Malonyl-CoA, fuel sensing, and insulin resistance

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[1]*Diabetes Unit, Section of Endocrinology and Departments of Medicine and Physiology, Boston University Medical Center, Boston, Massachusetts 02118; and* [2]*Endocrine-Metabolism Division, Department of Medicine and Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755*

Ruderman, Neil B., Asish K. Saha, Demetrios Vavvas, and Lee A. Witters. Malonyl-CoA, fuel sensing, and insulin resistance. *Am. J. Physiol.* 276 (*Endocrinol. Metab.* 39): E1–E18, 1999.—Malonyl-CoA is an allosteric inhibitor of carnitine palmitoyltransferase (CPT) I, the enzyme that controls the transfer of long-chain fatty acyl (LCFA)-CoAs into the mitochondria where they are oxidized. In rat skeletal muscle, the formation of malonyl-CoA is regulated acutely (in minutes) by changes in the activity of the β-isoform of acetyl-CoA carboxylase (ACC_β). This can occur by at least two mechanisms: one involving cytosolic citrate, an allosteric activator of ACC_β and a precursor of its substrate cytosolic acetyl-CoA, and the other involving changes in ACC_β phosphorylation. 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 keeping with a diminished need for fatty acid oxidation in these situations. Conversely, during exercise, when the need of the muscle cell for fatty acid oxidation is increased, decreases in the ATP/AMP and/or creatine phosphate-to-creatine ratios activate an isoform of an AMP-activated protein kinase (AMPK), which phosphorylates ACC_β and inhibits both its basal activity and activation by citrate. The central role of cytosolic citrate links this malonyl-CoA regulatory mechanism to the glucose-fatty acid cycle concept of Randle et al. (P. J. Randle, P. B. Garland. C. N. Hales, and E. A. Newsholme. *Lancet* 1: 785–789, 1963) and to a mechanism by which glucose might autoregulate its own use. A similar citrate-mediated malonyl-CoA regulatory mechanism appears to exist in other tissues, including the pancreatic β-cell, the heart, and probably the central nervous system. It is our hypothesis that by altering the cytosolic concentrations of LCFA-CoA and diacylglycerol, and secondarily the activity of one or more protein kinase C isoforms, changes in malonyl-CoA provide a link between fuel metabolism and signal transduction in these cells. It is also our hypothesis that dysregulation of the malonyl-CoA regulatory mechanism, if it leads to sustained increases in the concentrations of malonyl-CoA and cytosolic LCFA-CoA, could play a key role in the pathogenesis of insulin resistance in muscle. That it may contribute to abnormalities associated with the insulin resistance syndrome in other tissues and the development of obesity has also been suggested. Studies are clearly needed to test these hypotheses and to explore the notion that exercise and some pharmacological agents that increase insulin sensitivity act via effects on malonyl-CoA and/or cytosolic LCFA-CoA.

acetyl-CoA carboxylase; AMP-activated protein kinase; cytosolic citrate; glucose-fatty acid cycle; exercise; obesity; protein kinase C

The Malonyl-CoA Fuel-Sensing and Signaling Mechanism

In the liver, malonyl-CoA is both an intermediate in the de novo synthesis of fatty acids (135) and an allosteric inhibitor of carnitine palmitoyltransferase I (CPT I), the enzyme that regulates the rate at which long-chain fatty acyl(LCFA)-CoAs enter the mitochondria where they are oxidized (72). In contrast, in tissues such as skeletal and cardiac muscle, in which the synthesis of fatty acids de novo is minimal (6), regulation of CPT I is presumably its dominant role. The early studies of McGarry et al. (70)
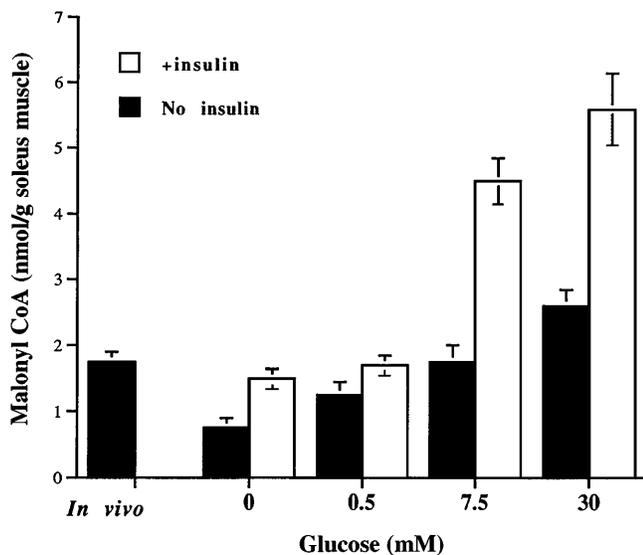
Fig. 1. Dependence of insulin-induced increase in malonyl-CoA on medium glucose concentration. Rat solei were incubated in media containing the indicated concentrations of glucose, with or without 10 mU/ml insulin, for 20 min. Value for a control rat fed ad libitum is also shown. Results are means ± SE for 6–10 muscles for each condition. (Adapted from Ref. 115).

review, we will explore how this mechanism operates and examine its relationship to other fuel-sensing mechanisms, such as those mediated by AMP-activated protein kinase (AMPK) (48) and the glucose-fatty acid cycle (96, 97). We will also examine the notion that malonyl-CoA can serve as a link between fuel metabolism and signal transduction in muscle and other tissues and that disturbances in this linkage contribute to the pathophysiology of insulin resistance and obesity. That changes in malonyl-CoA can play a pivotal role in the regulation of insulin secretion by glucose and possibly other insulin secretagogues has been reviewed elsewhere (84, 94, 152) and will be discussed briefly in *Link to cellular signaling*.

Acetyl-CoA Carboxylase: Isoforms

A major factor in the regulation of malonyl-CoA levels in muscle and other tissues is acetyl-CoA carboxylase (ACC), a cytosolic enzyme that catalyzes the carboxylation of cytosolic acetyl-CoA to form malonyl-CoA (Fig. 3). Two principal isoforms of ACC have been identified, a 265-kDa protein now referred to as ACC_{α} or ACC_1, which is found predominantly in lipogenic tissues such as liver, adipose tissue, and mammary gland (57); and a 275- to 280-kDa protein (ACC_{β} or ACC_2), which is the major isoform expressed in skeletal muscle and heart, although it is present to some extent in liver and probably other tissues (1, 11, 45, 64, 131, 133, 138, 143, 145) (Table 1).

Recent studies indicate that ACC_{α} and ACC_{β} are the products of distinct genes and that they have different affinities for their substrate, cytosolic acetyl-CoA (Table 1). However, excluding a 200-amino acid sequence unique to the NH_2 terminus of ACC_{β}, the two isoforms show ~75% amino acid identity, and they have their functional domains in homologous regions. Because of its predominant location in skeletal and cardiac muscle, it has been proposed that ACC_{β} is involved in the regulation of fatty acid oxidation rather than fatty acid biosynthesis (1, 11, 45, 64, 138). It has also been suggested that the NH_2-terminal sequence of ACC_{β} could be responsible for anchoring it to the mitochondrial outer membrane so as to control more closely the concentration of malonyl-CoA in the vicinity of CPT I (45). These notions remain to be proven, however.

demonstrated that the concentration of malonyl-CoA in skeletal muscle is diminished by 80% after 48 h of starvation, in keeping with the increased need for fatty acid oxidation in the fasting state. More recently, it has become apparent that malonyl-CoA levels in muscle can also be acutely (in minutes) regulated. Thus we have found that the concentration of malonyl-CoA increases two- to sixfold within 20 min when a rat soleus muscle is incubated with glucose and insulin (Fig. 1) and within 6 h when it is made inactive as a result of denervation [i.e., in situations in which the need for fatty acid oxidation is decreased (115)]. Conversely, during exercise (139) or electrically induced contractions (29, 115), or when a muscle is incubated in a medium devoid of glucose (115) (i.e., in situations in which the need for fatty acid oxidation to generate ATP is increased), malonyl-CoA levels are diminished within seconds to minutes. This rapid response of malonyl-CoA to changes 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. 2). In this

Fig. 2. 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. In the proposed mechanism, acetyl-CoA carboxylase functions as the sensor and malonyl-CoA is the signal (see also Fig. 3). (Reproduced from Ref. 116).
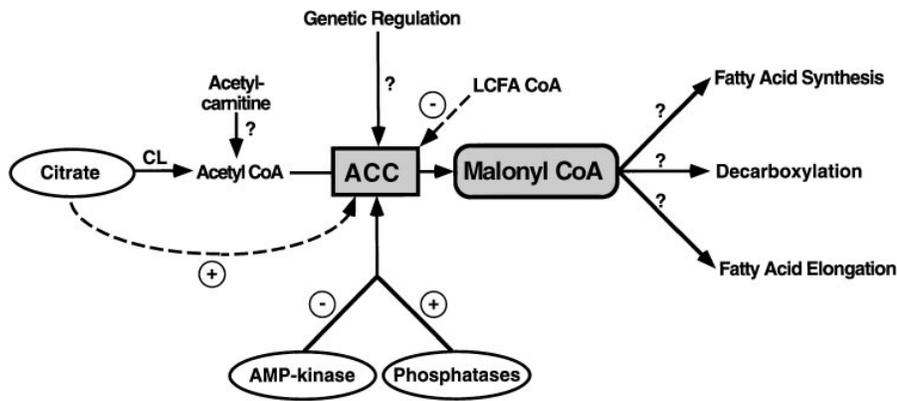
Until the past 2–3 yr, most of our knowledge of ACC regulation was of the ACC$_β$ isoform in liver. A large body of work had shown that its activity is modulated acutely (in minutes to hours) by changes in the phosphorylation state of specific serine residues by an AMP-activated protein kinase, cAMP-dependent protein kinase, and/or phosphatases (25, 44, 48, 57, 81, 146) (see also *ACC$_β$ Regulation During Exercise and Recovery*) and chronically (in hours to days) by changes in enzyme abundance due to alterations in gene expression at the level of transcription and mRNA stability (47, 57, 58). In keeping with these observations, hepatic ACC activity has been shown to decrease during starvation and to increase rapidly with refeeding (79, 81, 146), initially because of changes in phosphorylation state and later from changes in abundance. In general, insulin and glucose increase the activity of ACC$_α$ by diminishing its phosphorylation and by inducing its synthesis, and glucagon and catecholamines have the opposite effects (47, 57, 58, 147). Other potential regulators of ACC in liver include citrate, which is both the major precursor of the cytosolic acetyl-CoA from which malonyl-CoA is synthesized and an allosteric activator of ACC (2, 58) and LCFA-CoA and malonyl-CoA itself, both of which are allosteric inhibitors (1, 2, 47). In vitro, citrate counters the inhibition of ACC caused by malonyl-CoA and LCFA-CoA (2, 42). The physiological relevance of these effects of citrate is uncertain, however, because its concentration in liver does not appear to change markedly with nutritional state (124). The role of LCFA-CoA in regulating ACC$_α$ activity in liver, in vivo, has also been questioned (2, 42, 135).

Table 1. *Characteristics of ACC$_α$ and ACC$_β$*

	ACC$_α$	ACC$_β$
Apparent molecular mass, kDa	265	275–280
K_m acetyl-CoA, μM	74	167
Chromosomal location of gene	17	12
Possible mitochondria-binding sequence at		
NH$_2$ terminus	No	Yes
Role in fatty acid synthesis	Yes	?
Role in fatty acid oxidation	?	Yes
Activity altered by nutritional state	Yes (liver)	No (muscle)

K_m, Michaelis-Menten constant. [Adapted from Ha et al. (45) and Abu-Elheiga et al. (1).]

ACC$_β$ isolated from muscle, like ACC$_α$ from other tissues, is allosterically activated by citrate and inhibited by palmitoyl-CoA (LCFA-CoA) and malonyl-CoA (131). On the other hand, in contrast to ACC$_α$ in liver, it shows little if any change in assayable activity (11, 26, 138, 141) or abundance as a result of starvation or refeeding despite substantial changes in malonyl-CoA concentration (72, 141). This has caused a number of groups to reexamine how ACC in muscle is regulated in response to nutritional and hormonal perturbations. It also initiated studies of ACC regulation during exercise and other states in which fatty acid oxidation is altered.

Cytosolic Citrate: A Signal of the Fuel Status of the Muscle Cell and a Regulator of ACC$_β$

As already noted, decreases in assayable ACC activity do not account for the changes in malonyl-CoA in skeletal muscle caused by feeding or starvation (141). Likewise, we (116) have failed to observe an increase in assayable ACC activity in rat soleus muscles incubated with insulin or glucose for 20–30 min despite two- to sixfold increases in malonyl-CoA concentration (Fig. 1). In the latter studies (116), the increments in malonyl-CoA were associated with increases in the sum of the whole cell concentrations of citrate and of malate, an antiporter for citrate efflux from the mitochondria (20, 21, 52). Even greater increases in the concentrations of these metabolites and of malonyl-CoA were observed in muscles incubated with glucose and acetoacetate (116), a fuel long known to inhibit fatty acid oxidation in skeletal (104, 105) and cardiac (97) muscle (Fig. 4). Similar observations have been made by us (9) in rat and human muscle in vivo after sustained increases in plasma glucose and/or insulin (see *Operation of the Malonyl-CoA Fuel-Sensing and Signaling Mechanism in Muscle In Vivo*). Our interpretation of these findings is that ACC$_β$ is responding in these circumstances to an increase in the cytosolic concentration of citrate, which is reflected more closely by an increase in the sum of the whole cell concentrations of citrate and malate than of citrate alone (116). Although supported by correlative evidence, this assumption remains to be proven definitively.

Increases in the apparent concentration of cytosolic citrate, analogous to those in skeletal muscle, have
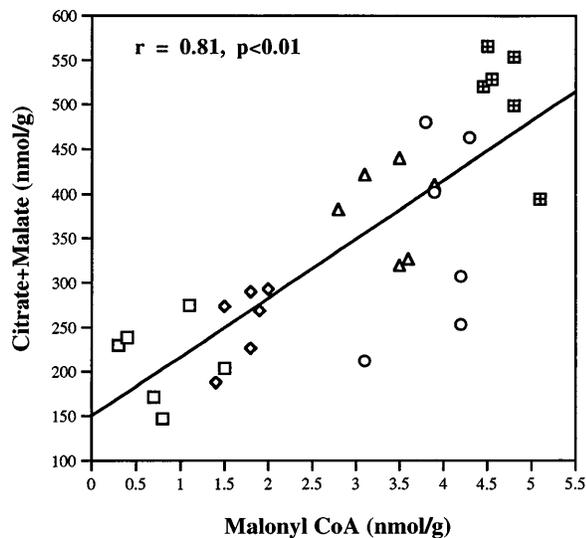
Fig. 4. Relationship between concentration of malonyl-CoA and the sum of concentrations of citrate and malate in rat soleus muscles incubated with no glucose (□), glucose (5.5 mM, ◇), glucose and insulin (10 mU/ml, ○), glucose and acetoacetate (1.7 mM, △), or glucose, insulin, and acetoacetate (⊞). Correlation of malonyl-CoA with whole cell citrate was weaker and in one group (glucose and insulin) was not observed at all (see Ref. 116).

been observed in heart (41, 113), brain (107), and the pancreatic β-cell (94) in response to changes in their fuel and hormonal milieu. They appear to require the presence of glucose at normal or high physiological concentrations (41, 66, 102, 113) and may be enhanced when ketone bodies or free fatty acids are also provided (41, 66, 107). An hypothetical scheme that takes into account these observations, as well as the relevance of malate, to explain how cytosolic citrate is regulated in skeletal muscle is presented in Fig. 5. Key events, as proposed by many others (20, 97, 113), include: *1*) increases in the concentrations of oxaloacetate and acetyl-CoA in the mitochondria, *2*) the condensation of this acetyl-CoA and oxaloacetate to form citrate, and *3*) the efflux of citrate into the cytosol, via the citrate transporter, in exchange for malate and possibly other anions. Malate functions in this scheme as the antiporter for citrate efflux from the mitochondria. In addition, oxaloacetate, generated in the cytosol by aspartate transamination and the ATP-citrate lyase reaction, is converted to malate to enter the mitochondria, where it repletes and expands the pool of tricarboxylic acid cycle intermediates (anaplerosis). Glucose is a major player in these events. It can provide acetyl-CoA for citrate synthesis; it is the principal source of pyruvate, required for the pyruvate carboxylase reaction in the mitochondria (134) and for the deamination of aspartate and glutamate (not shown) in the cytosol; and its metabolism in the glycolytic pathway results in the generation of the cytosolic NADH needed for the conversion of cytosolic oxaloacetate to malate (66, 106, 113) (Fig. 5). Similar lines of thinking have been put forth to explain how glucose increases the concentration of citrate in the pancreatic β-cell (94, 102) and the heart (41, 113). Ketone bodies can potentiate the effects

of glucose in these settings by serving as a source of mitochondrial acetyl-CoA; indeed, in the presence of glucose they increase the concentration of acetyl-CoA in rat skeletal muscle to levels well above those produced by glucose alone (104), even though they concurrently inhibit pyruvate dehydrogenase (46). Because acetyl-CoA is an activator of pyruvate carboxylase (148), the increase in acetyl-CoA generation caused by ketone bodies (104) could also increase oxaloacetate formation in the mitochondria.

In muscle, the question of whether citrate functions principally as an allosteric activator of ACC or a provider of the cytosolic acetyl-CoA from which malonyl-CoA is synthesized is still unresolved. On the one hand, we have found that incubation of muscle with hydroxycitrate, an inhibitor of ATP-citrate lyase (Fig. 3), markedly depresses the increase in malonyl-CoA caused by insulin and glucose, suggesting that citrate is the major source of cytosolic acetyl-CoA (116). On the other hand, studies with immunopurified ACC_β suggest that the observed changes in whole cell citrate, if they are reflected in cytosol, will cause at least a 1.5- to 2-fold increase in ACC activity (134). Furthermore, this may be an underestimate, because the magnitude of ACC_β activation by citrate is probably blunted by its phosphorylation during tissue processing (131, 134).

Cytosolic Citrate Links the Malonyl-CoA Fuel-Sensing and Signaling Mechanism to the Glucose-Fatty Acid Cycle

The apparently central role of cytosolic citrate in the malonyl-CoA fuel-sensing and signaling mechanism links it to the glucose-fatty acid cycle concept proposed by Randle and co-workers (96–98) on the basis of studies in heart muscle. According to the glucose-fatty acid cycle concept, increases in fatty acid or ketone body oxidation elevate the concentrations of acetyl-CoA and NADH in mitochondria, leading to inhibition of glucose metabolism at pyruvate dehydrogenase and, in the presence of glucose, to increases in the mitochondrial and subsequently the cytosolic concentration of citrate (46, 66, 97). The increase in cytosolic citrate in turn restrains glycolysis at the level of phosphofructokinase. This further diminishes the use of glucose as a fuel, although it may actually increase glucose incorporation into glycogen (66, 97).

The common involvement of cytosolic citrate in both the malonyl-CoA fuel-sensing system and the glucose-fatty acid cycle has led us to hypothesize that an increase in its concentration is not a unique feature of the glucose-fatty acid cycle, but rather a more general signal to the muscle cell that it has an excess of fuel for its immediate needs (116). According to the hypothesis proposed, the precise effect of such an increase in citrate will depend on the fuel(s) present in excess. When it is primarily glucose (e.g., in muscles incubated with high concentrations of glucose and insulin or in organisms infused with insulin and glucose), the increase in citrate restrains both fatty acid oxidation (via malonyl-CoA) (see also Ref. 6) and the further use of glucose itself as a fuel (Fig. 6). In other words, a glucose
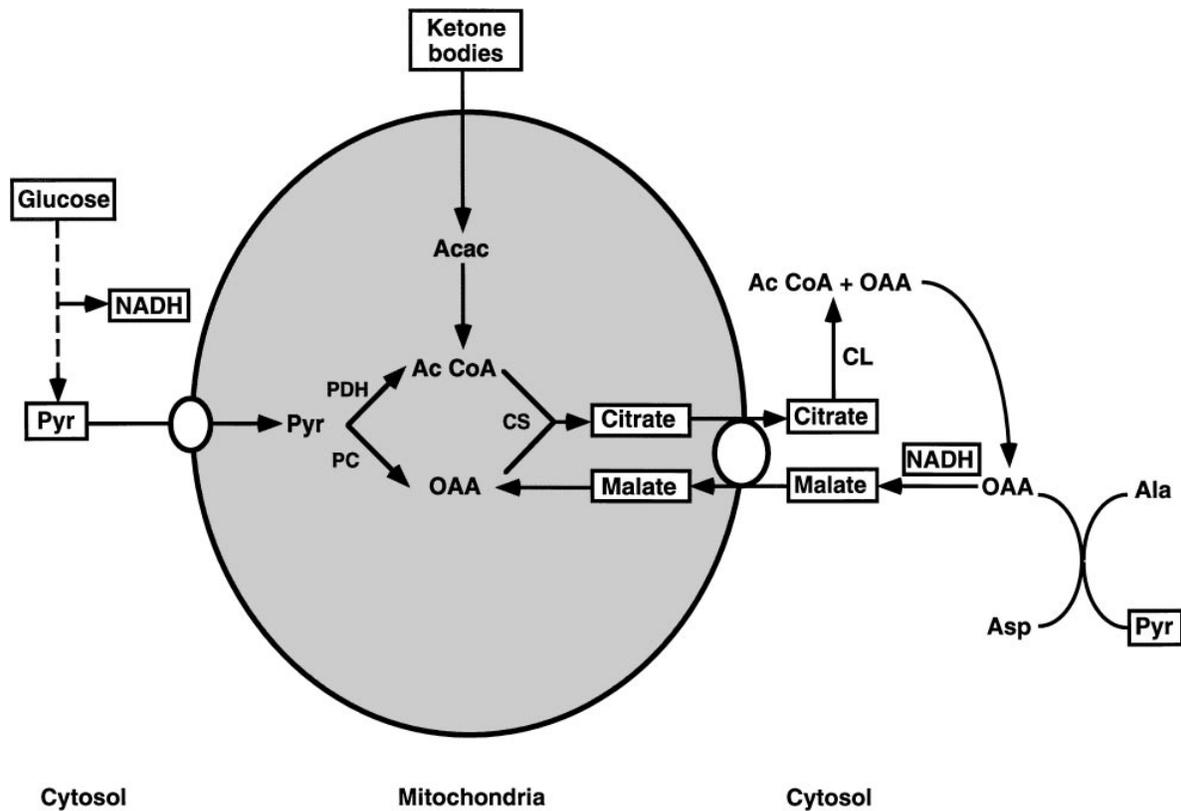
Fig. 5. Proposed scheme for regulation of cytosolic citrate in skeletal muscle by glucose and acetoacetate. Citrate is formed in the mitochondria by the reaction of acetyl-CoA and oxaloacetate (OAA). It enters the cytosol in exchange for malate via the tricarboxylic acid transporter (20). Acetyl-CoA for the citrate synthase (CS) reaction can be derived from pyruvate (Pyr) and/or acetoacetate (Acac) oxidation, and the OAA can be derived by pyruvate carboxylation and/or oxidation of malate. Glucose is necessary for these events because it generates both pyruvate and the NADH required to convert cytosolic OAA to malate. Cytosolic OAA can be derived from transamination of aspartate with pyruvate and as a product of the ATP CL reaction. PDH, pyruvate dehydrogenase; PC, pyruvate carboxylase. (See text and Ref. 66 for further details).

autoregulatory mechanism (106) accompanies the regulation of fatty acid oxidation by malonyl-CoA. In contrast, when muscle is presented with an excess of free fatty acid (FFA) (in the presence of glucose), we believe the putative glucose autoregulatory mechanism remains and may even be enhanced, but that inhibition of fatty acid oxidation by glucose will not be as marked. This is because the increase in cytosolic LCFA-CoA that accompanies an excess of FFA both allosterically inhibits ACC, thereby diminishing malonyl-CoA formation (131) (Fig. 3), and competes with malonyl-CoA for binding on CPT I (73). In keeping with this notion,

perfusion of a rat heart with FFAs and glucose, although it causes an increase in citrate (97), is associated with a decrease rather than an increase in the concentration of malonyl-CoA (6). To our knowledge, a similar study has not been carried out in skeletal muscle.

An interesting, but somewhat more complex, picture is observed when the fuels in excess are ketone bodies. Thus we have shown that, in the presence of glucose, the ketone bodies acetoacetate and β-hydroxybutyrate markedly inhibit the oxidation of fatty acids (10, 105), as well as glucose (10, 46, 66, 104, 105), in rat skeletal
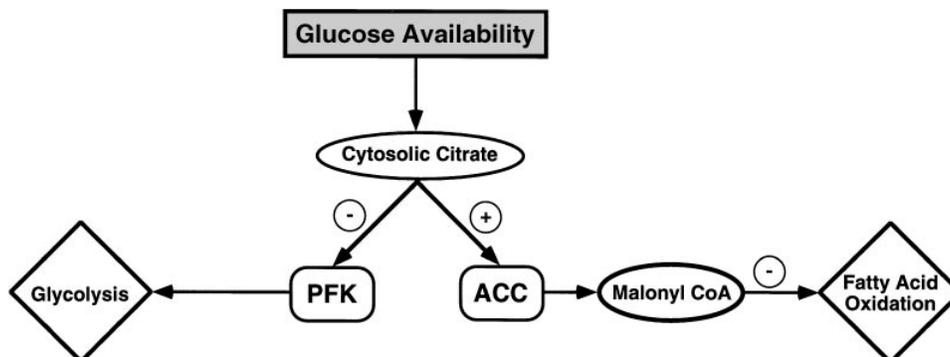

Fig. 6. Concurrent regulation of glycolysis and fatty acid oxidation by cytosolic citrate. As pictured, an increase in glucose availability would both restrain the increase in glycolysis that it causes and diminish fatty acid oxidation. PFK, phosphofructokinase.

muscle in vivo and in an isolated perfused hindquarter preparation. Furthermore, in incubated rat soleus muscle, acetoacetate causes the same inhibition of phosphofructokinase and stimulation of glycogen synthesis (66) that it does in heart, and it increases the concentrations of citrate, malate, and malonyl-CoA (66, 116) to an even greater extent than does glucose alone. In contrast, in the absence of glucose, or when the concentration of glucose is low, acetoacetate produces no increase in citrate or malate, and it causes only a small increase in malonyl-CoA (116), which could be accounted for by cytosolic acetyl-CoA generation from acetylcarnitine (Fig. 3). These findings suggest that acetoacetate by itself has little effect on the cytosolic concentrations of citrate and malonyl-CoA, but that it potentiates the effects of glucose on these metabolites. Put another way, acetoacetate appears to allow the glucose autoregulatory and malonyl-CoA fuel-sensing mechanisms to come into play at a lower glycolytic rate. Whether the ketone bodies inhibit fatty acid oxidation effectively when glucose availability is low is not known. That they may not do so is suggested by the observation that acetoacetate does not replace fatty acids as the major fuel of muscle in the isolated hindquarter preparation of a rat with diabetic ketoacidosis unless insulin, as well as glucose, is added to the perfusion medium (104).

Operation of the Malonyl-CoA Fuel-Sensing and Signaling Mechanism in Muscle In Vivo

As will be discussed later, the activity of an AMPK is increased when ACC_β activity is decreased during voluntary exercise (140), indicating that this component of the malonyl-CoA fuel-sensing and signaling mechanism operates in rat muscle in vivo. Recent studies (63, 86, 116, 117) suggest that regulation of ACC_β by cytosolic citrate also occurs in vivo. Thus we have found concurrent increases in the concentrations of malonyl-CoA, citrate, and/or malate in rat muscle after a euglycemic-hyperglycemic clamp (4–5 h) (86), a prolonged glucose infusion (1–4 days) (63), and brief (5 h) infusions of insulin (117). Likewise, similar increases in these metabolites have been found in hindlimb muscle of the hyperinsulinemic *fa/fa* rat and in rat muscle made inactive by denervation 24 h previously (109, 115, 117). In none of these situations was the assayable activity of ACC_β increased, suggesting that the observed change in cytosolic citrate was the principal determinant of the rate of malonyl-CoA formation.

One condition in which changes in malonyl-CoA in muscle are unexplained is the starved-fed transition. In rats starved for 48 h, we have observed 1.5- to 2-fold increases in the concentration of malonyl-CoA in various muscles after 3–24 h of refeeding, but modest increases in the concentrations of citrate or malate, if any, or in the activity of ACC_β (26). Possibly, during refeeding, the activity of ACC_β is increased allosterically by a decrease in the concentration of a negative effector such as LCFA-CoA (26, 117); however, this remains to be proven.

The existence of a malonyl-CoA fuel-sensing and signaling mechanism in humans is suggested by the observation of Bavenholm et al. (9) that the concentrations of malonyl-CoA, citrate, and malate increase concurrently in human leg muscle during a euglycemic-hyperinsulinemic clamp. In addition, whole body oxidation and presumably muscle fatty acid oxidation were markedly diminished in the subjects they evaluated. In an earlier study, Sidossis et al. (123) reported that decreases in oleate oxidation in humans undergoing a euglycemic-hyperinsulinemic clamp are accompanied by decreases in the concentration of long-chain fatty acylcarnitine in muscle, suggesting inhibition of CPT I. They attributed this to an increase in the concentration of malonyl-CoA, although malonyl-CoA itself was not measured. In toto, these reports suggest both that malonyl-CoA levels are regulated in human muscle and that, as in the rat, they play a role in the regulation of fatty acid oxidation.

Unanswered Questions About Malonyl-CoA in Skeletal Muscle

Does it regulate CPT I? Implicit in the malonyl-CoA fuel-sensing and signaling concept is the notion that changes in the concentration of malonyl-CoA in skeletal muscle regulate CPT I activity and, secondarily, fatty acid oxidation. Such a mechanism has been clearly demonstrated in liver (69, 151), where the whole cell concentration of malonyl-CoA is in the range at which it competitively inhibits purified CPT I and where alterations in malonyl-CoA concentration, in vivo, correlate closely with changes in CPT I activity and fatty acid oxidation (69, 151). Changes in the concentration of malonyl-CoA for the most part also correlate closely with changes in fatty acid oxidation in skeletal muscle. Thus, in the rat, malonyl-CoA levels are low in starvation (26, 76, 141) and during exercise (139), and they are high in the fed state (26, 141). Likewise, they are diminished and fatty acid oxidation is increased in muscles incubated with 5-aminoimidazole-4-carboxamide ribonucleoside [AICAR, an AMP analog that activates 5′-AMPK and secondarily diminishes ACC activity (74, 142)]. Still further evidence for an association is a remarkably close correlation ($r = 0.95$) between increases in whole body respiratory quotient and malonyl-CoA levels in muscle of 48-h-starved rats throughout the first 24 h of refeeding (26). Furthermore, these increases in malonyl-CoA were accompanied by decreases in the concentration of long-chain fatty acylcarnitine in muscle, suggesting inhibition of CPT I (26).

Despite these findings and similar observations in heart (6, 112), some questions persist about the relationship between malonyl-CoA concentration and fatty acid oxidation in skeletal muscle. One of these relates to the fact that CPT I is a different protein in skeletal muscle and liver and that the muscle isoform is more sensitive to inhibition by malonyl-CoA by two orders of magnitude (IC_{50} 0.03 vs. 2.7 mM) (70). Because the concentration of malonyl-CoA measured in intact rat muscle is 1–4 nmol/g and in human muscle, 0.1–0.3 nmol/g (9,

89), only a small fraction of this malonyl-CoA must be accessible to CPT I for fatty acid oxidation not to be suppressed at all times (72). As recently suggested by McGarry and Brown (69) "this paradox might be explained if the cytosol contains a binding protein that sequesters malonyl CoA when the tissue has a need for fatty acid oxidation" or if "a significant fraction of the malonyl CoA... measured in heart and skeletal muscle is present within the mitochondria (possibly produced there by the action of propionyl CoA carboxylase on acetyl CoA). In the latter event, it would not be accessible to CPT I, which is located in the outer mitochondrial membrane." Whatever the explanation, it is likely that the effective concentration of malonyl-CoA that interacts with CPT I in muscle is both lower than that measured in whole tissue and subject to greater variation. If so, an intriguing possibility is that the regulation of such a "microenvironment" of CPT I is related to the unique NH_2-terminal region of ACC_β (see *Acetyl-CoA Carboxylase: Isoforms*), which has been suggested to enhance its binding to mitochondria (45).

Are there other intracellular determinants of fatty acid oxidation? Changes in the concentration of malonyl-CoA are almost certainly not the sole intracellular determinant of the rate at which muscle oxidizes fatty acids. One circumstance in which malonyl-CoA does not appear to play a pivotal role is during intermediate periods of starvation, when ketone bodies are the major fuel of the muscle cell (105). Ketone body utilization accounts for at least 60–80% of the O_2 consumed by muscle in humans after 3–7 days (38, 90) and in the rat after 48 h of starvation (104), despite high plasma FFA levels. In the rat, this occurs even though the concentration of malonyl-CoA in muscle is low (26, 76, 141). Presumably acetoacetate and β-hydroxybutyrate are inhibiting fatty acid oxidation in this situation by a mechanism not involving malonyl-CoA (e.g., by competition for CoA in the mitochondria). The possibility that compartmentation of malonyl-CoA (see preceding section) masked an increase in its concentration in the cytosol cannot be ruled out, however.

How is malonyl-CoA utilized in muscle? The fact that the concentration of malonyl-CoA decreases by 50% within 20 min when an incubated soleus muscle is deprived of glucose (115), and even more rapidly during contraction (134), suggests that malonyl-CoA utilization, as well as synthesis, is regulated. In liver and other lipogenic tissues, the principal determinant of malonyl-CoA use is thought to be the rate of fatty acid synthesis, a process governed by the activity of fatty acid synthase (2). In nonlipogenic tissues, such as heart, fatty acid synthase activity is negligible, although not absent (6). Heart, like liver, contains a malonyl-CoA decarboxylase (61), a fatty acid elongation system (6), and possibly other enzymes that could utilize malonyl-CoA (1); however, essentially nothing is known about their role in skeletal muscle (Fig. 3). Adding to the perplexity is the fact that malonyl-CoA decarboxylase in all tissues studied, except the europygial gland of the goose, is predominantly a mitochondrial enzyme (53). Thus its role in degrading malonyl-

CoA produced by ACC, which is thought to be a cytosolic or at least an extramitochondrial enzyme, is unclear. One possibility is that malonyl-CoA generated in the cytosol is transported into the mitochondria; however, to date such an event has not been described.

Malonyl-CoA, LCFA-CoA, and Insulin Resistance

Association of high concentrations of malonyl-CoA with insulin resistance in skeletal muscle. A number of lines of evidence suggest an association between sustained elevations in the concentration of malonyl-CoA and insulin resistance (i.e., a less than normal biological effect of insulin) in skeletal muscle. 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 (117), the KKA[y] mouse (114), rats infused with glucose for 1–4 days (63), and the Goto-Kakizaki (GK) rat (109, and T. G. Kurowski, unpublished observations), as well as in muscle of normoinsulinemic-normoglycemic rats made insulin resistant by denervation (115) (Table 2). A high concentration of malonyl-CoA, by restraining the entrance of LCFA-CoA into the mitochondria, would in turn increase both their concentration in the cytosol and incorporation into glycerolipids (Fig. 7). Thus a high level of malonyl-CoA could contribute to the elevated concentrations of triglyceride, diacylglycerol, and LCFA-CoA observed in many insulin-resistant muscles (91) (Table 2). The effect of decreasing the concentration of malonyl-CoA on the concentrations of these lipid metabolites and on insulin resistance has received less attention, although prior exercise, which acutely decreases the concentration of malonyl-CoA in skeletal muscle (see *ACC$_\beta$ Regulation During Exercise and Recovery*), has been shown to increase the sensitivity and responsiveness of glucose transport and glycogen synthesis to stimulation by insulin (86, 101). Likewise, decreases in the concentration of malonyl-CoA have been observed in muscle and liver of the KKA[y] mouse (114), and decreases of triglycerides and diacylglycerol

Table 2. *Lipid metabolites and protein kinase C in muscle of insulin-resistant rodents*

	Plasma		Muscle				
	Insulin	Glucose	TG	DAG	LCFA-CoA	Malonyl-CoA	Altered PKC
KKAy mouse	+	+	+	+	ND	+	ND
fa/fa rat	+	0	+	+	ND	+	Yes
Glucose-infused rat	+	+	+	ND	+	+	Yes
Fat-fed rat	0	0	+	+	+	±	Yes
GK rat	0–+	+	+	+	ND	+	Yes
Denervated rat	0	0	ND	+	ND	+	Yes

TG, triglycerides; DAG, diacylglycerol; LCFA-CoA, long-chain fatty acyl-CoA; Altered PKC, altered protein kinase C distribution or activity; GK, Goto-Kakizaki. An increase in concentration is indicated by +. ND, not done. Recent studies suggest that alterations in the distribution of PKC$_\epsilon$ occur in the muscle of many of these rodents. [Compiled from data from our laboratory (51, 114, 115, 116), unpublished observations of Lin, Kurowski, Ruderman, and Yaney and of Kurowski, Saha, and Ruderman, and studies cited in Refs. 5, 63, 86, 87, 120, 121, and 132.]
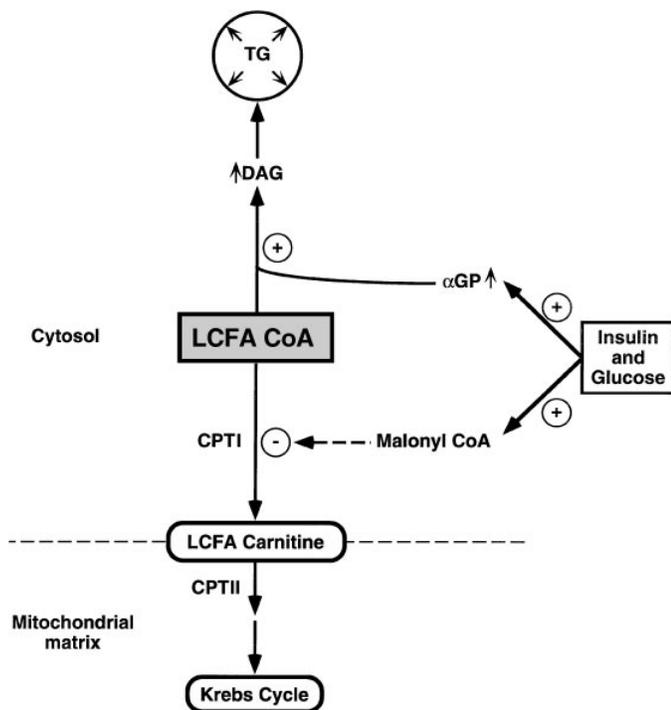
Fig. 7. Effect of insulin and glucose on the disposition of LCFA-CoA in skeletal muscle. By inhibiting carnitine palmitoyltransferase (CPT I), malonyl-CoA blocks conversion of LCFA-CoA to LCFA carnitine, thereby diminishing its uptake into mitochondria. LCFA incorporation into triglycerides and other glycerolipids is enhanced by this, as well as by increases in the concentration of α-glycerol phosphate (αGP) and the activities of enzymes involved in triglyceride synthesis. CPT II catalyzes the conversion of LCFA carnitine to LCFA-CoA in the mitochondrial matrix. DAG, diacylglycerol; TG, triglycerides.

have been demonstrated in muscle of fat-fed rats (87) when their insulin resistance is diminished by treatment with thiazolidinediones.

As recently reviewed (109), an increase in the concentration of malonyl-CoA by itself is not sufficient, or in some instances necessary, for the development of insulin resistance in muscle. Thus fat-feeding, which has been shown to produce insulin resistance (86), can do so without causing a significant increase in malonyl-CoA (86). Likewise, insulin resistance is either minimal or

not present in the male Dahl salt-sensitive rat (Dahl-S), a nonobese but hypertriglyceridemic rodent that has high levels of malonyl-CoA in both its liver and muscle (62). On the other hand, when the Dahl-S rat is fed a high-fat, high-sucrose diet, it becomes more hyperinsulinemic (and insulin resistant) and accumulates more retroperitoneal fat than does a Sprague-Dawley control rat. Whether its high tissue levels of malonyl-CoA predispose the Dahl rat to insulin resistance and abdominal obesity is an intriguing question (see *Fat partitioning, thrifty genes, and obesity*).

The LCFA-CoA/malonyl-CoA hypothesis. A scheme that both links malonyl-CoA to insulin resistance in skeletal muscle and accounts for the occasional presence of insulin resistance when malonyl-CoA levels are not increased (108, 114) is depicted in Fig. 8. The common denominator in the proposed model is an increase in the cytosolic concentration of LCFA-CoA, which can result from increases in malonyl-CoA or FFA, and especially the two in combination. Increases in LCFA-CoA could secondarily lead to increases in the concentration of diacylglycerol (DAG), phosphatidic acid, and triglycerides and activation of one or more protein kinase C (PKC) isoforms (Table 2). The PKC isoforms are attractive candidates for study, because changes in the distribution and/or activity of PKC_ϵ, and in some instances PKC_θ, have been demonstrated in insulin-resistant muscles by a number of investigators (5, 28, 63, 111, 120, 121). In addition, PKCs have been shown to phosphorylate and inhibit both the insulin receptor (80, 92) and glycogen synthase (13), and their activation at least in fat cells leads to inhibition of PKB/Akt (8), a distal component of the insulin-signaling cascade that appears to be involved in the regulation of glucose transport and glycogen synthesis (136). Other proposed mechanisms by which increases in LCFA-CoA could lead to insulin resistance include alterations in protein acylation, membrane fluidity (94, 108, 114), gene transcription and hexosamine synthesis (49), and direct inhibition of enzymes such as glycogen synthase (144). Increased hexosamine synthesis has been linked to the insulin resistance in skeletal muscle and adipose tissue caused by hyperglycemia (68). It has

Fig. 8. Hypothetical interrelations between concentration of cytosolic LCFA-CoA and development of insulin resistance in muscle. Factors that could increase the concentration of cytosolic LCFA-CoA by effects on malonyl-CoA and plasma free fatty acids (FFA), see boxes (*left*); possible mechanisms by which LCFA-CoA or a related metabolite could affect action of insulin, see boxes (*right*). See text for details.
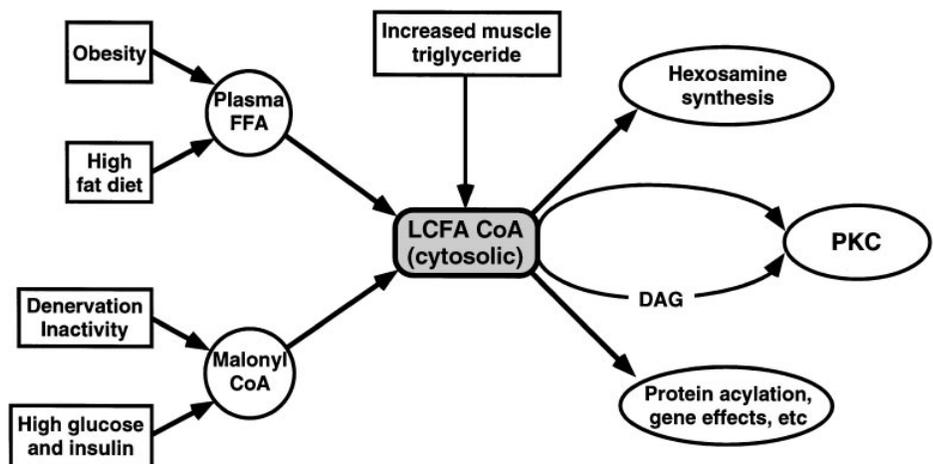
recently been shown that hexosamine accumulation observed in rat muscle during a euglycemic-hyperinsulinemic clamp is increased further, as is insulin resistance, if the rat is also infused with lipid (49). Even in the absence of extra lipid, incubation with glucosamine has been demonstrated to alter PKC distribution in rat adipocytes (32). Whether PKC is the common link in the insulin resistance attributed to the hexosamine and LCFA-CoA/malonyl-CoA mechanisms will clearly be the object of considerable investigation.

Another noteworthy feature of the scheme depicted in Fig. 8 is that it allows for the cytosolic concentration of LCFA-CoA in muscle to be increased, independently or in concert, by a number of mechanisms implicated in insulin resistance. Thus an increase in LCFA-CoA could occur if the muscle receives excess lipid from the circulation because of high plasma FFA or triglyceride levels, if the concentration of malonyl-CoA is increased and as a consequence less LCFA-CoA enters the mitochondria, or if the content of triglyceride in muscle is increased, resulting by mass action in an increased generation of LCFA and secondarily LCFA-CoA within the muscle cell. The latter could account for the well-established relationship between muscle triglyceride content and insulin resistance in both humans and experimental animals (91, 109, 128).

The LCFA-CoA/malonyl-CoA hypothesis, the glucose-fatty acid cycle, and insulin resistance in humans. Since the first description of the glucose-fatty acid cycle in the perfused rat heart, the notion that increases in plasma FFA, and secondary to this of fatty acid oxidation, lead to insulin resistance in skeletal muscle and other tissues has been both widely accepted and disputed (37, 43, 97, 98, 110). Recently, the role of this mechanism in causing insulin resistance has been examined in humans undergoing a euglycemic-hyperinsulinemic clamp, in which the usual decrease in plasma FFA was prevented by coinfusing a fat emulsion or a fat emulsion plus heparin (14, 15, 55). As reviewed by Boden and Jadal (15), the results clearly show that infusion of fat inhibits glucose oxidation within 1–2 h by inhibiting pyruvate dehydrogenase, as originally proposed (97). They also show that the fat infusion inhibits glucose utilization, but only after 4 h, suggesting that this effect is due to a mechanism that is not acutely related to fatty acid oxidation (15, 55). In support of this contention, impaired glucose incorporation into glycogen rather than diminished glycolysis [which should occur if increased fatty acid oxidation were the primary event (97)] accounted for most of the decrease in glucose utilization; indeed, in some studies (55) no decrease in muscle glycolysis was observed. Also, in the one investigation in which it was measured (15), no increase in muscle citrate was observed. Thus the classic glucose-fatty acid cycle mechanism, in which increased fatty acid oxidation inhibits glucose utilization by raising the cytosolic concentration of citrate and secondarily inhibiting glycolysis and glucose phosphorylation (98), does not appear to explain these findings. A noteworthy aspect of these studies in humans was that the experimental subjects were all undergoing a euglycemic clamp. This would raise the concentration of malonyl-CoA in muscle (9), leading to a decrease in the entrance of cytosolic LCFA-CoA into the mitochondria and an increase in its incorporation into glycerolipids (Fig. 7). Thus, when plasma FFA levels are concurrently increased, changes in DAG-PKC signaling would be more likely to occur (Figs. 7 and 8). As mentioned in *The LCFA-CoA/malonyl-CoA hypothesis*, such a mechanism has been put forth to explain insulin resistance in muscle in a variety of rodents.

Finally, increases in plasma FFA, induced by an intralipid infusion and heparin in a rat undergoing a euglycemic-hyperinsulinemic clamp, have also been shown to inhibit glucose utilization by skeletal muscle. In contrast to the findings in humans, glucose incorporation into glycogen was enhanced and glycolysis was inhibited, suggesting operation of a glucose-fatty acid cycle type of mechanism (56). Whether the apparently disparate results in humans and rats reflect species variability or differences in experimental design remains to be determined.

Fuel Sensing by Cellular Stresses: the 5'-AMPK

As pointed out in *Acetyl-CoA Carboxylase: Isoforms*, nutritional and hormonal regulation of ACC_β in skeletal muscle by hormones and nutrients has not been shown to involve phosphorylation, nor to date has genetic regulation been described except during differentiation (138). On the other hand, changes in the assayable activity of ACC_α and ACC_β due to phosphorylation have been observed in skeletal and cardiac muscle and liver in response to a variety of stressful stimuli, including ischemia/hypoxia, heat shock, inhibition of oxidative phosphorylation and glucose metabolism, and, in skeletal muscle, exercise (25, 48, 61, 65, 93, 112) (Fig. 9). In keeping with these observations, both ACC_α and ACC_β appear to be substrates for multisite protein phosphorylation by several cellular protein kinases (48, 57, 58). Furthermore, the major phosphorylation site motifs in both the NH_2 terminus and the middle of these molecules have all been demonstrated to be sites of actual phosphorylation in intact tissues (57, 58).

Although ACC is a multisite phosphorylated enzyme, it is clear that the major regulatory protein kinase that acts on it is the AMPK. AMPK phosphorylates ACC_α on at least three serine residues (S79, S1200, S1215), all of which are preserved on ACC_β (1, 44, 47, 48). As will also be discussed in ACC_β *Regulation During Exercise and Recovery*, phosphorylation is associated with marked enzyme inactivation and decreased sensitivity to the allosteric activator citrate (47, 48, 134). ACC isozymes in liver, adipose tissue, skeletal muscle, and heart are all identically affected after phosphorylation by AMPK (44, 47, 48, 61, 81, 134, 140). These changes in enzyme phosphorylation and activity are readily reversed on incubation with protein phosphatases; protein phosphatases 2A and 2C are the predominant phosphatases active on ACC in most tissues (47, 48).

AMPK was described originally as a protein kinase that phosphorylates and inhibits hydroxymethyl glu-
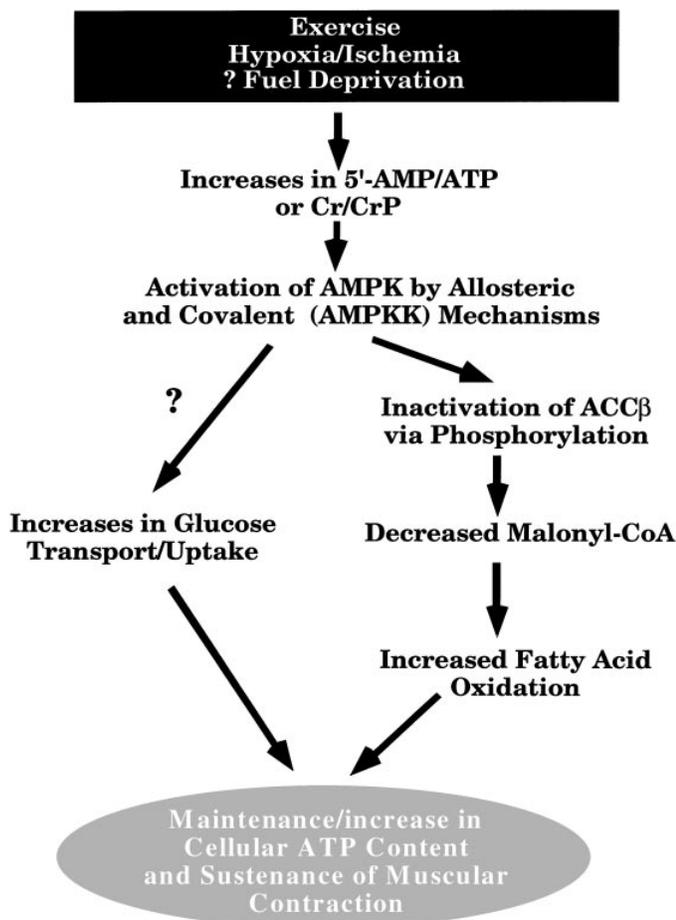
Fig. 9. AMP regulation of ACC$_\beta$ activity and fatty acid oxidation in skeletal muscle. Cr/CrP, ratio of creatine to creatine phosphate.

taryl-CoA reductase (47). It derives its name from the fact that it is potently activated by AMP (Michaelis-Menten constant $K_a = 20$ μM). As first shown by Hardie (47), alterations in AMP levels in intact cells and tissues are associated with reciprocal changes in AMPK and ACC activities. AMPK is also activated by phosphorylation of specific serine/threonine residues by an "upstream" kinase/kinase (AMPKK) (48). Increases in AMP concentration have been shown to activate AMPK in four distinct ways. These include direct allosteric regulation of AMPK, direct activation of AMPKK, enhancement of AMPK phosphorylation by AMPKK, and diminution of the susceptibility of AMPK to dephosphorylation by phosphatases (48). These multiple mechanisms permit substantial signal amplification with high sensitivity, even when changes in cellular AMP (or in the ratio AMP/ATP) are small. It is logical to assume that they come into play when ATP is depleted in response to substrate limitation (in some tissues), changes in oxidative phosphorylation, ischemia, hypoxia, and many other physiological and pathophysiological circumstances. In these conditions, AMP formation is increased as a result of the adenylate kinase reaction in which ATP and AMP are formed from two molecules of ADP in an effort to maintain ATP concentration. Thus, even with minimal reductions in cellular ATP,

changes in the concentration of AMP can cause AMPK to become a sensor and/or effector of the cell's energy state. Evidence has recently been presented that changes in the creatine phosphate-to-creatine ratio (CrP/Cr) in muscle may supply a second tier of allosteric regulation of AMPK (93) (see *ACC$_\beta$ Regulation During Exercise and Recovery*).

Purification, partial amino acid sequencing, and cDNA cloning have shown that AMPK consists of three subunits: the catalytic subunit α and two noncatalytic subunits β and γ (31, 39, 40, 48, 75, 77, 78, 125–127). The catalytic α-subunit of AMPK is a member of the SNF1 protein kinase subfamily that includes protein kinases of yeast, plants, *C. elegans*, and humans (39, 48, 78, 125–127). The structural relationship of AMPK$_\alpha$ to the *S. cerevisiae* protein kinase SNF1 is especially intriguing, because the latter is directly involved in fuel sensing. Thus it regulates the induction of invertase (SUC2) under conditions of nutritional stress (carbon catabolite derepression), as well as other glucose-responsive genes in yeast (39, 48, 78, 125). Recent cloning data indicate that, for each of the AMPK subunits, there exists at least one other mammalian protein isoform. For example, two different catalytic subunits, α-1 and α-2, that are the products of unique genes, have been identified (126). The AMPK γ-subunits (γ1, γ2, γ3) are homologous to the yeast protein Snf4p, and the AMPK β-subunits (β1, β2) are related to the yeast Sip1p/Sip2p/Gal83p family of proteins (39). Genetic evidence suggests that both of these yeast protein families positively regulate SNF1 protein kinase activity (149). The expression of all three subunits and the formation of an enzyme heterotrimer are necessary for optimal catalytic activity (31). Taken together, these observations indicate a high evolutionary conservation of this fuel-sensing protein kinase family that responds to nutrient signals in the absence of hormones. AMPK subunits (protein or mRNA) have been detected in virtually all mammalian tissues examined to date (39, 40, 48, 75, 126). Rat skeletal and heart muscles express the highest concentrations of both catalytic α-isoforms (75, 126).

Overall, the AMPK-ACC link appears to serve a sensor-effector function to alert the cell to changes in adenylate charge. As currently understood in skeletal and cardiac muscle (see *ACC$_\beta$ Regulation During Exercise and Recovery*), these adjustments are primarily compensatory, serving to alert the cell to diminished ATP and then to increase ATP generation through changes in fuel utilization or availability (Fig. 9). In liver, activation of AMPK leads not only to a compensatory increase in fatty acid oxidation through a malonyl-CoA-dependent mechanism, but also to diminutions in the rates of fatty acid and sterol biosynthesis. Thus it decreases flux through two pathways that use large amounts of ATP and are not necessary for immediate cell survival or function. It seems possible that AMPK might regulate similar adaptive events in muscle (e.g., diminished protein synthesis) during periods of hypoxia, ischemia, or exercise. It is also possible that AMPK activation, if sustained, in muscle (cardiac or

skeletal) during hypoxia, ischemia, or exercise might be maladaptive, resulting in muscle dysfunction.

ACC_β Regulation During Exercise and Recovery

Role of AMPK. As initially demonstrated by Winder et al. (139), the concentration of malonyl-CoA diminishes in rat skeletal muscle during exercise. Studies in which muscle has been made to contract by electrical stimulation of its nerve supply for 5 min have revealed that this decrease in malonyl-CoA (29, 134) is associated with a diminution in ACC_β activity that is evident within seconds and persists for upwards of an hour after the cessation of contraction (134, 140) (Fig. 10). Evidence from our laboratory that this decrease in assayable ACC_β activity is due to phosphorylation includes the following observations: *1*) A gel shift of immunopurified ACC_β, which parallels the decrease in activity, is observed in muscle sampled within seconds after the onset of contraction (134). *2*) Reversal of the gel shift and the decrease in ACC activity is induced by treatment of the immunopurified enzyme with phosphatases (134). *3*) The decrease in ACC_β activity during contraction and the increase in activity during recovery are associated with reciprocal changes in the activity of the $\alpha2$ (but not the $\alpha1$) isoform of AMPK (134) (Fig. 10). In addition, it has been shown that incubation of purified ACC_β with AMPK markedly diminishes its activity, whereas a variety of other protein kinases have no effect (142) (Vavvas, unpublished observations). Collectively, these findings suggest that activation of an AMPK, most likely the $\alpha2$ isoform, mediates the inhibition of ACC_β during contraction in rat muscle.

Similar changes appear to account for the decreases in ACC activity and malonyl-CoA as a result of voluntary exercise (140). Because free AMP levels are increased and CrP levels are decreased during contraction and exercise (3, 30), a logical conclusion is that changes in their concentrations are the initiating event (93, 134).

Our observation that only one ($\alpha2$-containing) isoform of AMPK is activated during contraction in skeletal muscle (134) implies differences in the regulatory control of different AMPK heterotrimers. Exercise-induced AMPK activation has been replicated by incubating rat muscle with the AMP-mimetic AICAR (or Z-riboside) (74). AICAR (as the riboside) can be taken up by several cell types and phosphorylated to ZMP, ZDP, and ZTP (48). ZMP, like 5′-AMP, is an allosteric regulator of AMPK and has been shown to lead to ACC inactivation in several cell types (24, 130). Although the effects of ZMP are most certainly nonspecific (likely mimicking other known effects of AMP on glycolysis and glycogenolysis) (150) and are not restricted to AMPK activation, these data provide additional evidence for a cause-effect link between AMPK activation and changes in malonyl-CoA content after exercise. Interestingly, AICAR also increases glucose transport in rat skeletal muscle (74), and, like exercise, it does so by a mechanism not involving phosphatidylinositol (PI) 3-kinase (50). If confirmed, this would suggest that AMPK is central to the utilization of both glucose and fatty acids as metabolic fuels in contracting muscle, perhaps through both malonyl-CoA-dependent and -independent mechanisms.
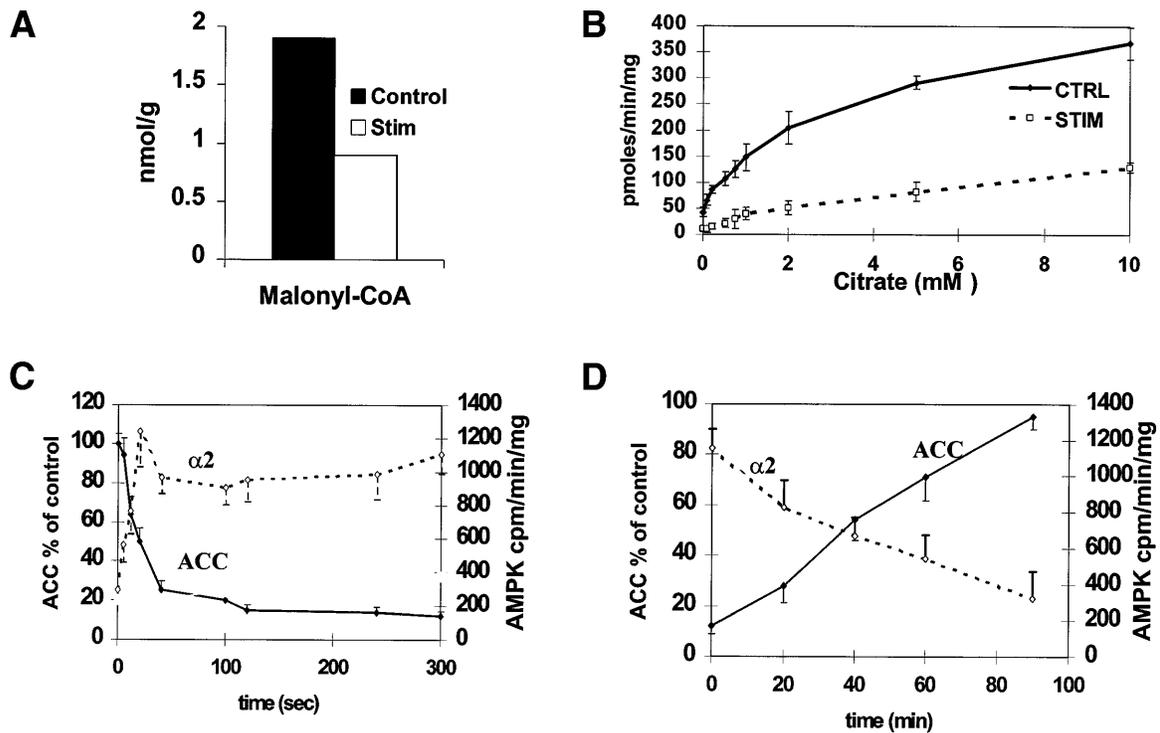

Fig. 10. Effect of electrically induced contractions of rat gastrocnemius-soleus muscles on concentration of malonyl-CoA (*A*) and activation of immunoprecipitated ACC_β by citrate (*B*). Time course of changes in activities of ACC_β and the $\alpha2$-isoform of AMPK during (*C*) and after (*D*) contraction. (Adapted from Ref. 134).

Although studies in rat muscle provide compelling evidence for the regulation of fatty acid oxidation by malonyl-CoA during exercise, it has not been established that malonyl-CoA plays a similar role in humans. Thus Odland et al. (89) found no decrease in the concentration of malonyl-CoA in muscle of normal volunteers after 10 and 20 min of leg exercise at 40 or 75% of maximal O_2 consumption. Possibly, small but significant decreases in malonyl-CoA were missed; however, this will require further study. Measurements of ACC_β and AMPK should be helpful in resolving this problem, because in the rat the activities of both enzymes change more dramatically than the concentration of malonyl-CoA during muscle contraction (Fig. 10).

Loss of ACC_β regulation by citrate. A noteworthy feature of the inhibition of ACC_β when it is phosphorylated by AMPK in rat muscle is that it can occur in the face of substantial increases in whole cell concentrations of both citrate and malate (134). In addition, the ability of citrate to activate ACC_β immunopurified from such muscles is substantially diminished (Fig. 11). These observations strongly suggest that, when the energy expenditure of the muscle cell and its need for fatty acid oxidation are increased, changes in the concentration of high-energy phosphate compounds overcome the effects of citrate on ACC_β activity and are the dominant mechanism for its regulation.

Dual regulation of ACC_β and phosphofructokinase-1. The dual regulation of ACC_β by citrate and AMP (ATP/AMP ratio) closely parallels the regulation by these substances of another key metabolic enzyme, phosphofructokinase-1 (PFK-1). PFK-1 is inhibited by ATP, and this inhibition is enhanced by citrate and diminished by AMP (85, 129). We have hypothesized that "by virtue of their dual effects on ACC_β and PFK, AMP and citrate complement each other in controlling the activities of these enzymes and, secondarily, the use of glucose and fatty acids as fuels for muscle" (134) (Fig. 11). Presumably changes in CrP/Cr, which also appear to alter the activity of AMPK (93) and possibly PFK (85) fit into this scheme.

AMPK and insulin sensitivity. As noted earlier, increases in the concentrations of malonyl-CoA and DAG and alterations in PKC distribution are associated with insulin resistance in skeletal muscle and could play a role in its development (see Table 2 and *Malonyl-CoA, LCFA-CoA, and Insulin Resistance*). Conversely, a single bout of exercise has been shown to increase insulin sensitivity in skeletal muscle in both humans (27) and experimental animals (101). An attractive notion is that exercise might exert this effect by activating AMPK, leading to changes in lipid metabolites and PKC contrary to those observed in insulin-resistant muscle. The observation that incubation of muscle with AICAR mimics the action of insulin on glucose transport (74) by a mechanism similar to that of exercise (50) is consistent with this possibility (74), as is the fact that exercise acutely lowers malonyl-CoA levels in rat muscle (139).
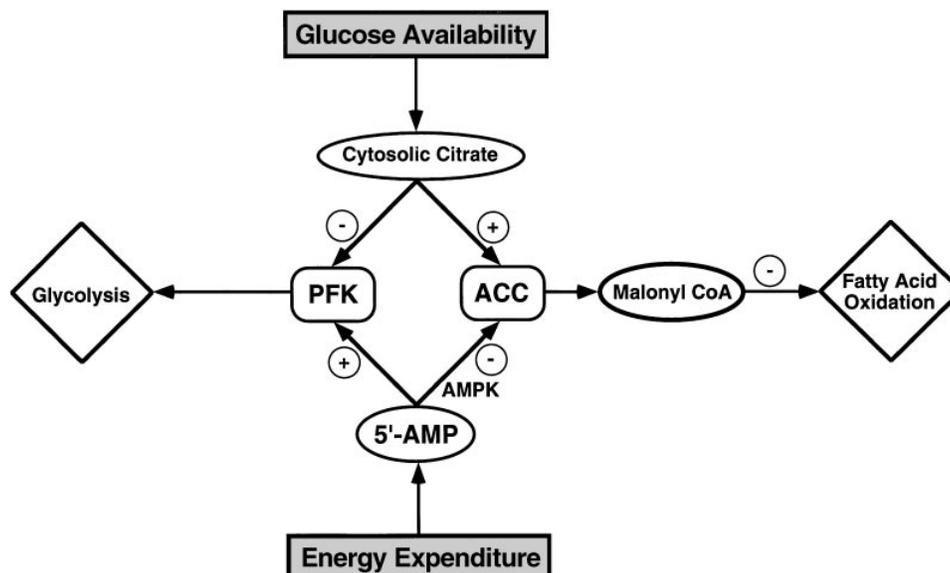

Fig. 11. Proposed dual mechanism for regulation of glycolysis and fatty acid oxidation in skeletal muscle and possibly other tissues. Increased glucose availability acutely activates ACC_β and inhibits PFK-1 by increasing the cytosolic concentration of citrate, whereas increased energy expenditure (exercise) activates PFK-1 and inhibits ACC_β by increasing concentration of free AMP. Phosphorylation and inhibition of ACC_β are catalyzed by an isoform of AMPK. According to proposed scheme, fatty acid oxidation will be restrained or enhanced, at least in part, by changes in concentration of malonyl-CoA, and glycolysis by changes in activity of PFK-1. During intense contraction, when both cytosolic citrate and AMP are increased, the AMP-mediated effects appear to dominate. How alterations in creatine PO_4-to-creatine ratio, which have recently been shown to activate AMPK, fit into this scheme remains to be determined. (Adapted from Ref. 134).

The Malonyl-CoA Fuel-Sensing and Signaling Mechanism: Other Implications

Link to cellular signaling. By definition, a malonyl-CoA fuel-sensing and signaling mechanism exists in all cells in which the concentration of malonyl-CoA is acutely regulated by the availability of glucose or other fuels and/or by changes in ATP/AMP or CrP/Cr (see *Fuel Sensing by Cellular Stresses: the 5'-AMPK and ACC$_\beta$ Regulation During Exercise and Recovery*). With respect to regulation by glucose, such a mechanism operating via cytosolic citrate appears to be present in skeletal (115) and cardiac (118) muscle, the pancreatic β-cell (23, 102), and brain (107). We would also predict that it will be found in neural cells in the hypothalamus that contain glucokinase or GLUT-4 glucose transporters. In addition to regulating fatty acid oxidation, changes in the concentration of malonyl-CoA could link fuel availability to signaling events and biological functions in these cells. As already discussed (see *Malonyl-CoA, LCFA-CoA, and Insulin Resistance*), one site where such a linkage to signal transduction appears to occur is skeletal muscle, where sustained high levels of malonyl-CoA are associated with insulin resistance (109). Another is the pancreatic β-cell, in which the stimulation of insulin secretion by glucose is associated with increases in the concentrations of malonyl-CoA, citrate, malate, and DAG (23, 95, 102), and insulin secretion is blocked by hydroxycitrate, an inhibitor of ATP-citrate lyase and secondarily of malonyl-CoA formation (22) (see Fig. 3) and by stable transfection with an ACC-specific antisense mRNA (152). It remains to be determined whether at a molecular level the links between malonyl-CoA and insulin resistance in muscle and insulin secretion in the β-cell are mediated by PKC isozymes (94, 108, 109) or by other signaling molecules. Nevertheless, these observations strongly suggest that malonyl-CoA plays a pivotal role in modulating the effects of glucose and possibly other fuels on the functions of these tissues.

Fat partitioning, thrifty genes, and obesity. One of the most intriguing implications of the malonyl-CoA fuel-sensing and signaling mechanism is its possible relationship to the pathogenesis of obesity. Obesity has classically been defined as a disorder in which, for a period of time, energy intake exceeds energy expenditure and the caloric excess accumulates as fat (33). As first suggested by Neel (83) in his "thrifty gene" hypothesis, factors that predispose to obesity, and to type 2 diabetes with which it is closely associated, may during evolution have improved survival in humans better able to store energy as fat. More specifically, he proposed that in the feast-famine environment of our ancestors, individuals "exceptionally efficient in the uptake and utilization of food" would have had a selective advantage. Also relevant to this issue is the notion that obesity is a disorder of fat partitioning. This conception is based on the observation that humans and experimental animals generally are able to adjust rates of carbohydrate and amino acid oxidation to the amounts of these nutrients in their diet, but they are less able to adjust fat oxidation to fat intake (34). It has been suggested that for this reason some humans and experimental animals are more prone to obesity than others when placed on a diet with a high-fat content (17, 35, 119). Although the notion of fat partitioning has led to many recommendations concerning the fat content of our diet, a mechanistic explanation for the adipogenic effect of a high-fat, high-calorie diet has not come forth, nor is it clear why certain individuals and animals are more likely to become obese than others when ingesting it (17, 35, 119). It is our premise that dysregulation of the malonyl-CoA fuel-sensing and signaling mechanism, resulting in an inappropriately high concentration of malonyl-CoA (i.e., high for a given cytosolic LCFA-CoA level) in muscle and other tissues, could be a contributory factor. The following characteristics of humans and experimental animals at risk for obesity are supportive of this view: *1*) a decreased ability to oxidize fatty acids as reflected by a high respiratory quotient (RQ) (4, 17, 19, 99); *2*) decreased physical activity (99, 103), which would be expected to raise the concentration of malonyl-CoA in muscle; *3*) hyperinsulinemia and insulin resistance (in children and some adults) (16, 88); and *4*) high tissue levels of malonyl-CoA (in the Dahl-S rat, a lean rodent that becomes more obese than a control rat when fed a high fat-high sucrose diet) (62). That skeletal muscle may be a specific site of malonyl-CoA dysregulation is suggested by the finding of a much higher RQ across leg muscle of humans with established obesity than in lean control subjects, despite twofold higher plasma levels of FFA in the obese group (67).

Yet another connection between malonyl-CoA and the pathophysiology of obesity is its apparent relationship to leptin, the product of the *ob* gene. Although classically thought of as an appetite suppressant by virtue of its effects on the arcuate and perhaps other nuclei in the hypothalamus, leptin has also been demonstrated to increase total body energy expenditure (36) and carbohydrate metabolism (54). In addition, it increases fat oxidation in muscle (82, 124) and the pancreatic β-cell (122) and inhibits acetyl-CoA carboxylase in fat cells (7). Especially intriguing is a preliminary report in which a leptin infusion was shown to lower the concentration of malonyl-CoA in rat liver by 50% within 6 h (137). These findings raise many questions, including the following: *1*) How is the effect of leptin on malonyl-CoA mediated? *2*) Does leptin have a similar effect on malonyl-CoA in the hypothalamic nuclei considered to be its principal target? *3*) Does a defect in leptin production or action account for the high RQ in preobese subjects? *4*) Do changes in malonyl-CoA secondarily (e.g., by effects on DAG-PKC) affect activation of JAK kinase and other signaling events set in motion when leptin binds to its receptor? Whatever the answers to these questions, the interrelationships between malonyl-CoA and leptin and their physiological implications are clearly exciting areas for further study (36).

The insulin resistance syndrome. The combination of hyperinsulinemia, insulin resistance in skeletal muscle

and possibly liver, and an increase in abdominal adiposity has been shown to antedate a cluster of disorders that include type 2 diabetes, essential hypertension, endogenous hypertriglyceridemia, and premature coronary artery disease. This association has been referred to as the insulin resistance syndrome or syndrome X (18, 60, 100, 109). Despite the unquestioned clinical importance of this syndrome, it is still uncertain whether a β-cell defect leading to hyperinsulinemia or insulin resistance in muscle is the primary event (71) or whether they occur together (94, 114). Also unclear is what role increases in intra-abdominal fat play in its pathogenesis (12, 59). As noted earlier, a mechanism for the regulation of malonyl-CoA and LCFA-CoA by glucose and fatty acids, similar to that observed in muscle, has been described in the pancreatic β-cell, where it may have an important role in the regulation of insulin secretion (22, 84, 94, 102). It has been suggested that such a mechanism will also be found in adipocytes (94), glucose-sensing cells in the central nervous system, and other cells in which the use of glucose as a fuel is a function of its availability (108). The notion that concurrent alterations in malonyl-CoA and cytosolic LCFA-CoA in these cells could produce signaling abnormalities that in turn cause hyperinsulinemia, impaired insulin action, and other manifestations of the insulin resistance syndrome, as well as obesity itself, has also been proposed (94, 108, 114) (see Fig. 8 and Table 2). As pointed out by Prentki and Corkey (94), such an hypothesis offers a novel explanation both for the presence of the multiple alterations of the insulin resistance syndrome in some individuals and for the fact that it has been difficult to determine the nature of the primary event. The numerous observations that exercise, caloric restriction, and thiazolidinediones, all of which improve insulin sensitivity, can concurrently diminish malonyl-CoA levels and many manifestations of the insulin resistance syndrome support this contention.

Concluding Remarks

The elucidation of how the concentration of malonyl-CoA is regulated in muscle and other cells has broad implications. Apart from enhancing our understanding of the intracellular control of fatty acid oxidation, it offers a potential mechanism by which fuels, and in particular glucose, create signals that regulate cellular function. In addition, an increasing body of evidence 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. How changes in malonyl-CoA concentration 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, as is the possible therapeutic value of exercise, pharmacological agents, and other therapies that lower the concentration of malonyl-CoA and diminish glycerolipid synthesis.

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