Metabolic Syndrome: Adenosine Monophosphate-activated Protein Kinase and Malonyl Coenzyme A

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Abstract
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The metabolic syndrome can be defined as a state of metabolic dysregulation characterized by insulin resistance, central obesity, and predisposition to type 2 diabetes, dyslipidemia, premature atherosclerosis, and other diseases. An increasing body of evidence has linked the metabolic syndrome to abnormalities in lipid metabolism that ultimately lead to cellular dysfunction. We review here the hypothesis that, in many instances, the cause of these lipid abnormalities could be a dysregulation of the adenosine monophosphate-activated protein kinase (AMPK)/malonyl coenzyme A (CoA) fuel-sensing and signaling mechanism. Such dysregulation could be reflected by isolated increases in malonyl CoA or by concurrent changes in malonyl CoA and AMPK, both of which would alter intracellular fatty acid partitioning. The possibility is also raised that pharmacological agents and other factors that activate AMPK and/or decrease malonyl CoA could be therapeutic targets.

Key words: acetyl coenzyme A carboxylase, atherosclerosis, diabetes, fatty acyl coenzyme A, insulin resistance

Introduction
The metabolic syndrome can be defined as a state of metabolic dysregulation characterized by insulin resistance, inflammation, and predisposition to type 2 diabetes, dyslipidemia, premature atherosclerosis, and other disorders (Figure 1). Obesity, in particular central obesity, and physical inactivity are contributing factors, as are incompletely understood genetic determinants. At a molecular level, abnormalities in cellular lipid metabolism, as reflected by increases in intracellular triglycerides, are thought to be an early event (1,2). The importance of the metabolic syndrome stems from the fact it antedates and probably contributes both to the pathogenesis of the many disorders with which it is associated (3,4) and to the increased incidence of coronary heart disease that accompanies these disorders (5,6). In addition, the metabolic syndrome is extremely common: an estimated 65,000,000 people in the United States are affected, according to a recent report (7). Furthermore, its prevalence is likely to increase further as obesity and inactivity become more endemic in younger populations.

An increasing body of evidence has led us to hypothesize that a common feature underlying the metabolic syndrome and a target for its therapy, in many instances, could be dysregulation of the adenosine monophosphate-activated protein kinase (AMPK)1/malonyl coenzyme A (CoA) fuel-sensing and signaling network (8). As will be discussed later, such dysregulation could consist of an isolated increase in malonyl CoA concentration, an increase in malonyl CoA together with a decrease in AMPK activity, or a failure of one or both of these molecules to change appropriately in a given setting. The evidence for and against this hypothesis will be examined in this review.

Insulin Resistance: The Lipid Theory (Figure 2)
The notion that insulin resistance could be the result of an increase in plasma free fatty acid (FFA) levels was first
proposed by Randle et al. (9) based on studies in the rat heart. Nearly 30 years later, Boden et al. (10) showed that raising plasma FFA levels during a euglycemic clamp for 4 to 6 hours causes insulin resistance in human skeletal muscle. Subsequent studies by many laboratories, most notably that of Shulman (1) and Ruderman (4), showed that the insulin resistance in these individuals was associated with decreased glucose transport into the muscle cell, abnormalities in insulin signaling, and an increase in intramyocellular triglyceride. Later it was shown that these changes were in turn associated temporally with 2- to 3-fold increases in the concentration of diacylglycerol (DAG) and activation of protein kinases C\(\beta\) and C\(\delta\) (PKCs) and nuclear factor-\(\kappa\)B (NF-\(\kappa\)B) (11). Similar, although not identical, findings in muscle have been described in rats in which plasma FFAs are elevated during a clamp or insulin resistance is produced by fat feeding (12–14).

Insulin resistance in muscle, even when accompanied by alterations in intracellular lipid metabolism, can occur in the absence of an increase in plasma FFAs. One example of this is denervated rat muscle. It had long been known that 24 hours after a sciatic-nerve section, the ability of insulin to simulate glucose incorporation into glycogen is almost totally inhibited in hind limb muscles of these rats (15). Thus, it was of considerable interest when we found that both PKC activity and the concentration of the PKC activator, DAG, were increased several-fold in these muscles (16). Furthermore, although the ability of insulin to enhance glucose incorporation into glycogen was markedly diminished, the stimulation of glucose incorporation into diacylglycerol and other glycerolipids by insulin was increased 2- to 3-fold, suggesting a disturbance in fatty acid partitioning.

**Malonyl CoA Fuel-sensing and Signaling Mechanisms**

In searching for a cause of altered fatty acid partitioning in denervated muscle, our attention turned to malonyl CoA. Malonyl CoA was originally identified as an intermediate in the de novo synthesis of fatty acids (e.g., FA-CoA) increase in the cytosol and this leads to elevations in DAG, oxidative stress (Reactive Oxygen Species), NF-\(\kappa\)B activation, etc. As depicted, four factors, individually and collectively, could contribute to this 1) increases in plasma FFA caused by obesity or lipodystrophy, 2) enhanced de novo synthesis of cytosolic FA-CoA, 3) inhibition or lack of a compensatory increase in FA-CoA entrance into the mitochondria where they are oxidized, and 4) an increase in the extramitochondrial mechanisms leading to DAG, Reactive Oxygen Species, etc. An increase in malonyl CoA by inhibiting CPT1 and diminishing FA-CoA entrance into mitochondria affects mechanism (3). A decrease in AMPK could affect all four mechanisms. Not shown in the diagram is that an increased release of certain cytokines from adipose tissue (TNF-\(\alpha\), resistin) causes insulin resistance in muscle and liver, as does a decrease release of another cytokine–adiponectin. AMPK activation has been shown to inhibit TNF-\(\alpha\)-mediated events in vascular endothelium (48). Whether it does so in other tissues or affects the release of the various cytokines from adipose tissue is not known.
revealed that malonyl CoA also plays a central role in mediating the regulation of fatty acid oxidation by glucose. Thus, in incubated rat muscle, the acute inhibition of fatty acid oxidation caused by an increase in glucose availability was associated with an increase in the concentration of malonyl CoA, and the enhancement of fatty acid oxidation caused by glucose deprivation was accompanied by a decrease in its concentration (19). At a cellular level, these events were shown to be mediated, at least in part, by changes in the activity of acetyl CoA carboxylase (ACC), the enzyme that catalyzes the synthesis of malonyl CoA (8,20). Based on these studies in skeletal muscle, the existence of a malonyl CoA fuel-sensing and signaling mechanism was proposed (19,20), in which ACC acts as the sensor and malonyl CoA as the signal (Figure 4). A similar role for malonyl CoA has been proposed in the pancreatic cells (21) and in hypothalamic nuclei (22,23), in which changes in its concentration have been linked to the regulation by glucose availability of insulin secretion and food intake, respectively.

As already noted, by preventing fatty acyl CoA from entering the mitochondria, malonyl CoA would theoretically make more of the FACoA in the cytosol available for TG, DAG, and ceramide synthesis, and possibly for lipid peroxidation. AMPK could inhibit these events and increase fatty acid oxidation, acutely by phosphorylating or otherwise inhibiting ACC and GPAT and activating MCD, and chronically by diminishing the expression of SREBP1c and activating PGC1α and PRARα (not shown). In addition, AMPK could independently decrease the cytosolic concentrations of FA-CoA by inhibiting lipolysis in the fat cell. The basis for its ability to diminish oxidant stress is not known. Whether AMPK activation results in enhancement of inhibition of a process is denoted in the figure by plus and minus signs, respectively. Available evidence indicates that a decrease in AMPK activity has opposite effects.

Table 1. Metabolic and enzymic changes in muscle of insulin-resistant organisms

| Model              | TG | DAG | Malonyl CoA | PKC activity |
|--------------------|----|-----|-------------|--------------|
| fa/fa rat          | +  | +   | +           | +            |
| Glucose-infused rat| +  | +   | +           | +            |
| Fat-fed rodents    | +  | +   | +/-         | +            |
| Fat-infused humans | +  | ND  | ND          | +            |
| Obese insulin-resistant humans | +  | ND  | ND          | +            |

TG, triglyceride; DAG, diacylglycerol; PKC, protein kinase C; ND, not determined.

Data are from the laboratories of the authors and of Turinsky, Kraegen, Caro, Biden, Boden, Shoelson, and Shulman. Many of these changes have also been shown in liver of insulin-resistant obese humans and fat-fed, fat-infused, and glucose-infused rats and fa/fa rats.
humans (26,27); however, malonyl CoA was not measured. As reviewed elsewhere (28), the insulin resistance in these situations has also been associated with inflammatory changes as evidenced by increases in cellular NF-κB activation and oxidative stress (29,30).

**Malonyl CoA as a Target for Treating Insulin Resistance**

A number of reports suggest that decreasing the concentration of malonyl CoA diminishes insulin resistance. Thus, mice in which ACC2 (the isoform thought to generate the malonyl CoA that regulates CPT1) was deleted by homologous recombination are leaner, have less hepatic and muscle lipid, and are more insulin sensitive than control mice. Likewise, the administration of a novel ACC inhibitor, CP040 186, has been shown to diminish ectopic lipid deposition and insulin resistance in fat-fed rats (mice) (31). A similar effect has been observed in fat-fed rats when malonyl CoA decarboxylase is overexpressed in liver (32).

**AMPK Regulation and Physiology**

The acute (minutes) changes in the concentration of malonyl CoA observed in skeletal muscle in response to altered glucose availability have been attributed to increases and decreases in the cytosolic concentration of citrate, an allosteric activator of ACC and a precursor for its substrate cytosolic acetyl CoA (20,21). In contrast, the acute decrease in malonyl CoA caused by exercise and by various hormones (e.g., leptin, adiponectin, glucagon) is secondary to activation of AMPK, a fuel-sensing enzyme that phosphorylates and inhibits ACC (8,33–35).

AMPK was the first kinase shown to respond to changes in cellular energy state. As recently reviewed (33,34), increases in the AMP/ATP ratio, such as occur in response to hypoxia, glucose deprivation in some tissues, and in muscle exercise, leads to conformational changes in the AMPK molecule that make it susceptible to phosphorylation and activation by an upstream kinase (AMPK). The activated AMPK in turn attempts to restore energy balance by stimulating processes that generate ATP (e.g., fatty acid oxidation and in some tissues glucose transport or glycolysis) and inhibiting others that consume ATP, but are not acutely necessary for survival (e.g., glycerolipid and protein synthesis). AMPK also seems to protect cells in these settings by increasing the abundance of peroxisome proliferator-activated receptor (PPAR)γ coactivator 1α (PGC1α), which among its other effects, enhances the expression of genes regulating mitochondrial biogenesis and oxidative phosphorylation (36–38) and increases the expression of uncoupling proteins (UCP2 and UCP3) (39) that diminish the generation of superoxide by mitochondria (40). It is highly likely that a great many additional actions of AMPK will be delineated in keeping with its apparently central role in responding to cellular stresses.

**AMPK as a Target for Treating Insulin Resistance**

In addition to exercise, AMPK is activated by the fat-cell derived hormones adiponectin and leptin and by two therapeutically used antidiabetic agents, metformin and the thiazolidinediones (TZDs) (8). All of these agents, as well as exercise, have proven useful in treating insulin resistance (Table 2) and other abnormalities associated with the metabolic syndrome.

**Table 2. Effect of factors that increase AMPK and/or decrease malonyl CoA on disorders associated with the metabolic syndrome**

| Factor          | Pancreatic β-cell dysfunction | Endothelial dysfunction | Coronary heart disease | NAFLD/NASH syndrome |
|-----------------|------------------------------|-------------------------|------------------------|---------------------|
| Exercise        | --                           | --                      | --                     | --                  |
| Calorie/weight reduction | --                           | --                      | --                     | --                  |
| Adiponectin     | --                           | --                      | --                     | --                  |
| Leptin          | --                           | --                      | ND                     | --                  |
| AICAR           | --                           | --                      | ND                     | ND                  |
| Metformin       | --                           | --                      | --                     | --                  |
| TZDs            | --                           | --                      | --                     | --                  |

AMPK, adenosine monophosphate-activated protein kinase; CoA, coenzyme A; --, decrease; NAFLD/NASH, non-alcoholic fatty liver disease/non-alcoholic steatotic hepatitis; ND, not determined; AICAR, 5 aminoimidazole 4-carboxamide riboside; TZD, thiazolidinedione. Where studied, these factors also alter ectopic lipid deposition and diminish insulin resistance. Inactivity, caloric excess (glucose), and deficiencies of leptin or adiponectin, where studied, have been shown to have opposite effects.
Abolic syndrome, including non-alcoholic fatty liver disease and endothelial cell dysfunction (Table 3). Furthermore, where these agents were studied in intact rodents (41,42) and in cultured liver cells (43,44) and endothelium (43), their action paralleled activation of AMPK. Conversely, resistin, a hormone produced by adipocytes in the mouse and macrophages in humans, causes insulin resistance and diminishes AMPK activity in liver, as evidenced by a reversal of these changes in resistin knock-out mice (45).

The most widely used AMPK activator to date has been 5-aminoimidazole-4-carboxamide riboside (AICAR), an agent that enters the cell and is phosphorylated to form AICAR-P, an AMP mimetic. AICAR administration in vivo has been shown to increase insulin sensitivity in rat muscle (46,47) and liver (46). In addition, it inhibits the activation of NF-κB and NF-κB-mediated genes in endothelial cells incubated with the fatty acid palmitate or the pro-inflammatory cytokine, tumor necrosis factor α (TNF-α) (48). Importantly, these effects were mimicked by infecting the cells with a constitutively active AMPK-adenovirus, indicating they were not a side effect of the drug. Still to be determined is whether the effects of these agents are predominantly a reflection of the decrease in malonyl CoA caused by AMPK or one or more of its other actions (see Figure 3).

### Table 3. Some studies in which AMPK activation has been linked to increases in insulin sensitivity

| AMPK activator   | Tissues (cells)                                      | Finding                                                                                   | Reference |
|------------------|------------------------------------------------------|-------------------------------------------------------------------------------------------|-----------|
| AICAR            | Muscle and liver                                     | Increases insulin sensitivity in control and fat-fed rats                                  | 46        |
| AICAR            | Cultured endothelium                                | Prevents inhibition of insulin-induced Akt activation caused by high glucose             | 67        |
| Metformin        | HepG2 hepatocytes                                   | Prevents inhibition of insulin-induced Akt activation caused by high glucose             | 44        |
| Metformin,       | Rat liver in vivo                                    | Prevents insulin resistance when plasma FFA levels are increased during a euglycemic-hyperinsulinemic clamp | 42        |
| Rosiglitazone    |                                                      |                                                                                          |           |
| Troglitazone,    | Liver, pancreatic β cells, muscle                    | Prevents development of diabetes, insulin resistance and ectopic lipid deposition in the Zucker diabetic fatty rat | 49, 68    |
| AICAR, exercise  |                                                      |                                                                                          |           |
| Exercise         | Muscle in vivo                                       | Increases insulin sensitivity for many hours in various species                           | 69        |
| Adiponectin      | Fat-fed rat (whole body)                            | Oral administration of globular subunit increases insulin sensitivity                     | 70        |
| Resistin         | Mice deficient in resistin (whole body)              | Hepatic AMPK activity and insulin sensitivity assessed by clamp are increased             | 45        |

AMPK, adenosine monophosphate-activated protein kinase; AICAR, 5-aminoimidazole-4-carboxamide riboside; FAcOA, fatty acyl coenzyme A.

As noted in the text and in Figure 3, AMPK could exert these effects by actions on multiple processes affecting the generation or use of cytosolic FAcOA.

### Depressed AMPK Activity as a Cause of Insulin Resistance

In addition to being a therapeutic target for the metabolic syndrome, a decrease in AMPK may be involved in its pathogenesis. Thus, spontaneous decreases in AMPK activity have been found in a variety of rodents with aspects of the metabolic syndrome, including obese insulin-resistant mice deficient in leptin (ob/ob) (49); rats with functionally deficient leptin receptors (fa/fa and ZDF) (49); the Dahl-salt sensitive rat, a mildly insulin-resistant rodent with hypertriglyceridemia (41); and the IL6 KO mouse (50), which, as it ages, becomes obese, glucose intolerant, and hypertriglyceridemic (51) (Table 4). The effects of AMPK activation on insulin resistance and other parameters has been studied in a number of these animal models. For instance, treatment with AICAR has been shown to prevent the development of diabetes and diminish ectopic lipid deposition in liver, mus-
cle, and pancreatic β cells of the ZDF rat (49). Treatment increases insulin sensitivity, decreases plasma triglycerides, FFAs, and blood pressure, and improves glucose tolerance in fa/fa rats (52). Likewise, treatment of the Dahl-salt sensitive rats with pioglitazone increases low hepatic AMPK activity to control values or higher and decreases elevated concentrations of malonyl CoA in liver and triglycerides in plasma (41,53). Finally, infusion of the ob/ob mouse with leptin (54) has recently been reported to activate AMPK and increase mitochondrial number in the adipocyte at the same time it diminishes adipose tissue mass.

Decreases in AMPK activity and insulin resistance have also been observed in liver and muscle of rats infused with glucose for 5 hours or more (24). As in other insulin-resistant models, increases in malonyl CoA and DAG content and ACC activity are also found. Similar changes, as well as impaired Akt activation by insulin, have been observed in incubated muscles (55,56) and cultured HepG2 hepatocytes (44) exposed to an elevated glucose concentration for 4 and 24 hours, respectively. This suggests that such changes can be attributable to glucose oversupply; however, it remains to be determined whether hormonal alterations (insulin, glucagon) play a role in vivo. Also of note, the impaired ability of insulin to activate Akt in the hepatocytes was prevented by coincubating the cells with metformin (Table 2). This effect of the metformin correlated with an increase in AMPK activity and was inhibited by infecting these cells with a dominant negative AMPK-adenovirus and mimicked by infecting them with a constitutively active AMPK-adenovirus. Thus, it seemed to be mediated by AMPK.

In liver, changes in AMPK, ACC, and malonyl CoA similar to those induced by a glucose infusion have been observed within an hour in rats fed a carbohydrate meal after a 48-hour fast. In addition, alterations in glycerol phosphate acyltransferase (GPAT), malonyl-CoA decarboxylase (MCD), fatty acid synthase and PGC1α were observed (57), raising the possibility that a decrease in AMPK mediates many of the metabolic, enzymic, and genetic changes that characterize the transition between the starved and fed states in liver.

Finally, the mechanism responsible for the decrease in AMPK activity in the glucose-infused and starved-refed rat and in the comparable in vitro models remains to be determined. In muscle incubated with a high concentration of glucose, no change in creatine phosphate or adenine nucleotides has been reported (56), and, to our knowledge, significant changes in these high-energy phosphate molecules have not been observed in either liver or muscle during the starved to fed transition in vivo. If so, novel mechanisms for regulating AMPK may be operative in these circumstances.

### Table 4. Characteristics of rodents in which decreased AMPK activity antedates or is associated with aspects of the metabolic syndrome

|                  | Obese | Insulin resistant | Ectopic lipid | Hepatic malonyl CoA | Dyslipidemia | Hyperglycemia |
|------------------|-------|------------------|---------------|--------------------|--------------|--------------|
| fa/fa rat        | ++    | +                | +             | +                  | +            | +            |
| ZDF rat          | ++    | +                | +             | +                  | +            | +            |
| ob/ob mouse      | ++    | +                | +             | +                  | +            | +            |
| Glucose-infused rat | No  | +                | +             | +                  | Check        | +            |
| Dahl-S rat       | No    | +/-              | -             | +                  | +            | +            |
| IL6-KO mouse     | 3 months | No  | ND            | ND                | ND          | No          | -            |
|                  | 9 months | +      | ND            | ND                | ND          | +            |

AMPK, adenosine monophosphate-activated protein kinase; CoA, coenzyme A; ZDF, Zucker diabetic fatty; IL6-KO, interleukin-6 knockout; ND, not determined.

Increases in malonyl CoA and ectopic lipid, where indicated, were found in liver and, where studied, in skeletal muscle. Indicated references are for decreased AMPK.

Mitochondrial Dysfunction

Studies by Kelley et al. (58) and Petersen et al. (59,60) have linked insulin resistance in human skeletal muscle to mitochondrial dysfunction. Thus, impaired mitochondrial function (or diminished mitochondrial number) together with an increase in muscle triglycerides have been found in patients with type 2 diabetes (58), elderly individuals (59), and offspring of diabetic parents (60). AMPK was not assayed in these studies; however, it is noteworthy that AMPK activation has been shown to increase mitochondrial biogenesis, at least in part, by increasing the expression of the transcriptional coactivator, PGC1α (8).
Genetically Modified Mice: A Conundrum

If a sustained decrease in AMPK activity antedates and contributes to the pathogenesis of altered cellular lipid metabolism and insulin resistance as we have proposed, one would expect to observe these abnormalities in mice made deficient in AMPK by genetic manipulations. However, in neither mice expressing a muscle-specific dominant-negative AMPK (61) nor mice globally deficient in either α1 or α2 AMPK has evidence of insulin resistance in muscle been observed, although a decrease in voluntary exercise was noted in the former group (61). Whether insulin resistance and altered cellular lipid metabolism occur at a later time in either of these mice remains to be determined. In this context, a decrease in AMPK activity (phosphorylation) in muscle and adipose tissue has been observed in lean, normoglycemic IL6 KO mice at age 3 months (50), but by 9 months of age, these mice were obese, glucose intolerant, and dyslipidemic (51). Interestingly, at age 3 months, the respiratory exchange ratio of these mice was elevated (0.9 vs. 0.8 in control mice), suggesting decreased fatty acid oxidation and that their capacity to sustain exercise was diminished. Studies of mitochondria in these mice will be of interest, because decreases in mitochondrial function and PGC1α-mediated gene expression have been reported in humans with type 2 diabetes and their offspring (36,37) as well as in individuals with impaired glucose tolerance (36,62).

Whether overexpressing a constitutively active AMPK or diminishing AMPK activity in vivo by genetic tools other than those already used (e.g., siRNA) affects insulin action, to our knowledge, has not been reported. In contrast, as already noted, overexpressing malonyl CoA decarboxylase in liver (using adenovirus) diminishes insulin resistance in the fat-fed rat (32).

Concluding Remarks and Unanswered Questions

Over the past decade, AMPK and malonyl CoA fuel-sensing mechanisms have been implicated in the regulation of insulin secretion (63), the control of food intake at the level of the hypothalamus (23,64,65), and even tumorigenesis (66). An even larger body of evidence suggests a likely relationship between the AMPK/malonyl CoA fuel-sensing network and the metabolic syndrome (4,8). Whether dys-regulation of this network plays a pathogenetic role, is a target for therapy of this syndrome, or both remains to be determined. The evidence supporting these possibilities, although compelling, has been obtained mainly from studies in rodents and cultured cells, and data from humans are limited. Also, studies in genetically modified mice have not yet yielded conclusive answers.

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