Interleukin-6 Regulation of AMP-Activated Protein Kinase

Potential Role in the Systemic Response to Exercise and Prevention of the Metabolic Syndrome

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Interleukin (IL)-6 is a pleiotropic hormone that has both proinflammatory and anti-inflammatory actions. AMP-activated protein kinase (AMPK) is a fuel-sensing enzyme that among its other actions responds to decreases in cellular energy state by enhancing processes that generate ATP and inhibiting others that consume ATP but are not acutely necessary for survival. IL-6 is synthesized and released from skeletal muscle in large amounts during exercise, and in rodents, the resultant increase in its concentration correlates temporally with increases in AMPK activity in multiple tissues. That IL-6 may be responsible in great measure for these increases in AMPK is suggested by the fact it increases AMPK activity both in muscle and adipose tissue in vivo and in incubated muscles and cultured adipocytes. In addition, we have found that AMPK activity is diminished in muscle and adipose tissue of 3-month-old IL-6 knockout (KO) mice at rest and that the absolute increases in AMPK activity in these tissues caused by exercise is diminished compared with control mice. Except for an impaired ability to exercise and to oxidize fatty acids, the IL-6 KO mouse appears normal at 3 months of age. On the other hand, by age 9 months, it manifests many of the abnormalities of the metabolic syndrome including obesity, dyslipidemia, and impaired glucose tolerance. This, plus the association of decreased AMPK activity with similar abnormalities in a number of other rodents, suggests that a decrease in AMPK activity may be a causal factor. Whether increases in IL-6, by virtue of their effects on AMPK, contribute to the reported ability of exercise to diminish the prevalence of type 2 diabetes, coronary heart disease, and other disorders associated with the metabolic syndrome remains to be determined. *Diabetes* 55 (Suppl. 2):S48–S54, 2006

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ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; CPT-1, carnitine palmitoyltransferase 1; FA-CoA, fatty acyl-CoA; IL, interleukin; MCD, malonyl-CoA decarboxylase.

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Interleukin (IL)-6 is "a pleiotropic cytokine with a wide range of biological activities including immune regulation, hematopoiesis, inflammation, and oncogenesis" (1). It was initially viewed as a proinflammatory cytokine and because of its elevated plasma concentration in people with obesity and type 2 diabetes, it is generally thought to contribute to the low-grade inflammation present in these disorders. However, IL-6 may also protect against inflammation (2–7). AMP-activated protein kinase (AMPK) was first identified as a fuel-sensing enzyme, whose principal task is to assist cells in restoring cellular ATP when they are energy depleted as a result of glucose or O_2 deprivation or other stresses (8). However, like IL-6, its actions now appear to be somewhat more complex. In this article, we will review an increasing body of evidence that suggests a link between these molecules. In particular, we will address the notion that under certain circumstances, AMPK is activated by IL-6 and may mediate or modify some of its biological actions. We will also review the evidence that decreased AMPK activity could contribute to the development of obesity, dyslipidemia, and glucose intolerance (i.e., a metabolic-like syndrome) in IL-6 knockout mice.

AMPK

AMPK is a heterotrimer composed of a catalytic α-subunit and regulatory β- and γ-subunits. In humans, each subunit is encoded by either two or three distinct genes (α1, α2, β1, β2, γ1, γ2, γ3), so that there are 12 possible α, β, and γ combinations. All three subunits are necessary for full activity (9–11). AMPK is activated by decreases in the energy state of a cell as reflected by an increase in the AMP-to-ATP ratio. According to the most widely held view, increased binding of AMP to specific domains on the γ-subunit produces a conformational change that allows upstream kinases (AMPK kinases) to phosphorylate the threonine-172 residue of the α-subunit and activate the enzyme (10,11). Two AMPK kinases have recently been identified: LKB1, a tumor suppressor that is deficient in patients with the Peutz-Jegher syndrome (12,13), and a Ca^{2+}-dependent calmodulin-dependent protein kinase ki-

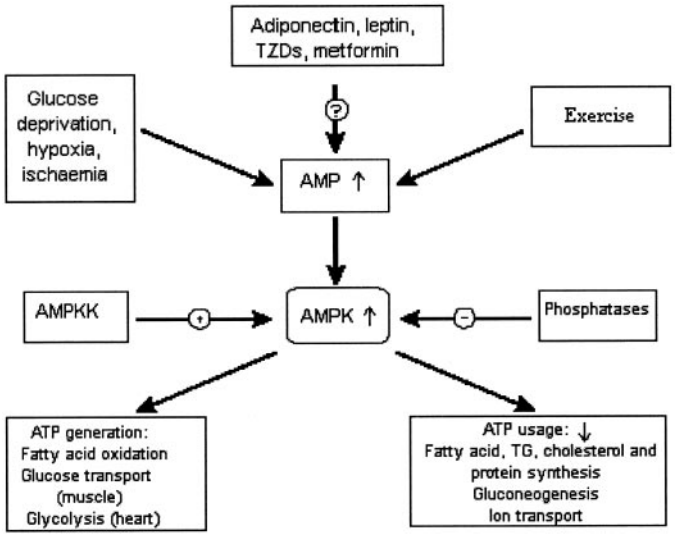

FIG. 1. AMPK activation and its effects on cellular energy state. AMPK activation concurrently leads to an increase in cellular processes that generate ATP and a decrease in processes that use ATP, but are not immediately necessary for cell survival. AMPK activation has been attributed to ATP depletion that leads to changes in the AMP-to-ATP ratio. Recent studies suggest that AMP binds to the γ-subunit of AMPK and produces a conformational change in the enzyme that makes it more susceptible to phosphorylation by an AMPK kinase (see text for details). Whether leptin and adiponectin, and pharmacological agents such as the thiazolidinediones, activate AMPK by altering the energy state of a cell, as does exercise (in skeletal muscle), is uncertain. The two AMPK kinases that have been identified to date are the tumor suppressor LKB1 and a calcium-dependent enzyme CAMKKβ. The precise role of changes in the activity of these enzymes and the role of phosphatases in this scheme are unknown. AMPKK, AMPK kinase; TZDs, thiazolidinediones. Adapted from Ruderman and Prentki (17).

nase, CAMKK (14–16). Numerous stimuli have been shown to activate AMPK (Fig. 1), including exercise, glucose deprivation, and hypoxia; various hormones including leptin, adiponectin, and catecholamines; and drugs such as metformin and thiazolidinediones (11,17). Likewise, a lack of leptin or its receptor, a surfeit of glucose, and the hormone ghrelin decrease AMPK activity in peripheral tissues (11). In many instances, it is unclear whether these hormones and drugs act by altering the energy state of the cell, by altering the functional activity of one of the AMPK kinases, or by an as yet undescribed mechanism.

Once it is activated, AMPK helps to restore the cell's energy state by acutely and chronically enhancing processes that generate ATP, such as fatty acid oxidation, and inhibiting others that consume ATP, but are not acutely necessary for survival (Fig. 1). The latter include protein, fatty acid, and triglyceride synthesis (9,10,17).

To illustrate how AMPK functions, a schema depicting some of its many effects on cellular fatty acid partitioning and metabolism is depicted in Fig. 2. AMPK increases fatty acid oxidation by diminishing the concentration of malonyl CoA, an allosteric inhibitor of carnitine palmitoyltransferase 1 (CPT-1), the enzyme that governs the transfer of long-chain fatty acyl-CoA (FA-CoA) from the cytosol into mitochondria. It accomplishes this acutely (minutes) by phosphorylating and inhibiting acetyl-CoA carboxylase (ACC), the rate-limiting enzyme for malonyl-CoA synthesis, and phosphorylating (most likely) and activating malonyl-CoA decarboxylase (MCD), which catalyzes malonyl-CoA degradation (18). In addition, AMPK subacutely (hours) represses the expression of the transcriptional

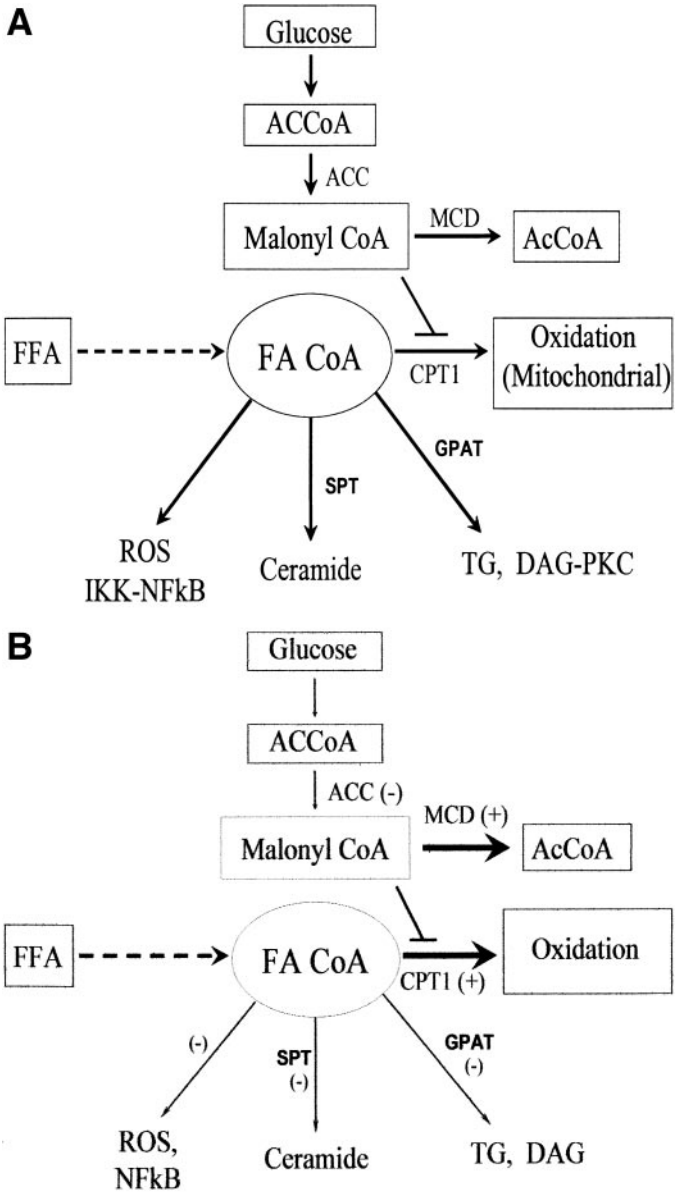

FIG. 2. Regulation of cellular fatty acid partitioning and metabolism by AMPK. *A*: AMPK neutral. By inhibiting CPT-1, malonyl-CoA, which is derived from glucose, diminishes the entrance of cytosolic FA-CoA into mitochondria where they are oxidized. This makes more cytosolic FA-CoA available for triglyceride (TG), diacylglycerol, and ceramide synthesis; lipid peroxidation; and possibly other events that lead to NKκB activation. *B*: AMPK activated: AMPK increases fatty acid oxidation acutely by phosphorylating and inhibiting ACC and activating MCD, leading to a decrease in malonyl-CoA. It also does this subacutely by effects on ACC, MCD, and CPT-1 abundance at the level of transcription. In addition, AMPK inhibits serine palmitoyltransferase, the first committed enzyme in the de novo pathway for ceramide synthesis and glycerophosphate acyltransferase, which plays a similar role in glycerolipid synthesis. The basis for the ability of AMPK to inhibit oxidant stress (ROS generation) and nuclear factor (NF)-κB activation (inflammation) is not known. Whether AMPK activation enhances or inhibits a process or an enzyme in this scheme is denoted by plus and minus signs, respectively (see full text for details). ACC, acetyl-CoA carboxylase; CPT1, carnitine palmitoyltransferase 1; DAG, diacylglycerol; FA CoA, cytosolic long-chain fatty acyl-CoA; FFA, free fatty acid; GPAT, glycerophosphate acyltransferase; MCD, malonyl-CoA decarboxylase; ROS, reactive O_2 species. Adapted from Ruderman and Prentki (17). See text for additional details.

activator SREBP1C, leading to decreases in ACC abundance, and it increases the expression of the transcriptional coactivator peroxisome proliferator–activated

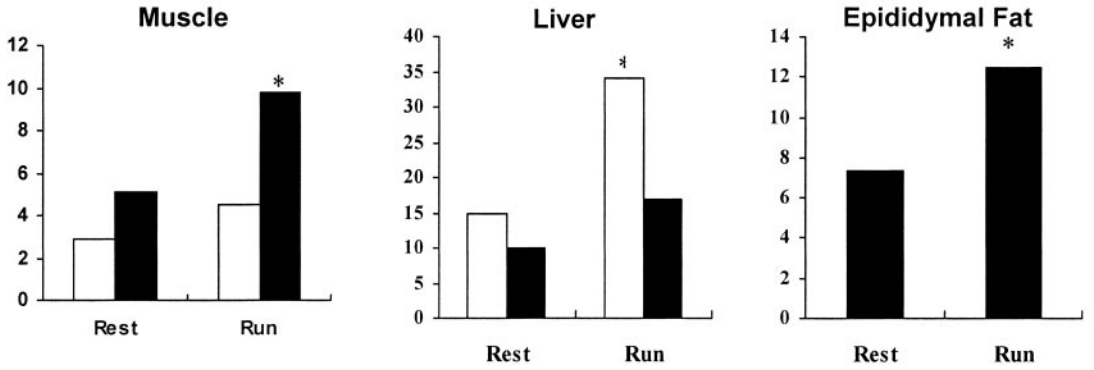

FIG. 3. Effects of 30 min of treadmill running on AMPK activity in muscle, liver, and adipose tissue of normal rats. AMPK activity is expressed in nanomoles per minute per gram protein and was determined in tissue taken from anesthetized rats ~30 min after the run and in control rats that did not run. Activity was determined after immunoprecipitation with α1 (□) and α2 (■) AMPK antibody in muscle and liver and after ammonium sulfate purification in adipose tissue. Reproduced with permission from Park et al. (34).

receptor-γ coactivator 1, PGC1α, and, secondary to this, the transcription factor peroxisome proliferator–activated receptor-α, leading to increases in the synthesis of MCD and CPT-1 itself. Thus, when it is activated, AMPK increases fatty acid oxidation by a number of mechanisms and it does so both acutely and subacutely, underscoring how closely it governs this process.

AMPK also appears to exert multiple controls over the use of cytosolic FA-CoA for other purposes. For instance, by enhancing FA-CoA transfer into mitochondria, it makes less of it available for other processes in the cytosol. In addition, AMPK specifically diminishes the use of cytosolic FA-CoA for the synthesis of diacylglycerol, triglycerides, and phospholipids by decreasing the transcription of glycerophosphate acyltransferase, the first committed enzyme in the glycerolipid synthesis pathway; it diminishes the generation of lipid peroxides and the activation of nuclear factor κB (inflammation) in cells incubated with the fatty acid palmitate (19), and it inhibits the incorporation of palmitate into ceramide, a molecule implicated in causing insulin resistance, oxidative stress, and apoptosis (11,20). Finally, AMPK increases the expression of PGC1α, which in addition to regulating MCD and CPT-1 mRNA, enhances the expression of genes regulating mitochondrial biogenesis and oxidative phosphorylation (21–23), an effect that may be impaired in people with type 2 diabetes and their overweight offspring (21,24,25). It was in large part based on these effects, and the finding that diminished AMPK activity is associated with changes in the opposite direction, that we proposed that dysregulation of AMPK and malonyl-CoA could be pathogenetic factors for the metabolic syndrome as well as targets for its therapy (rev. in 17,26–29).

AMPK ACTIVATION DURING AND AFTER EXERCISE

During exercise, AMPK is activated in contracting skeletal muscle (30,31) in response to increases in the AMP-to-ATP ratio, and it is well established that this contributes to the changes in muscle fuel metabolism that occur during and after physical activity (32,33). Surprisingly, however, in rats studied 30 min after a treadmill run, we also found increases in AMPK activity (Fig. 3) and associated changes in malonyl-CoA content and ACC, MCD, and glycerophosphate acyltransferase activity in liver and adipose tissue (34). Because the energy state of these tissues was not presumably altered, this raised the possibility that AMPK was activated by a systemic hormonal factor.

IL-6: A HORMONE RELEASED FROM SKELETAL MUSCLE DURING EXERCISE

As noted earlier, IL-6 has long been recognized for its effects on the immune system (1), and sustained but modest increases in its plasma concentration have been found in proinflammatory insulin-resistant states. The latter association has been attributed to the presence of obesity, since adipose tissue is perhaps the major contributor to plasma IL-6 in most conditions (4,5). This view of IL-6 has recently been altered by the demonstration of Pedersen and coworkers (35,36) that IL-6 is also a myokine, for which concentration in plasma can rise dramatically during and after exercise as a result of increases in its synthesis and release by contracting skeletal muscle (37). In humans, plasma concentrations of IL-6 may be increased by as much as 100-fold during exercise, to levels far in excess of those observed in obese and diabetic humans (Fig. 4). In general, such increases in IL-6 have paralleled the intensity and duration of exercise, and they were greatest when muscle glycogen levels were low (38). It has been suggested that this increase in IL-6 plays a role in stimulating adipose tissue lipolysis and possibly hepatic glucose production during sustained exercise to provide for the fuel needs of muscle as its endogenous fuel stores (e.g., glycogen) are depleted (35,36). In keeping with this

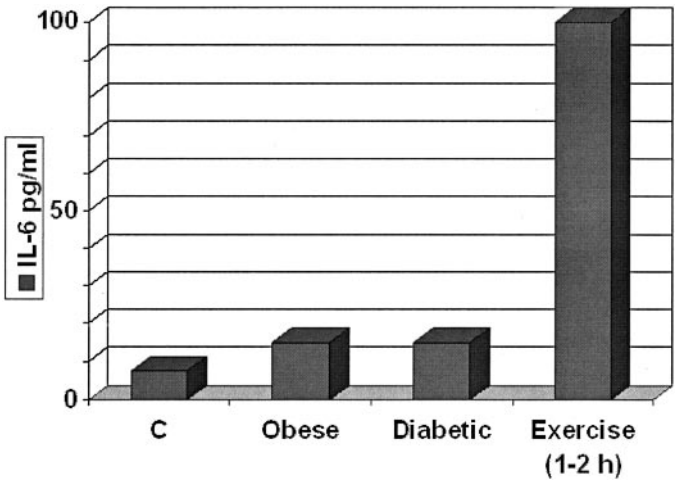

FIG. 4. Plasma levels of IL-6 in obese, type 2 diabetic, and normal control humans and control humans after 1–2 h of moderately intense exercise. Based on data in literature (5).

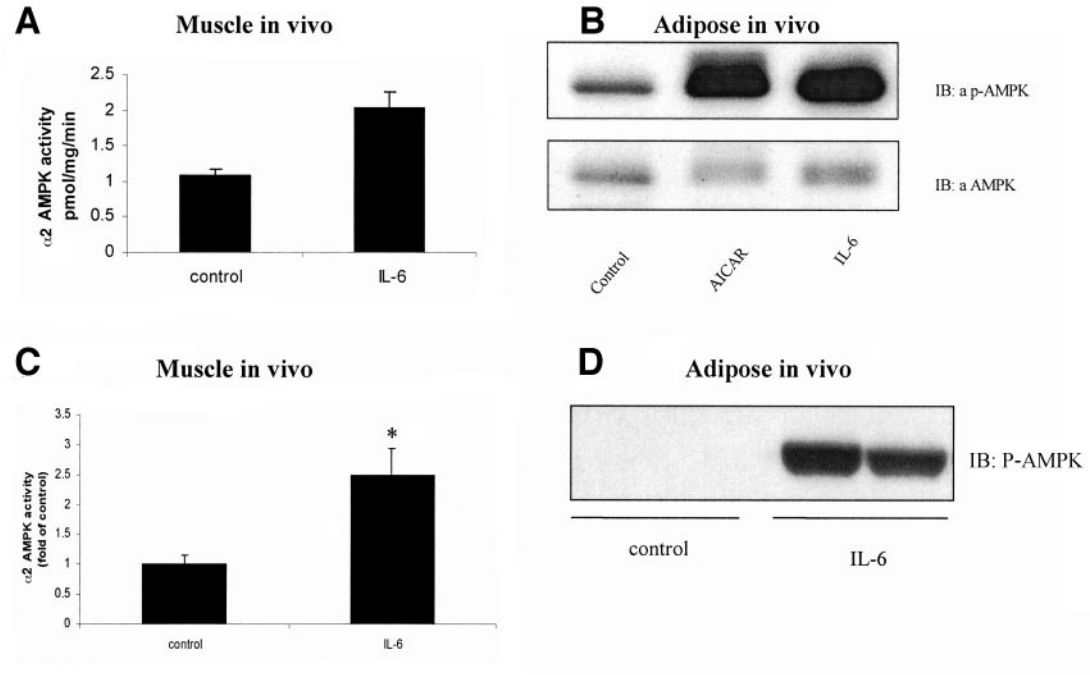

FIG. 5. AMPK activation by IL-6 in (*A*) incubated rat extensor digitorum longus muscle, (*B*) cultured 442A adipocytes, (*C*) rat gastrocnemius muscle, and (*D*) rat epididymal adipose tissue in vivo 1 h after an intraperitoneal injection of IL-6 (2.5 μg). Results are means ± SE (*A*, *C*, and *D*) of four measurements. Blots in *B* are representative of three studies. Adapted from Kelly et al. (41).

notion, IL-6 increases lipolysis upon infusion into humans at rest (39), and it further increases exercise-induced endogenous glucose production (40).

IL-6 ACTIVATES AMPK IN MUSCLE AND ADIPOSE TISSUE

The temporal correlation between changes in plasma IL-6 and tissue AMPK during exercise led us to examine whether the two events are related. As a first test of this notion, we assessed the effect of IL-6 on AMPK activity in rat muscle and F442A adipocytes in vitro. IL-6 markedly increased AMPK in both tissues, with the increase in phospho-AMPK (P-AMPK) abundance (an index of activity) in the fat cells comparable to that produced by the classic AMPK activator 5-aminoimidazole-4-carboxamide riboside (AICAR) (Fig. 5). We have found similar IL-6–induced increases in AMPK in incubated pancreatic islets (A.-M.R., M.K., unpublished data) and in rat muscle and adipose tissue after an intraperitoneal injection of IL-6 (Fig. 5). Also of note, the increase in AMPK activity in incubated muscle was transient, and values below those of control muscles were observed by 60 min (41). Whether this secondary decrease in AMPK activity persists during longer periods of incubation with IL-6 and whether a similar pattern occurs in vivo and in other tissues remains to be determined.

STUDIES IN THE IL-6 KO MOUSE

To assess more directly whether IL-6 causes the increase in tissue AMPK during and after exercise, we compared AMPK activity in muscle, adipose tissue, and liver of control mice and IL-6 knockout (IL-6 KO) mice after 1 h of swimming (41). In keeping with previous studies in the rat (34), exercise increased the abundance of both P-AMPK and phospho-ACC (P-ACC) in muscle, liver, and adipose tissue of the control mice. No obvious difference in the ability of the control and IL-6 KO mice to perform exercise was observed. Despite this, in the IL-6 KO mice at rest, the abundance of P-AMPK and P-ACC was diminished by 75–90% in muscle (Fig. 6) and to a somewhat lesser extent in adipose tissue. After exercise, similar percentage increases in P-AMPK and P-ACC abundance were observed in muscle and adipose tissue of the control and IL-6 KO mice; however, because of their lower initial values, the absolute levels of P-AMPK and P-ACC achieved were lower in the IL-6 KO mice. For reasons unknown, P-AMPK and P-ACC were only minimally diminished in liver of the mice lacking IL-6, and the increments in their abundance produced by exercise were indistinguishable from those of control mice. Collectively, these data strongly suggest that IL-6 is a significant regulator of the basal activity of AMPK in muscle and adipose tissue. They also suggest that it is a key factor regulating the increase in tissue P-AMPK and P-ACC caused by exercise, but not the only factor. As discussed elsewhere (41), another likely regulator of the effects of exercise on AMPK is the sympathetic nervous system, which itself may be activated in part by a central action of IL-6 (42). Perhaps relevant to this discussion, it has recently been observed that transgenic mice overexpressing IL-6 do not develop obesity and insulin resistance as do control mice when fed a high-fat diet (M.F. White, personal communication).

IL-6 KO MOUSE AS A MODEL OF THE METABOLIC SYNDROME

At 3 months of age, the IL-6 KO mouse is not obese, whereas by 9 months of age, it is obese, hypertriglyceridemic, and glucose intolerant (42), which by definition gives it a diagnosis of the metabolic syndrome (43). Decreases in AMPK activity have been observed in a number of rodents that either have or subsequently develop manifestations of the metabolic syndrome. As listed

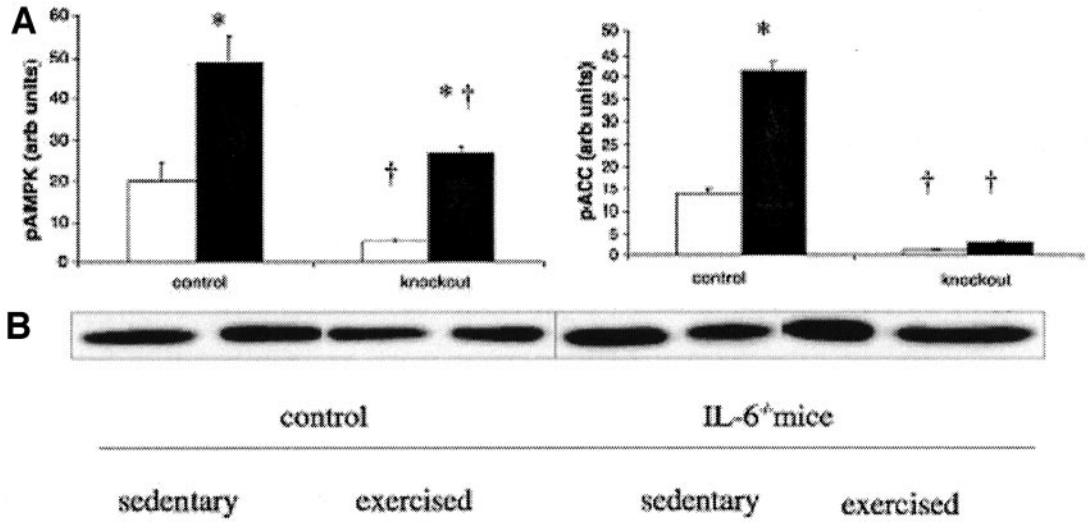

FIG. 6. AMPK and ACC phosphorylation and AMPK abundance in gastrocnemius muscle of control and IL-6 KO mice and the effect of exercise (1 h of swimming). *A*: P-AMPK and P-ACC abundance in sedentary (□) and exercised (■) control and IL-6 KO ($n = 4$) mice. Results are means ± SE. *$P < 0.05$ vs. resting value; †$P < 0.01$ vs. control value. *B*: Abundance of total AMPK (α1- and α2-subunit) protein in control and IL-6 KO mice at rest and after exercise. Results are representative of four blots in each condition. Similar findings after exercise were observed in adipose tissue but not in liver. Adapted from Kelly et al. (41).

in Table 1, they include rodents that are obese and not obese, diabetic and nondiabetic, and hypertriglyceridemic and normoglyceridemic, with insulin resistance a common denominator. In one of these rodents, the glucose-infused rat, insulin resistance, ectopic lipid accumulation, and decreased AMPK activity in liver all first appeared between 3 and 5 h, suggesting they are early events (44). AMPK activation has been demonstrated to diminish insulin resistance and other abnormalities in a number of these animals, suggesting it plays a causal role. For instance, treatment of the ZDF rat with AICAR (45,46) or regular exercise (46) has been shown to prevent the development of diabetes, as has treatment with thiazolidinedione therapy and calorie restriction (47), both of which have also been reported to activate AMPK in rats (48,49). Similar benefits of exercise (50) and AICAR (51) have been reported in rats made insulin resistant by fat feeding.

Several characteristics of the IL-6 KO mouse suggest that the early decrease in AMPK activity in muscle and adipose tissue of these mice could contribute to the metabolic syndrome phenotype observed at 9 months. Thus, Faldt et al. (52), based on measurements of respiratory exchange ratio at 3 months of age (respiratory exchange ratio 0.92 vs. 0.82 in control mice), concluded that the ability of the IL-6 mouse to oxidize fatty acids is impaired. Interestingly, they also noted that by 9 months of age, the respiratory exchange ratio of these mice had decreased to 0.82 (same as control mice). Whether this reflected an increased availability of fatty acids as a result of obesity and/or a restoration of AMPK activity remains to be determined. Also of note, Faldt et al. (52) found that the ability of both 3- and 9-month-old IL-6 KO mice to sustain exercise was diminished, suggesting impaired cardiovascular, pulmonary, or muscle function. In keeping with the latter possibility in a very preliminary study (in 3-month-old IL-6 KO mice), we found decreased levels of cardiolipin, a mitochondrial lipid whose concentration diminishes when mitochondria are damaged (e.g., as seen when apoptosis and impaired mitochondrial function are caused by incubation of cells with the fatty acid palmitate) (53) (Table 2). In addition, we found a reduced (50%) abundance in muscle of the mRNA of mitochondrial uncoupling protein (UCP)-3. Diminished size and efficiency of mitochondria have been observed in muscle of patients with type 2 diabetes (54). Whether decreased AMPK activity, by virtue of its effects on cellular lipid metabolism, mitochondrial genes, or other factors, causes these abnormalities to our knowledge has not been studied. On the other hand, it has been demonstrated that AMPK activation prevents both the apoptosis and mitochondrial dysfunction observed in cultured human umbilical vein endothelial cells incubated in a high-glucose medium (55) and the apoptosis, inflam-

TABLE 1
Characteristics of rodents in which decreased AMPK activity precedes or is associated with aspects of the metabolic syndrome (see text for details)

	Obese	Insulin resistant	Ectopic lipid	Dyslipidemia	Hyperglycemia
fa/fa rat	++	+	+	+	−
ZDF rat	++	+	+	+	++
Fat-fed rat	++	+	+	+	+
Glucose-infused rat	−	+	+	ND	+
Dahl-S rat	−	+/−	−	+	−
IL-6 KO mouse					
3 months	−	ND	ND	−	−
9 months	+	ND	ND	+	+

ND, no data.

TABLE 2
Decreased cardiolipin and UCP3 mRNA in white, but not red, gastrocnemius muscle of 3-month-old IL-6 KO mice

	Cardiolipin (μg/mU creatine kinase)		NADH oxidase (units/mU creatine kinase)		UCP3 content (arbitrary units)
	White	Red	White	Red	White
Control	114	134	0.75	1.24	0.72 ± 0.05
	118	130	0.68	1.41	
IL-6 KO	67	156	0.63	1.48	0.373 ± 0.05*
	77	180	0.78	1.55	

Similar changes were not observed for the NADH oxidase. In keeping with previous reports, NADH oxidase activity was higher in red muscle, which is richer in mitochondria. Cardiolipin and NADH oxidase results are for muscles from two mice. UCP3 data are means ± SE (n = 7–10). *$P < 0.005$. UCP3, uncoupling protein 3. See text for additional details.

mation, and oxidative stress observed when human umbilical vein endothelial cells are incubated with a modestly elevated concentration of palmitate (19).

IL-6 AND THE METABOLIC SYNDROME: A CONUNDRUM

In addition to being predisposed to obesity, dyslipidemia, and glucose intolerance, IL-6 KO mice demonstrate more advanced atherosclerosis when bred on an Apo $E^{-/-}$ background than do Apo $E^{-/-}$ mice in which IL-6 is not lacking (56,57). On the other hand, humans with modest elevations of IL-6, attributable to obesity (5), are also predisposed to diabetes and atherosclerotic vascular disease (58). It is tempting to speculate that differences in AMPK activity in response to IL-6 in various tissues could contribute to this paradox; however, there is as yet no evidence for or against this notion. In this context, studies of both the mechanism by which AMPK is activated by IL-6 and the effects of AMPK activation on different components of the IL-6 signaling pathway could prove interesting (Fig. 7).

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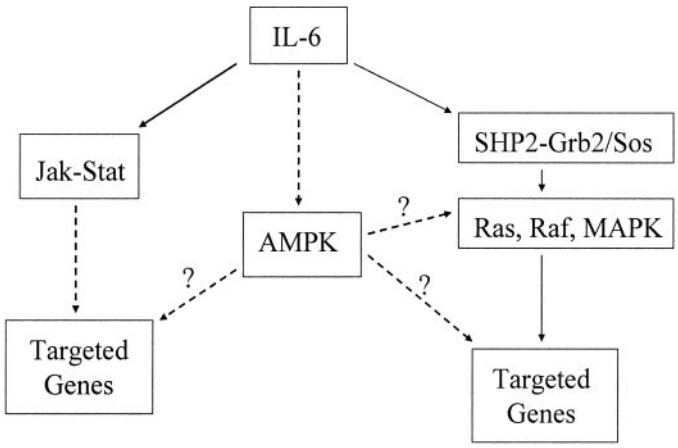

FIG. 7. Hypothetical scheme to explain how AMPK activation by IL-6 could influence its actions on the cell. AMPK is known to inhibit NFκB activation and JNK, mTOR, and other cellular enzymes by multiple means (see Fig. 2 and accompanying text). Whether it selectively exerts an effect on some of the signals or genes altered by IL-6 is presently under study.

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