

Minireview: Adenosine 5'-Monophosphate-Activated Protein Kinase Regulation of Fatty Acid Oxidation in Skeletal Muscle

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AMP-activated protein kinase (AMPK) is master regulator of energy balance through suppression of ATP-consuming anabolic pathways and enhancement of ATP-producing catabolic pathways. AMPK is activated by external metabolic stresses and subsequently orchestrates a complex downstream signaling cascade that mobilizes the cell for efficient energy pro-

duction. AMPK has emerged as a key kinase driving lipid oxidation in skeletal muscle, and this function has important implications for exercise adaptations as well as metabolic defects associated with obesity. (Endocrinology 149: 935–941, 2008)

EMPHASIS ON SIGNALING pathways involved in energy metabolism is of tremendous physiological relevance today, because an imbalance of energy intake and energy expenditure leads to obesity. Regular physical exercise clearly maintains a healthy metabolic profile and largely prevents the development of obesity and metabolic disorders. AMP-activated protein kinase (AMPK) is a key enzyme that has emerged as a master regulator of energy balance and a common denominator between the two opposing states of metabolic fitness, characterized by physical activity or obesity. At the molecular level, AMPK is a serine/threonine kinase activated by any metabolic stress that generates an increase in the AMP/ATP ratio (1–3). Upon activation, AMPK orchestrates intracellular events to suppress ATP-consuming anabolic pathways and enhance ATP-producing catabolic pathways to drive more efficient energy production in response to hormonal, nutritional, and external stimuli (2). Here we will review the role of AMPK in the regulation of fatty acid (FA) oxidation in skeletal muscle, a key tissue important for lipid metabolism and whole-body glucose homeostasis. In particular, we will review recent findings demonstrating how AMPK activation coordinates lipid oxidation in differing metabolic circumstances. Finally, we will highlight additional molecules emerging as putative factors that impact AMPK regulation of lipid oxidation.

Historical Perspective of AMPK Discovery

The first experimental observation underscoring a role for a kinase in lipid metabolism was realized concordantly in

1973 by two groups who independently demonstrated that

the same kinase inactivates two enzymes involved in liver fat metabolism, 3-hydroxy-3-methylglutaryl coenzyme A (CoA) reductase and acetyl-CoA carboxylase (ACC) (4, 5). Subsequently, this kinase was later named AMPK due to its responsiveness to alterations in AMP levels after changes in cellular energy levels (6–9). The parallel discovery of the SNF kinases, the *Saccharomyces cerevisiae* yeast homolog of AMPK (10) has provided substantiation of this protein family in energy regulation and suggests evolutionary conservation (11). In the past decade, AMPK has been a cornerstone molecule for investigation of energy balance through its involvement in glucose/lipid metabolism and the Randle cycle (12) as well as enhancements of mitochondrial biogenesis (13, 14) and regulation of protein synthesis and gene expression (15). AMPK regulation of lipid oxidation remains a key focus in studies of metabolism because physical exercise, currently one of the most efficacious antiobesity treatments, is a potent activator of AMPK in skeletal muscle (16–20).

AMPK is a heterotrimeric protein complex composed of one α -subunit ($\alpha 1$ and $\alpha 2$), one β -subunit ($\beta 1$ and $\beta 2$), and one γ -subunit ($\gamma 1$, $\gamma 2$, and $\gamma 3$) (21). The α -subunit is the catalytic subunit containing the kinase domain, and among the many phosphorylation sites, T172 must be phosphorylated for activity (3). The β -subunit serves primarily as the structural core of the heterotrimer through interactions with both the α - and γ -subunits and also possesses a glycogen-binding domain. The regulatory γ -subunit contains two pairs of cystathionine β -synthase (CBS) domains, each pair known as a Bateman domain, that bind one AMP molecule (22). The γ -subunit is the central functional component responsible for the AMP response, because binding of AMP at CBS domains allosterically activates AMPK, rendering it a better substrate for upstream kinases and a worse substrate for phosphatases (23–25). The first crystal structure resolution of an AMPK heterotrimeric complex has been achieved (26, 27). The struc-

tural data have clarified the interaction sites between subunits and defined the location of the kinase domain on the α -subunit and the phosphate tunnel for AMP binding on the γ -subunit. Complementary to this recent structural examination, *in vitro* deletion analysis has further defined domains on the γ -subunit that are critical to kinase activity (28). These studies have provided new insights regarding competitive AMP/ATP binding and also offer insight into why mutations in the γ -subunit influence activation of the heterotrimeric kinase.

AMPK Expression in Skeletal Muscle

The initial identification of AMPK and its functional kinase activity was achieved in liver. Subsequently, mRNA and protein expression analysis of AMPK provided evidence that AMPK had a likely role in other tissues, because the highest expression of the AMPK mRNA was found in skeletal muscle, seven-fold higher than liver (29). In recent years, expression analysis of particular isoforms of AMPK subunits in skeletal muscle has been investigated extensively in both rodents and humans but is not entirely resolved (20, 30–33). Current perspectives of the roles and functions of specific isoforms have been comprehensively reviewed (34). Several isoforms, $\alpha 1$, $\beta 1$, and $\gamma 1$, are ubiquitous, whereas $\alpha 2$, $\beta 2$, $\gamma 2$, and $\gamma 3$ are preferentially expressed in skeletal muscle, further implicating a function for AMPK in this tissue. Of the 12 possible subunit combinations, only three exist in human skeletal muscle, namely $\alpha 1/\beta 2/\gamma 1$, $\alpha 2/\beta 2/\gamma 1$, and $\alpha 2/\beta 2/\gamma 3$ (35). Unique roles of specific isoforms have begun to emerge. For example, $\alpha 2$ and $\gamma 3$ isoforms have been implicated to be essential for AMPK activity during exercise (18, 20, 36, 37), and the combination of $\alpha 2/\beta 2/\gamma 3$ confers the heterotrimeric complex most associated with exercise-induced AMPK activation in human skeletal muscle (35). However, further investigation is required to fully elucidate the activation capacity of individual kinase isoforms. Whether specific isoforms or subunit combinations provide preferential control of lipid oxidation *vs.* glucose metabolism is unknown.

Molecular Regulation of Lipid Oxidation in Muscle by AMPK

Even before the discovery of skeletal muscle-specific isoforms, the realized putative importance of AMPK in skeletal muscle made possible the connection between AMPK as an energy sensor controlling glucose and lipid metabolism and the creatine kinase (CK)-phosphocreatine system (38). The CK/phosphocreatine system functions to provide a sensitive and fast-responding mechanism to prevent an imbalance of the AMP/ATP ratio, such that the energy supply to the working muscle is maintained (39). The discovery that AMPK phosphorylates and inactivates the skeletal muscle-specific isoform of CK provided a link between the two systems (38). Thus, AMPK responds to depletions in available stores of high-energy phosphate from both phospho-CK and ATP and drives a consequent shift to lipid oxidation, by which the muscle can acquire a persistent and a potentially unlimited source of energy (19, 40).

Canonical activation of AMPK in skeletal muscle occurs

after a metabolic stress such as exercise, hypoxia, starvation, or other external stimuli that elicit an increase in the AMP/ATP ratio. In response to an intense energy demand, AMPK promotes an orchestration of energy-conserving events geared toward prolonging energy availability by phosphorylation of at least 20 target genes (34). On one hand, AMPK activity promotes glucose metabolism by stimulating glucose uptake via glucose transporter 4 transporters and glycogen storage, likely by allosteric activation of glucose 6-phosphate-induced glycogen synthase activity (41). However, glucose stores represent a finite source of ATP, particularly in states of exercise or fasting, and endogenous fat stores are recruited for energy production. Thus, by driving lipid oxidation, the working muscle can harvest energy from fat, which provides a 3-fold greater source of ATP than carbohydrate sources. AMPK activation favors lipid metabolism under such conditions (5, 42, 43).

FA oxidation occurs in skeletal muscle when cytosolic long-chain fatty acyl-CoA is transported into the mitochondria where it can undergo β -oxidation and enter the citric acid cycle for ATP production (44). This process is regulated allosterically by malonyl-CoA, a cytosolic glucose-derived species that inhibits carnitine palmitoyltransferase-1 (CPT-1), a rate-limiting enzyme in mitochondrial FA uptake, thereby restricting the entry of acyl-CoA into mitochondria (45). The regulatory enzyme ACC catalyzes the conversion of acetyl-CoA to malonyl-CoA, which diminishes FA CoA entrance into mitochondria and increases availability for synthesis of triglycerides, diacylglycerol, and ceramides, a mechanism that favors glucose metabolism (16). AMPK enhances FA oxidation in skeletal muscle, as in the liver, by inactivating ACC via phosphorylation, thereby reducing the synthesis of malonyl-CoA (46) and possibly by activation of malonyl-CoA decarboxylase, the enzyme catalyzing the decarboxylation of malonyl-CoA to acetyl-CoA (47, 48). This idea is further supported by the finding that ACC2-deficient mice exhibit increased fat oxidation and reduced fat storage (49). Thus, AMPK activity (Fig. 1) rectifies energy imbalances via inhibition of ACC to consequently relieve the inhibition of CPT-1 and permit uptake and subsequent β -oxidation of acyl-CoA in the mitochondria (45, 50). However, malonyl-CoA does not exclusively account for the regulation of all lipid oxidation that occurs. Other factors hypothesized to influence FA oxidation include the FA concentration (51), carnitine availability (52), and the availability of CoA in the mitochondrial matrix (53, 54).

Genetic Mutations of AMPK

Mutations in AMPK have shed light on the role of AMPK activity in energy maintenance. In particular, naturally occurring mutations in the PRKAG3 locus, which encodes the γ -subunit, highlight the functional contribution of the γ -subunit to total AMPK activity in glucose and lipid metabolism. Rodents and swine harboring an R225Q or R220Q mutation, respectively, in the first CBS domain of the $\gamma 3$ -subunit, the region responsible for AMP binding, have increased muscle glycogen (31, 32, 55). Further investigation of genetic mouse models with this mutation demonstrated protection against diet-induced insulin resistance, lower im triglycerides, in-

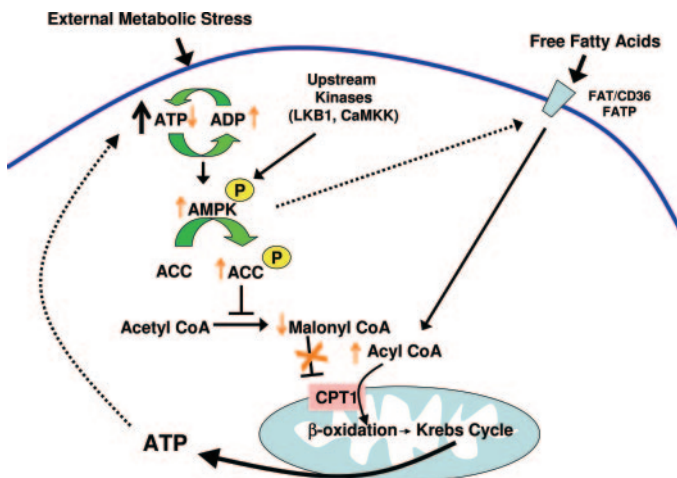


FIG. 1. AMPK regulation of lipid oxidation in skeletal muscle. External metabolic stresses (exercise, fasting, hypoxia, etc.) cause an increase in AMP/ATP ratio. AMPK is activated by AMP binding and a phosphorylation event by upstream kinases. Phospho-AMPK phosphorylates and inhibits ACC activity, thereby inhibiting malonyl-CoA synthesis. This relieves the inhibition of CPT-1 activity and increases mitochondrial import and β -oxidation of FAs in muscle. ATP production by mitochondria satisfies the cellular energy need.

creased ACC phosphorylation, and increased lipid oxidation in these animals after a high-fat diet (37). A human mutation in the γ 3-subunit at the homologous site has been identified (56). Subjects carrying the γ 3 R225W mutation had a 2-fold increase in AMPK activity, a 90% increase in skeletal muscle glycogen content, and a 30% decrease in intramuscular triglycerides. The first single-nucleotide polymorphism analysis of the PRKAG3 genetic locus reveals an association between low-density lipoprotein cholesterol and apolipoprotein B-100 serum levels in a nondiabetic population (33). Clearly, these genetic reports underscore the role of the γ 3-subunit in glucose and lipid metabolism.

AMPK and Metabolic Fitness

From a metabolic health perspective, much that is known about AMPK-dependent energy metabolism has been revealed through examination of different states of metabolic fitness. For example, the metabolic state of a highly trained athlete is characterized by extreme flexibility in the transition between glucose and lipid oxidation to achieve the most advantageous balance of glucose sparing and energy efficiency (57). Conversely, the sedentary, obese individual has lost the ability to recruit energy stores in the same manner (58, 59). Accumulating evidence suggests that opposing regulation of an AMPK fuel-sensing signaling cascade could be a key feature underlying these distinct states of metabolic fitness (Fig. 2).

AMPK and exercise

The benefits of exercise on human health can be partly attributed to an increased reliance on lipid oxidation in response to training adaptation (43, 60–62). During acute and prolonged exercise, skeletal muscle sustains a persistent depletion of ATP, and activation of AMPK allows an adequate response to the changes in energy need (63). However, AMPK activation, as well as substrate selection, is a function

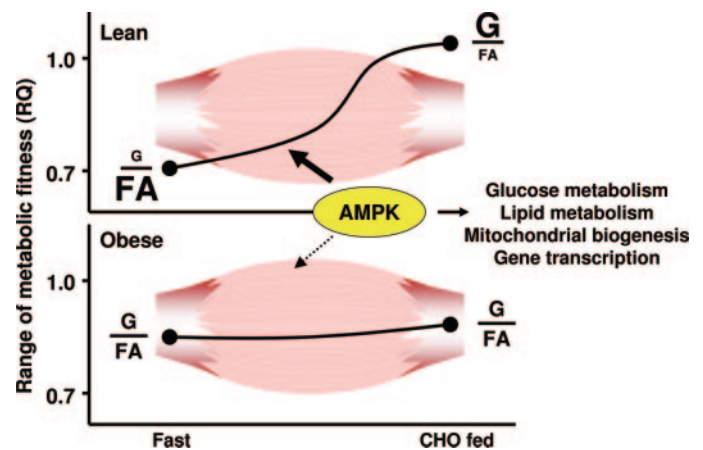


FIG. 2. Oposing states of metabolic fitness impact skeletal muscle metabolism. At rest, a fasted lean muscle relies primarily on fat oxidation (FA) for energy, which is associated with a lower RQ value (~ 0.7). After a high-carbohydrate (CHO) meal, a lean muscle uses glucose (G) metabolic pathways for energy, resulting in an increased RQ value, close to 1. This capability, termed metabolic flexibility, is further enhanced with endurance training. A greater reliance on glucose metabolism under fasting conditions is a hallmark of obesity. States of obesity heavily alter the substrate-switching capacity of skeletal muscle and render the tissue more metabolically inflexible. This is reflected by a narrow RQ range after consumption of a meal. Defects in AMPK signaling could contribute to this impairment due to the importance of AMPK action on glucose and lipid metabolism, mitochondrial biogenesis, and transcription of genes along these pathways. Whether and how endurance training can improve the metabolic RQ range of obese individuals is a topic of intense investigation.

of the intensity and length of the exercise bout (19, 35, 64). Longer and/or low-intensity exercise facilitates greater reliance on fat oxidation, whereas an increase in the exercise intensity elicits a switch from lipid-based to carbohydrate-based fuels (65, 66). Beyond this crossover point, carbohydrates are the primary energy source, because glucose metabolism is more tightly regulated to the energy requirements and can more precisely meet the needs of the working muscle. However, during exercise, an increased lipid oxidation enables a better match between energy supply and demand and delays consumption of muscle glycogen. This transition to an enhanced reliance on lipid oxidation spares use of plasma glucose but provides a need for an enzyme to control such a metabolic switch. The relationship between exercise and lipid oxidation at the molecular basis was realized by the finding that malonyl-CoA was decreased after an exercise bout (67, 68), a finding that provides additional evidence for a contribution by AMPK. Although the behavior of AMPK signaling appears to vary with exercise intensity and skeletal muscle fiber type and across species, AMPK activation clearly plays an important role, and future investigation will bring further clarity.

A multitude of studies have addressed the role of exercise-induced AMPK activation on lipid oxidation using a variety of experimental systems and protocols that, together, do not point to a single clear mechanism of action. At high-intensity exercise, the activation of AMPK responds to the depletion of ATP and increases glucose transport (69), whereas at lower intensities, contracting muscle experiences persistent AMPK activation

that favors lipid metabolism. Furthermore, in a trained state, changes occur that make the muscle primed for lipid oxidation. This is represented by a perpetual decrease in the concentration of both malonyl-CoA and phosphorylated ACC (19, 70), which facilitates greater uptake and oxidation of FA in mitochondria after each single and subsequent bout of exercise. Although evidence for the importance of AMPK activity in transducing exercise adaptations continues to accumulate, further investigation is essential to clarify cross-species differences and subunit contribution at varying exercise intensities.

For the past several years, research groups have attempted to understand differential AMPK response to exercise. Several subunit isoforms of the kinase have emerged as being more exercise activated than others. In humans, $\alpha 2$ - rather than $\alpha 1$ -containing AMPK complexes appear to mediate metabolic responses to exercise in skeletal muscle (18). However, maximal sprint exercise over 30 sec activates both AMPK $\alpha 1$ and $\alpha 2$ (43), whereas only AMPK $\alpha 2$ activity is coupled with an increase in ACC β phosphorylation and increased FA oxidation during moderate-intensity exercise (71). Additional studies hypothesize that exercise training results in reduced activation of AMPK after a single bout of exercise in rats (72) and humans (73), indicating that perhaps repeated AMPK signaling renders an endurance-trained muscle more metabolically fit so that each subsequent bout of exercise creates less metabolic stress. Also, much attention has been directed toward the particular contribution of the γ -subunit, because mutations of the $\gamma 1$ - of $\gamma 3$ -subunit have been associated with enhancements associated with an exercise response and increased energy metabolism (31, 74). Conversely, a decrease in $\gamma 3$ -subunit expression characterizes the adaptations to exercise training (35).

In addition to mediating positive effects on lipid oxidation with exercise training, AMPK is also implicated in the induction of mitochondrial biogenesis. A transcription factor, peroxisome proliferator-activated receptor- γ coactivator (PGC)-1 α has emerged as a key orchestrator of transcriptional pathways that induce mitochondrial biogenesis (75–77) and has been associated with exercise training adaptation (78) and skeletal muscle fiber type transformation (79). Exercise increases mitochondrial enzyme activity (60, 62, 80). Similarly, AMPK activation has been linked with increases in mitochondrial genes such as cytochrome C, ALA-S, malate dehydrogenase, and succinate dehydrogenase in glycolytic muscle (13). Because AMPK activation through exercise is hypothesized to initiate a coordinated sequence of events that favor lipid oxidation, enhanced mitochondrial function, and a more energy-efficient state of metabolic fitness, the influence on PGC-1 α activity has naturally been addressed (14, 81, 82). More recently, AMPK has been shown to directly interact with, and phosphorylate, PGC-1 α , indicating that many AMPK-induced mitochondrial gene expression changes occur through PGC-1 α (83). Further detail regarding the interaction between AMPK and PGC-1 α will undoubtedly become evident in the near future.

AMPK and obesity

Metabolic flexibility is described as the ability to switch between carbohydrate use in the insulin-stimulated prandial

state and lipid use in the fasting state to spare glucose for other organs such as the brain (58, 84). Substrate selection in the muscle can be expressed in terms of the respiratory quotient (RQ), where a value of 1.0 correlates with use of carbohydrate as an energy source and a lower value of about 0.7 is associated with FA oxidation. Lean subjects exhibit a dramatic rise in RQ after a meal, as the body switches from lipid to carbohydrate substrates, in response to an increase of blood glucose. The idea of metabolic inflexibility reflects the observation that obese and insulin-resistant diabetic people maintain a nearly constant RQ value, demonstrating that a switch between substrate use does not occur (85). Thus, the inability of skeletal muscle to preferentially switch to FA oxidation could contribute to the impairments in lipid metabolism observed in obese and insulin-resistant people (58, 86). In addition, mitochondrial dysfunction has been observed in inflexible subjects, indicating these defects contribute to the deficiencies in lipid oxidation ability (87).

AMPK activation enhances FA breakdown for energy and drives mitochondrial biogenesis, and thus potential impairments in AMPK activity in states of metabolic inflexibility are quite feasible. Insulin-dependent glucose uptake pathways are impaired in type 2 diabetes, but the capacity for AMPK modulation of glucose metabolism in diabetic muscle remains intact (88). Nevertheless, a recent study demonstrates that obese and type 2 diabetic subjects exhibited an attenuated exercise-induced activation of AMPK, after a 4-month low-intensity exercise protocol. Although RQ was not measured, this finding nevertheless represents another model of inflexibility and suggests that perhaps a more intense exercise protocol may be required to achieve the same benefits compared with lean counterparts (89). AMPK-dependent decreases in malonyl-CoA and FA are associated with improvements in insulin sensitivity, because excess fatty acyl-CoA, ceramides, and diacylglycerol, common lipid metabolites, have a negative impact on lipid and glucose metabolism (90). AMPK activators have been shown to normalize states of metabolic inflexibility in obese rats (91–93). Interestingly, exercise intervention exerts similar improvements (94). Thus, in light of these findings, chronic activation of AMPK could allow the recovery of a flexible metabolism and hold great potential as an exercise intervention therapy or drug target for metabolic disorders.

Current Perspectives of AMPK Signaling

Clearly, AMPK FA signaling in muscle is differentially modulated by the exercised or diabetic phenotype, but AMPK also responds to endocrine hormones such as leptin and adiponectin, which are secreted from adipocytes. Leptin acts centrally on the hypothalamus and peripherally on skeletal muscle to increase insulin sensitivity by promoting lipid oxidation and reducing fat accumulation in nonadipose tissues. Adiponectin lowers circulating glucose and lipid levels after a high-fat meal. The insulin-sensitizing action of these molecules is partly mediated through AMPK activation. Another adipokine, resistin, promotes insulin resistance and appears to exhibit a negative impact on AMPK signaling. These adipocyte-derived hormones also represent novel

therapeutic targets for the treatment of insulin resistance and type 2 diabetes (50).

Recently, the canonical picture of AMPK regulation of FA oxidation has been broadened further by the emergence of additional downstream targets. In addition to its effects on ACC, AMPK was shown to regulate FA uptake into the cell via plasma membrane FA transporters FAT/CD36 (95, 96) and FABPpm (97) in cardiac muscle. Contraction increases levels of FAT/CD36 as well as FA oxidation (98, 99) and is hypothesized to coordinately function with CPT-1 to regulate acyl-CoA. Stearoyl-CoA desaturase (SCD1), an enzyme that catalyzing the synthesis of monounsaturated FAs, has also emerged as a possible target of AMPK and mechanism to mediate FA oxidation in skeletal muscle. Mice lacking the SCD1 gene show an increased AMPK phosphorylation and CPT-1 activity, reduced ceramide synthesis, and enhanced FA β -oxidation (100). The authors hypothesize that absence of SCD1 permits greater activation of AMPK and up-regulation of genes involved in lipid oxidation, possibly through interaction with the leptin signaling pathway. Resolving the true contribution by these proteins on AMPK regulation of lipid metabolism requires further insight.

Summary

Accumulating evidence points to an important role of AMPK in lipid metabolism. Due to the considerable health burden associated with obesity and type 2 diabetes, interest of this relationship continues to grow. Although physical exercise remains a principal anti-obesity intervention strategy, pharmacological activators of AMPK, including metformin, a long-standing antidiabetic agent, mimic many of the effects observed with exercise. Furthermore, this review has primarily focused on AMPK action in skeletal muscle, but without doubt, AMPK signaling in other metabolic tissues including liver and adipose serves a concomitant and important function in the favorable regulation of glucose and lipid metabolism (50). In concert with physical exercise, a coordinated AMPK activation will perhaps emerge as a management strategy to counteract obesity and related metabolic diseases, where impairments in lipid oxidation contribute to disease pathogenesis.

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