therapies that lower the concentration of malonyl CoA and development of specific AMPK inhibitors will be an important area for future investigation.

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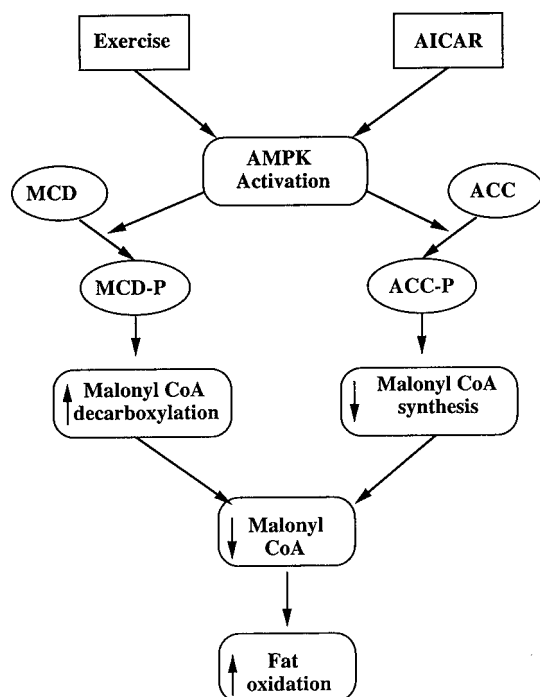


Fig. 4. Dual regulation of malonyl CoA decarboxylase and acetyl CoA carboxylase by AMP-activated protein kinase. Increases in AMPK activity caused by exercise and AICAR lead to the phosphorylation of both ACC and MCD. According to the proposed schema this results in inhibition of ACC_β and activation of MCD. Such dual regulation could magnify the effects of changes in AMPK activity on the malonyl CoA concentration with resulting enhanced fat oxidation and energy production (adapted from [43]).

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