

AMP-activated protein kinase, a metabolic master switch: possible roles in Type 2 diabetes

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Winder, W. W., and D. G. Hardie. AMP-activated protein kinase, a metabolic master switch: possible roles in Type 2 diabetes. *Am. J. Physiol.* 277 (Endocrinol. Metab. 40): E1–E10, 1999.—Adenosine 5'-monophosphate-activated protein kinase (AMPK) now appears to be a metabolic master switch, phosphorylating key target proteins that control flux through metabolic pathways of hepatic ketogenesis, cholesterol synthesis, lipogenesis, and triglyceride synthesis, adipocyte lipolysis, and skeletal muscle fatty acid oxidation. Recent evidence also implicates AMPK as being responsible for mediating the stimulation of glucose uptake induced by muscle contraction. In addition, the secretion of insulin by insulin secreting (INS-1) cells in culture is modulated by AMPK activation. The net effect of AMPK activation is stimulation of hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis, stimulation of skeletal muscle fatty acid oxidation and muscle glucose uptake, and modulation of insulin secretion by pancreatic β -cells. In skeletal muscle, AMPK is activated by contraction. Type 2 diabetes mellitus is likely to be a disease of numerous etiologies. However, defects or disuse (due to a sedentary lifestyle) of the AMPK signaling system would be predicted to result in many of the metabolic perturbations observed in Type 2 diabetes mellitus. Increased recruitment of the AMPK signaling system, either by exercise or pharmaceutical activators, may be effective in correcting insulin resistance in patients with forms of impaired glucose tolerance and Type 2 diabetes resulting from defects in the insulin signaling cascade.

adenosine 5'-monophosphate-activated protein kinase; fatty acid oxidation; glucose uptake; GLUT-4; insulin insensitivity; malonyl-coenzyme A

THE BASIC DEFECTS IN TYPE 2 DIABETES are insulin resistance of skeletal muscle, hyperinsulinemia in the early stages of the disease followed by insulin deficiency in the later stages, elevated hepatic glucose production, hyperglycemia, and dyslipidemia (4, 25, 27). The sequence of development of these conditions has recently been debated (25, 27). These abnormalities in glucose and fat metabolism are often associated with obesity. Individuals with Type 2 diabetes are typically ketosis resistant, as opposed to the propensity for ketoacidosis in patients with Type 1 diabetes. Despite a considerable amount of effort and resources allocated to determining etiology, the basic cellular defect for the

vast majority of patients with this disease remains undefined. Recent data collected in several laboratories indicate that AMP-activated protein kinase (AMPK) plays a key role in regulation of carbohydrate and fat metabolism (30, 31, 52, 53, 84), serving as a metabolic master switch in response to alterations in cellular energy charge. In this review, we suggest the hypothesis that defects in this signaling pathway could give rise to many of the metabolic dysfunctions of Type 2 diabetes. The review will focus on the known metabolic regulatory effects of AMPK on fatty acid and carbohydrate metabolism in liver, adipose tissue, pancreatic β -cells, and skeletal muscle and on how inadequate functioning of the AMPK signaling system could possibly give rise to several of the multiple metabolic defects of Type 2 diabetes. The activation of this kinase as a means of prevention and treatment of patients with Type 2 diabetes and with intact AMPK signaling systems will also be considered.

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STRUCTURE AND TISSUE DISTRIBUTION OF AMPK

AMPK phosphorylates numerous target proteins at serine residues in the context of a characteristic sequence recognition motif (11, 15). Phosphorylation may result in increases or decreases in the rate of the metabolic pathway in which the protein target plays a regulatory role (30, 31). One form of AMPK is expressed in the cell nucleus (67), and recent evidence suggests that AMPK can also influence metabolism by regulating gene expression (26, 45). AMPK is a heterotrimer of three subunits, i.e., α , β , and γ (31). The 63-kDa α -subunit contains the kinase domain and also contributes to the AMP-binding site (9). Two isoforms of both the α - and β -subunits have been described and are designated α_1 , α_2 , β_1 , and β_2 (31, 71, 72, 88). Searching of the human expressed sequence tag databases suggests that there are also multiple forms of the γ -subunit (72). Coexpression of all three subunits is required for kinase activity (20, 88). Heterotrimers containing the α_2 -subunit are more AMP dependent, being stimulated five- to sixfold by AMP as opposed to twofold for complexes containing the α_1 -subunit (68). The liver expresses predominantly the β_1 -subunit and an approximately equal mix of α_1 and α_2 , whereas skeletal muscle expresses predominantly the α_2 - and β_2 -isoforms (71, 74, 88). Human chromosomal locations of the genes encoding α_1 , α_2 , β_1 , and γ_1 are 5p11-p14, 1p31, 12q24.1-24.3, and 12q12-q14, respectively (7, 72).

REGULATION OF AMPK ACTIVITY

The AMPK system is activated by 5'-AMP not only via an allosteric mechanism (13) but also via phosphorylation of a key threonine residue on the catalytic subunit catalyzed by a distinct upstream kinase [AMPK kinase (AMPKK)] (32). Because the latter effect involves a covalent change in the kinase, by use of appropriate conditions it can be preserved during preparation of cell-free extracts, unlike the allosteric effect. Activation is achieved by no less than four mechanisms, i.e., 1) allosteric activation of AMPK by AMP (68); 2) binding of AMP to AMPK, making it a better substrate for AMPKK (33); 3) binding of AMP to AMPK, making it a worse substrate for protein phosphatases, especially protein phosphatase-2C (17); and 4) allosteric activation of the upstream kinase, AMPKK, by AMP (33). The combined effect of these mechanisms allows >200-fold activation and results in the system being exquisitely sensitive to small changes in AMP concentration in the cell. All of the activating effects of AMP are antagonized by high concentrations of ATP (14, 17, 32) so that the system responds to the AMP-to-ATP ratio (AMP/ATP) rather than just to the level of AMP. AMP and ATP vary reciprocally in cells because of the action of adenylate kinase (myokinase), and this may be another device to increase the sensitivity of the system (30, 31). Recently, it has also been found that physiological concentrations of phosphocreatine allosterically inhibit AMPK (56). Because it decreases during muscle contraction, phosphocreatine rather than AMP may be the key regulator of the AMPK system during

short-term exercise. Unlike AMP, phosphocreatine does not appear to regulate phosphorylation by the upstream kinase (S. Hawley and D. G. Hardie, unpublished observations). Because the effects of phosphocreatine on AMPK are entirely allosteric, they would therefore be expected to be lost on preparation of cell-free extracts.

AMPK REGULATION OF LIVER METABOLISM

Numerous studies have characterized the effects of AMPK activation on liver metabolism (30, 31). Two of the classical targets for the system are acetyl-CoA carboxylase (ACC) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), catalyzing the key regulatory steps in fatty acid and sterol synthesis, respectively. Activation of AMPK in isolated hepatocytes by cellular stresses that deplete ATP (e.g., heat shock, arsenite) leads to phosphorylation of HMGR and inhibition of fatty acid and sterol synthesis (13). These lipid biosynthetic pathways, as well as triglyceride synthesis (54), are also inhibited if AMPK is activated by incubating hepatocytes with 5-aminoimidazole-4-carboxamide (AICA)-riboside, a nucleoside that is taken up into cells, probably by the adenosine transport system, and then converted by adenosine kinase to the monophosphorylated form ZMP (14, 36, 67). ZMP is a normal intermediate in the pathway of purine nucleotide synthesis; however, at least in short-term incubations (<1 h) with isolated hepatocytes, it accumulates to millimolar concentrations without any change in the content of AMP, ADP, or ATP (14, 36). ZMP is an AMP analog, and it mimics the effects of AMP on the phosphorylation of AMPK by AMPKK (14), as well as on the allosteric activation of AMPK (14, 37). ZMP is not a completely specific activator of AMPK in that it also mimics effects of AMP on other AMP-regulated enzymes, such as glycogen phosphorylase (90) and fructose-1,6-bisphosphatase (80). Long-term incubation with AICA-riboside can also lead to the appearance of the triphosphorylated form ZTP, possibly by reversal of the 5-phosphoribosyl-1-pyrophosphate synthetase reaction (67). The effects of this compound are uncertain, but if incubation times are kept at <1 h, its formation is negligible. There are no current indications that AICA-riboside treatment causes activation of protein kinases other than the AMPK cascade, and it is certainly a more specific method of activating the latter than stress treatments such as arsenite, which can stimulate other stress-activated protein kinases. Although there is a need to develop more specific activators (and inhibitors) of the AMPK system, effects of AICA-riboside remain a very useful initial indication for an involvement of the AMPK system. Intriguingly, AICA-riboside treatment of cultured rat hepatocytes inhibits expression of the L-type pyruvate kinase and fatty acid synthase genes (26, 45), suggesting that AMPK inhibits lipogenesis by effects on gene expression as well as by direct effects on metabolism.

Inactivation of ACC in the liver cell also leads to decreases in the concentration of the product of ACC, i.e., malonyl-CoA, which has marked effects on fatty

acid oxidation. Malonyl-CoA is a potent inhibitor of carnitine palmitoyltransferase-1 (CPT-1) (50, 51), the "gatekeeper" for entry of fatty acids into the mitochondria. Consistent with this, incubation of hepatocytes with AICA-riboside leads to increases in fatty acid oxidation and CPT-1 activity, and the change in CPT-1 can largely, although not entirely, be ascribed to decreases in malonyl-CoA content (79). In the liver, fatty acid oxidation can be considered to be an essential component of the pathway for synthesis of ketone bodies: increases in fatty acid oxidation lead to increased hepatic ketogenesis.

The overall effects of AMPK activation in the liver would therefore be decreases in fatty acid, triglyceride, and sterol synthesis and increases in fatty acid oxidation and ketogenesis. The same effects are produced by a decrease in the insulin-to-glucagon ratio, although the molecular mechanisms for AMPK involvement in these hormonal effects are not well understood. In rat liver, both fatty acid and cholesterol synthesis exhibit a diurnal rhythm, being high in the dark period when rats are eating. This can be explained by changes in the phosphorylation states of ACC (16) and HMGCR (22), which exhibit the inverse rhythm. At least in the case of HMGCR (21), the phosphorylation state appears to be largely under the control of insulin, with glucagon having a smaller, secondary effect. Although all available evidence points to AMPK as the protein kinase responsible for phosphorylation of ACC and HMGCR, the activity of AMPK does not appear to vary during the normal diurnal cycle in rat liver (16). The effect could be due perhaps to regulation of protein phosphatases or to modulation of ACC and HMGCR through unidentified mechanism(s) that alter their ability to act as substrates for AMPK. It should be pointed out that malonyl-CoA levels may also be regulated independently of AMPK, for example via changes in the cellular content of the allosteric effectors of ACC, citrate, and long-chain acyl-CoA (LCA-CoA).

In rats, liver AMPK can be activated in response to high-intensity treadmill exercise (10). This activation of AMPK is accompanied by an increase in the plasma glucagon concentration. The constant for citrate activation (K_a) of liver ACC is increased, and the maximum velocity (V_{max}) is decreased in response to exercise. No detectable activation of liver AMPK occurs during prolonged low-intensity running. The mechanism of activation of AMPK during the higher-intensity exercise is not known.

AMPK REGULATION OF FAT CELL METABOLISM

Hormone-sensitive lipase (HSL) is a target for AMPK in adipose tissue (30, 31). Studies on isolated rat adipocytes have demonstrated that activation of AMPK with AICA-riboside inhibits lipogenesis by phosphorylation of ACC (73) and also inhibits isoprenaline-stimulated lipolysis (14, 73). This effect on HSL has been postulated to be important in minimizing recycling of fatty acids into triacylglycerols during catecholamine-stimulated lipolysis, thus conserving ATP (30).

AMPK REGULATION OF INSULIN SECRETION

Previous studies have demonstrated that incubation of insulin-secreting cells (INS) in medium containing high glucose concentrations results in an increase in ACC activity and an increase in malonyl-CoA (12, 46–48). INS-1 cells (derived from pancreatic β -cells) with reduced levels of ACC (expressing ACC-specific antisense mRNA) exhibited a reduced insulin secretory response to glucose (91), implying that malonyl-CoA, the product of the ACC reaction, is involved in stimulation of insulin secretion in response to glucose. This was previously postulated to be a result of malonyl-CoA inhibition of fatty acid oxidation, with secondary accumulation of cytosolic LCA-CoA. The LCA-CoA was in turn proposed to be the mediator of glucose stimulation of insulin release (57). Supporting this hypothesis are data indicating that artificial suppression of circulating free fatty acids in 24- and 48-h-fasted human subjects decreased the insulin response to intravenous glucose (19). This hypothesis has recently been questioned as a result of the observation that INS-1 cells transfected with the malonyl-CoA decarboxylase gene (which results in a decrease in malonyl-CoA response to glucose) respond to increasing glucose concentrations with normal increases in insulin release (5). In addition, treatment of INS-1 cells and pancreatic islets with triacsin C to inhibit LCA-CoA synthesis had no effect on glucose-stimulated insulin secretion (5).

Recent studies by Salt et al. (69) on INS-1 cells demonstrated that AMPK may play an important role in regulation of insulin secretion. Incubation of INS-1 cells in medium containing low glucose concentration results in an increase in the AMPK activity and a decrease in ACC activity. Artificial activation of AMPK by incubation of the INS-1 cells with AICA-riboside inhibited insulin secretion. In isolated rat islets incubated in medium with glucose concentrations of 3.3 and 5.5 mM, insulin secretion was stimulated by incubation with AICA-riboside (69). At a glucose concentration of 16.7 mM, insulin secretion was inhibited. Akkan and Malaisse (1) and Malaisse et al. (48) previously reported AICA-riboside stimulation of insulin release in incubated islets and in the perfused pancreas. Insulin secretion by isolated islets was increased by AICA-riboside at glucose concentrations ranging from 5.6 to 20 mM (1). These authors attributed the increase in insulin release to nonspecific effects of the drug, citing increases in ZTP as one possibility (48). The results in isolated islets may not present a true picture of the action of AMPK on the cells, because paracrine control mechanisms (e.g., increased glucagon release by α -cells with consequent stimulation of insulin release) may mask the direct action of AMPK activation. The only physiological stimulus identified to date for AMPK activation in INS cells is low glucose. It is likely, therefore, that the most important physiological effect of AMPK activation is inhibition of insulin release at low glucose concentrations. ACC in the pancreatic β -cell is likely to be one target for phosphorylation by AMPK, but the possibility of other unidentified protein

targets that are also important in modulating insulin release cannot be ruled out. Malonyl-CoA- and LCA-CoA-independent mechanisms are certainly possible.

CONTRACTION AND HYPOXIA PRODUCE INSULIN-LIKE EFFECTS ON GLUCOSE UPTAKE IN MUSCLE

A very large number of studies demonstrate that contraction and hypoxia both produce an insulin-like effect on skeletal muscle (8, 28, 35, 38). Studies in isolated muscle incubations (i.e., epitrochlearis, soleus) clearly show an enhancement of uptake of nonmetabolizable glucose analogs in response to contraction induced by electrical stimulation (8, 28, 35, 38). Studies using rat hindlimb perfusions also demonstrate an increase in glucose uptake or uptake of glucose analogs in response to contraction (8, 28, 35, 38). Numerous studies have demonstrated that the contraction-stimulated glucose uptake mechanism is distinctly different from the insulin stimulation of glucose uptake in skeletal muscle (28, 35, 38). Effects of maximal insulin stimulation and of contraction have for the most part been reported to be additive. The effect of insulin is blocked by wortmannin, an inhibitor of the phosphatidylinositol 3-kinase signaling pathway, but the contraction-induced increase in glucose uptake is not (89). As with insulin stimulation of glucose uptake, the effect of contraction is mediated by triggering of translocation of GLUT-4 from vesicles in the sarcoplasm to the sarcolemma of the muscle fibers. Chronic daily bouts of exercise result in an increase in the total amount of GLUT-4 in the muscle (28, 38). This effect may be seen within 16 h after a single prolonged bout of exercise and is accompanied by an increase in mRNA for GLUT-4 (38, 61).

AMPK REGULATION OF MUSCLE METABOLISM AND GLUCOSE UPTAKE

The study of AMPK activation in skeletal muscle was prompted by the observation that malonyl-CoA decreases rapidly in hindlimb muscles of rats running on the treadmill (85). It was postulated at that time that this decrease in malonyl-CoA (also an inhibitor of the skeletal muscle isoform of CPT-1) served as the signal for increasing the rate of fatty acid oxidation as exercise continued. A negative correlation between fatty acid oxidation and malonyl-CoA concentration had previously been reported for rat heart muscle (44). The muscle isoform of ACC was subsequently isolated and characterized (76). The purified muscle isoform was found to be phosphorylated *in vitro* by AMPK with a consequent increase in K_a for citrate activation and an increase in the Michaelis constant for the substrates ATP, acetyl-CoA, and bicarbonate (86, 87). The net effect of phosphorylation was a marked decrease in rate of malonyl-CoA synthesis at physiological concentrations of citrate. Subsequent studies on rats running on the treadmill demonstrated that AMPK activity, measured in ammonium sulfate precipitates of muscle homogenates, was markedly increased after short bouts of submaximal exercise (83, 86). This observation pro-

vides evidence for AMPK phosphorylation by AMPKK, because the allosteric effect of AMP would have been lost during ammonium sulfate precipitation. There was also a concurrent increase in K_a and decrease in V_{max} for muscle ACC, kinetic changes similar to those seen in the *in vitro* phosphorylation studies (86, 87). Additional evidence for phosphorylation was obtained by Vavvas et al. (78), who demonstrated that the effect of contraction on muscle ACC could be reversed by treatment with phosphatases. ACC isolated from previously contracting muscle also exhibited an electrophoretic mobility shift compared with that isolated from resting muscle (78). Recent studies have also demonstrated that AMPK activity is increased and ACC activity and malonyl-CoA content decreased in skeletal muscle in response to electrical stimulation (42, 78). An increase in estimated free AMP concentration in the muscle was also observed in the electrically stimulated muscle (42). Vavvas et al. also demonstrated that only the α_2 -isoform of AMPK showed an increase in activity that survived immunoprecipitation, although allosteric activation of the α_1 -isoform could not be ruled out. However, it might be expected that the effect on α_1 would be smaller than that on α_2 , because α_2 -complexes purified from rat liver are more AMP dependent than α_1 complexes (6-fold stimulation vs. 2.5-fold), both for phosphorylation by AMPKK and allosteric activation (68). In fact, when muscles were stimulated at a lower frequency (1/s) for longer periods (30 min), activation of the α_1 -isoform was also observed (C. Thornton, A. Hutber, W. W. Winder, and D. Carling, unpublished data). In the study by Hutber et al. (42), the decrease in ACC activity appeared, if anything, to precede the increases in AMPK activity and the AMP/ATP. A potential explanation for this might be that the initial rapid effect was due to the relief of inhibition of AMPK by phosphocreatine, the latter being an allosteric effect that would not persist into the AMPK assays (56).

A follow-up study was undertaken to determine whether a decrease in muscle malonyl-CoA would be accompanied by an increase in fatty acid oxidation. Perfused rat hindlimb muscles were exposed to AICA-riboside for the purpose of activating AMPK. In this respect, treatment of muscle with AICA-riboside mimics the effect of contraction on this control system. In these rat hindlimb perfusion studies, it was demonstrated that inclusion of AICA-riboside in the perfusion medium at a concentration of 2 mM would increase ZMP in the muscle and activate AMPK (52, 53). The activation of AMPK was accompanied by kinetic changes in muscle ACC similar to those seen after exercise or after phosphorylation of purified ACC *in vitro*, that is, a marked increase in K_a for citrate activation and decrease in V_{max} . Malonyl-CoA was markedly depressed in the AICA-riboside-infused muscles. Oxidation of [14 C]palmitate to $^{14}\text{CO}_2$ was increased 3- to 10-fold (depending on the palmitate concentration) by the inclusion of AICA-riboside in the perfusion medium (52, 53). This provided the first direct evidence that a decrease in malonyl-CoA would result in an increase in fatty acid oxidation in skeletal muscle. The stimulating

effects of AICA-riboside on fatty acid oxidation (consequence of a decrease in malonyl-CoA) have been recently confirmed in incubated soleus muscles by Alam and Saggerson (2) in isolated mouse soleus and in C₂C₁₂ myoblasts or differentiated C₂C₁₂ myotubes by Muoio et al. (54).

A surprising finding in the first AICA-riboside study on perfused skeletal muscle (52) was the observation that glucose uptake across the hindlimb was increased approximately twofold in response to AICA-riboside. It was anticipated on the basis of previous reports on the fatty acid cycle (cf. Ref. 39) that the increased rates of fatty acid oxidation would result in decreased rates of glucose uptake. Our unexpected observation led to the hypothesis (52, 53, 82) that a contraction-induced increase in AMPK in muscle has the dual effect of increasing intramitochondrial availability of fatty acids and of increasing glucose uptake to meet the increasing energy demands accompanying contraction (Fig. 1). The effect of contraction on glucose uptake in muscle has been well documented (28, 35, 38), but the mechanism of this insulin-like effect of contraction had not been elucidated. Ongoing studies (34) have demonstrated that AICA-riboside stimulates uptake of 3-*O*-methylglucose into isolated epitrochlearis muscle in the absence of insulin and that this activation is similar in nature to that induced by contraction. The effect is additive with the effect of maximal insulin stimulation. Insulin stimulation of glucose uptake into muscle is mediated by activation of phosphatidylinositol 3-kinase (PI 3-kinase), evidenced by the complete inhibition of the effect by the PI 3-kinase inhibitor wortmannin (34, 89). In contrast, the effect of contraction and the effect of artificial activation of AMPK by AICA-riboside are not influenced by wortmannin (34). AMPK activity is stimulated by both contraction and AICA-riboside treatment. The effects of AICA-riboside and contraction on 3-*O*-methylglucose transport are not additive, providing evidence that the same signaling mechanism is involved (34). These initial observations on the characteristics of AICA-riboside stimulation of glucose uptake

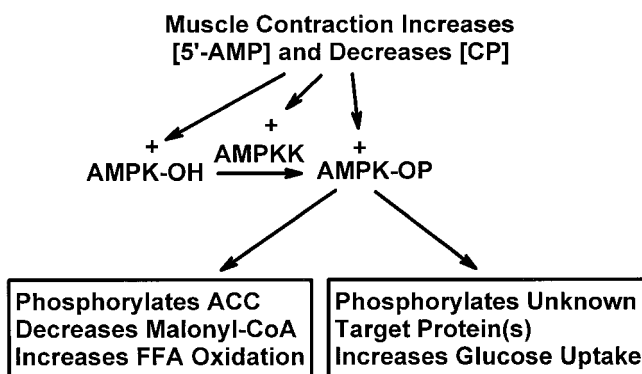


Fig. 1. Postulated mechanisms of increase in fatty acid oxidation and on glucose uptake in skeletal muscle in response to contraction. AMPKK, AMP-activated protein kinase kinase; [5'-AMP] and [CP], 5'-AMP and creatine phosphate concentrations, respectively; AMPK-OH and AMPK-OP, nonphosphorylated and phosphorylated AMPK, respectively; ACC, acetyl-CoA carboxylase; FFA, free fatty acids.

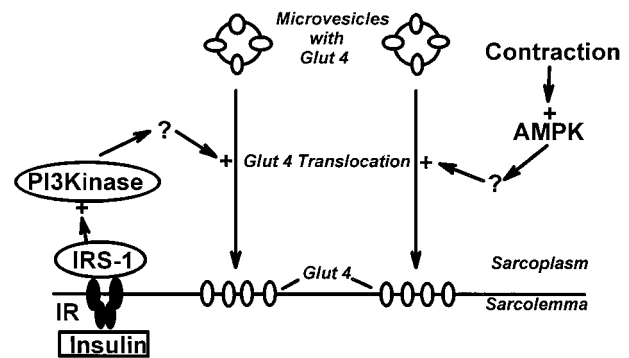


Fig. 2. Two mechanisms for stimulation of glucose uptake in skeletal muscle, one mediated by insulin and one triggered by muscle contraction. The hypothesis of mediation of the contraction effect by AMPK is based on the observations that exercise and electrical stimulation increase AMPK activity and glucose uptake and that glucose uptake is increased by chemical activation of AMPK with AICA-riboside. IR, insulin receptor; IRS-1, insulin receptor substrate 1; PI 3-kinase, phosphatidylinositol 3-kinase.

in isolated epitrochlearis have recently been confirmed and extended (6). Infusion of AICA-riboside into conscious rats stimulated a greater than twofold increase in uptake of labeled 2-deoxyglucose into soleus and into the lateral and medial gastrocnemius muscles (6). Recent data from rat hindlimb perfusion studies demonstrates an increase in GLUT-4 translocation from microvesicles to sarcolemmal membranes in muscle in response to a 45-min exposure to 2 mM AICA-riboside (E. Kurth-Kraczek, M. F. Hirshman, L. J. Goodyear, and W. W. Winder, unpublished data) (Fig. 2).

Although it is clear that AMPK is activated in response to muscle contraction concurrently with the stimulation of glucose uptake, and that chemical activation of AMPK with AICA-riboside induces an increase in glucose uptake in isolated epitrochlearis, with characteristics strikingly similar to those induced by contraction, additional studies are needed to firmly establish a role of the kinase in this regard. Development of a highly specific inhibitor of AMPK or of mouse knock-outs will be essential for providing more direct evidence of its involvement in mediation of the contraction stimulation of glucose uptake and of the increase in fatty acid oxidation. In studies using AICA-riboside to activate AMPK, the possibility of effects not mediated by AMPK must be considered. In addition to the effect of ZMP on phosphorylase mentioned earlier, there is the possibility that AICA-riboside-treated muscles would produce more adenosine. The effect of adenosine on contraction- and insulin-stimulated glucose uptake is controversial, but one recent report by Han et al. (29) provided evidence that extracellular adenosine is responsible in part for mediating both these mechanisms for increasing glucose uptake into skeletal muscle. However, our studies in perfused skeletal muscle indicate no change in ATP, ADP, or AMP in response to AICA-riboside (52). Because adenosine is generated from AMP, we consider it unlikely that adenosine production was increased. Han et al. reported that incubation of muscles with an adenosine agonist did not further increase the glucose transport response to

maximally effective insulin concentrations. If the effects reported by Hayashi et al. (34) were due to increased adenosine production, an additive effect of insulin and AICA-riboside treatment would not have been observed. It is clear, however, that the adenosine effect requires additional study, particularly in terms of whether or not 5'-AMP and AMPK activity are altered by the adenosine receptor antagonists or by depletion of adenosine in the incubation medium using adenosine deaminase. One recent study clearly demonstrated that 10 μ M 8-(p-sulfophenyl)-theophylline, an adenosine receptor antagonist, failed to block AICA-riboside-stimulated 2-deoxyglucose uptake in isolated epitrochlearis muscles (6).

Additional properties of the AMPK signaling system in skeletal muscle have recently been elucidated in studies on rats exercised on a treadmill. The extent of activation of muscle AMPK during exercise is dependent on intensity. In general, higher exercise intensities produce greater activation of AMPK in the muscle (60). In addition, a recent study indicates that in response to 5-min bouts of high-intensity exercise, AMPK peaks \sim 5 min postexercise and is back to baseline within 15 min after the cessation of exercise (59). Muscle AMPK also increases in response to moderate intensity exercise of 30-min duration and returns to baseline within 15–30 min postexercise (59).

COULD DEFECTS IN THE AMPK SIGNALING SYSTEM RESULT IN TYPE 2 DIABETES?

The rapidly accumulating data identifying the importance of this protein kinase in the control of carbohydrate and fat metabolism led us to consider whether AMPK deficiency or disuse (due to a sedentary lifestyle) could possibly result in insulin insensitivity and Type 2 diabetes (Fig. 3). We emphasize that, at this point, we are unaware of any data on the properties of the AMPK signaling system in animal models of insulin resistance or in diabetic patients. Our hypothesis is based solely on data about actions of AMPK obtained from experiments on normal rats, on perfused or incubated rat skeletal muscle, on cells in culture, and on isolated enzymes. We also recognize that many of these metabolic processes are subject to multiple regulatory mecha-

nisms in addition to putative effects of AMPK. The net effect of AMPK activation is stimulation of hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis, stimulation of skeletal muscle fatty acid oxidation and muscle glucose uptake, and modulation of insulin secretion by pancreatic β -cells. We consider a defect in the AMPK signaling cascade to be a feasible primary cause of the insulin resistance, dyslipidemia, ketosis resistance, and possibly other metabolic derangements of some Type 2 diabetes patients.

A diminished functioning of the AMPK signaling system could result from mutations in the gene for the upstream kinase AMPKK or from genetic defects for any one of the individual subunits of AMPK. Defects in downstream target proteins for AMPK (yet undefined) are also possible candidates for explaining the decreased insulin sensitivity of skeletal muscle. The usual time course for development of Type 2 diabetes would not necessarily be explained by a genetic defect in one of the AMPK signaling proteins, unless this primary defect chronically altered the metabolite milieu (elevated serum triglycerides, and fatty acids with consequent elevated cellular fatty acyl-CoA), which has been postulated (65) to contribute secondarily to insulin resistance. The etiology of insulin deficiency in Type 2 diabetes is not understood but could be secondary to increased demands for secretion of the β -cells caused by insulin insensitivity (cf. Refs. 25, 27). The possibility of downregulation of the AMPK signaling system in response to diet (e.g., high fat feeding) and/or lack of physical activity and to chronic hyperglycemia is also conceivable, although no data are available to support this speculation. It is clear that, even in normal individuals, the reduction in muscular activity that accompanies bed rest triggers insulin resistance and reduced glucose tolerance (43). The decrease in insulin sensitivity that occurs with aging has also been attributed to a decrease in physical activity (40).

Because of the numerous proteins involved in stimulation of glucose uptake into muscle, it should be clear that Type 2 diabetes is not likely to be one disease, but a disease of numerous etiologies, all resulting in decreased glucose disposal and/or increased hepatic glucose production. The insulin insensitivity in Type 2 diabetics could conceivably result from defects in the insulin signaling pathway (via PI 3-kinase) or from defects in the contraction-signaling pathway (hypothesized to be mediated by the AMPK activation) for inducing increases in glucose uptake by the muscle. For those patients with defects in the insulin signaling pathway, manipulation of the AMPK pathway (using exercise or AICA-riboside-like drugs, which at least in isolated perfused hindlimbs and isolated epitrochlearis muscles increase glucose uptake) may prove beneficial in treatment of the disease. It is well documented that some Type 2 diabetics respond favorably to acute bouts of exercise and to exercise training (3, 18, 23, 28, 35, 38, 40, 41, 43, 62, 75, 77, 81). However, patient selection may bias the results of such experiments. Patients with

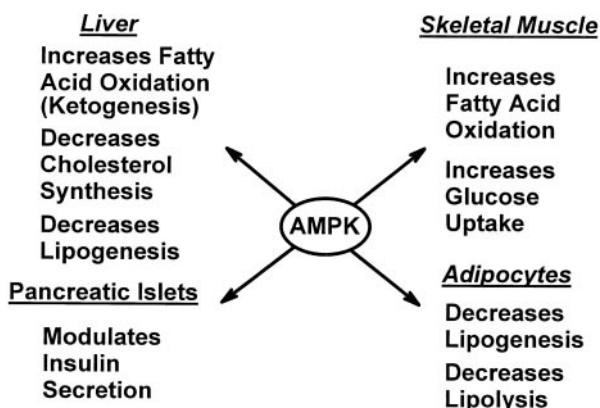


Fig. 3. Multiple effects of AMPK on liver, adipose tissue, muscle metabolism, and pancreatic islets.

an AMPK defect might be expected to have a low exercise tolerance. It has already been demonstrated (cf. Ref. 62) that patients with Type 2 diabetes have a lower maximum O₂ consumption than do normal subjects. If our hypothesis is correct, AMPK-deficient individuals would be expected to obtain a greater proportion of their energy for muscle contraction from stored glycogen. This would cause a more rapid depletion of muscle glycogen, thereby causing early onset of fatigue and reducing capacity for prolonged exercise. These patients may be less likely to become involved in an exercise intervention study because of past experience with low exercise tolerance. In at least one study, muscle glycogen depletion was accelerated and total body fat oxidation was reduced during exercise in lean Type 2 diabetic patients compared with healthy control subjects (49). In the fatty Zucker rat, an animal model of Type 2 diabetes, the defect does not appear to be in the contraction-induced pathway. Muscles from these rats respond to contraction by increasing translocation of GLUT-4 to the sarcolemma, and endurance training induces an increase in muscle total GLUT-4 and restores insulin sensitivity (24). Likewise, insulin-resistant muscles from male offspring resulting from a cross between BTBR and C57BL/6J mice appear to respond to hypoxia (which is likely to activate AMPK, although this was not measured) by increasing glucose transport (58).

RELATION OF THIS HYPOTHESIS TO PREVIOUSLY POSTULATED ETIOLOGIES OF TYPE 2 DIABETES

A role for malonyl-CoA and LCA-CoA in producing insulin resistance in muscle and abnormal insulin secretion by the pancreatic islets was proposed and has been discussed in detail previously (55, 57, 63–65). Elevated muscle malonyl-CoA resulting from inactivity or abundant carbohydrate substrate (increases in malonyl-CoA via citrate activation of ACC) (66) was proposed to increase cytoplasmic LCA-CoA as a result of inhibition of CPT-1. This elevated cytoplasmic LCA-CoA or products synthesized from LCA-CoA (such as phosphatidic acid or diacylglycerol) were postulated to activate specific protein kinase C (PKC) isozymes (70). Activated PKC was then proposed to phosphorylate the insulin receptor, thereby decreasing tyrosine kinase activity. Other mechanisms, such as direct inhibition of glycogen synthase and activation of the hexosamine pathway were also suggested. Muscle malonyl-CoA has been reported to be elevated in soleus muscle of three insulin-resistant rodent models, including the KKA^y mouse, the GK rat, and the denervated rat soleus (63, 64). Treatment of obese, hyperinsulinemic rodents with pioglitazone decreases muscle malonyl-CoA, plasma triglyceride concentrations, and plasma glucose (63, 64). To our knowledge, malonyl-CoA, ACC, and AMPK data on muscle from Type 2 diabetic patients are not yet available. Our hypothesis is compatible with these previous proposals and in fact suggests a feasible primary defect that would give rise to the increase in malonyl-CoA with consequent accumulation of LCA-CoA.

FUTURE DIRECTIONS

Additional studies are needed to verify the hypothesis of AMPK mediation of the stimulation of glucose uptake induced by muscle contraction. Development of specific AMPK inhibitors will be essential for these studies. Data on expression of AMPK genes and on AMPK activity in resting and contracting muscle in the various animal models of insulin resistance will be important. Development of models of AMPKK and or AMPK deficiency using gene knockout mice will prove useful, although interpretation may be difficult because of the multiple isoforms of the AMPK subunits. Finally, basic studies must be extended to examination of this signaling system in normal human subjects and in diabetic populations. Initially it may be worthwhile for geneticists to probe for mutations in the AMPK and AMPKK genes in diabetic populations.

SUMMARY

In summary, evidence has been presented indicating that AMPK influences many metabolic processes that become deranged in response to diabetes. These include lipogenesis, triglyceride synthesis, fatty acid oxidation, ketogenesis, and cholesterol synthesis in the liver. Recent data implicate AMPK as being important in control of fatty acid oxidation and glucose uptake in skeletal muscle, and possibly in modulating insulin secretion by the pancreatic β -cell. It is recognized that Type 2 diabetes is not one disease but a syndrome with numerous possible etiologies. This review provides the rationale for examining the function of the AMPK control system in diabetic populations as one possible cellular mechanism for explaining several of the metabolic derangements of Type 2 diabetes. Activation of this control system either with exercise or by pharmacological manipulation may partially correct the metabolic perturbations of the forms of Type 2 diabetes resulting from defects in the insulin signaling cascade. We are hopeful that this hypothesis can be tested quickly by the numerous laboratories devoted to determination of the etiologies of this disease, which is now occurring in epidemic frequency throughout the world.

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