0013-7227/08/$15.00/0
Printed in U.S.A.

Endocrinology 149(3):935–941

doi: 10.1210/en.2007-1441

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

Megan E. Osler and Juleen R. Zierath

Karolinska Institute, Department of Molecular Medicine and Surgery, Integrative Physiology, SE-171 77 Stockholm, Sweden

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 production. 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).

Structural Characteristics of AMPK

AMPK is a heterotrimeric protein complex composed of one α-subunit (α1 and α2), one β-subunit (β1 and β2), and one γ-subunit (γ1, γ2, and γ3) (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-

First Published Online December 13, 2007

Abbreviations: ACC, Acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; CBS, cystathionine β-synthase; CK, creatine kinase; CoA, coenzyme A; CPT-1, carnitine palmitoyltransferase-1; FA, fatty acid; PGC, peroxisome proliferator-activated receptor-γ coactivator; RQ, respiratory quotient; SCD1, stearoyl-CoA desaturase.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

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, α1, β1, and γ1, are ubiquitous, whereas α2, β2, γ2, and γ3 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 α1/β2/γ1, α2/β2/γ1, and α2/β2/γ3 (35). Unique roles of specific isoforms have begun to emerge. For example, α2 and γ3 isoforms have been implicated to be essential for AMPK activity during exercise (18, 20, 36, 37), and the combination of α2/β2/γ3 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 γ3-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-

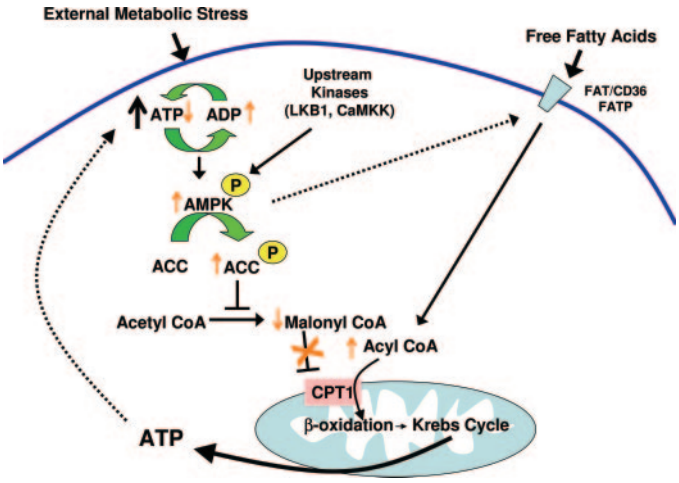

FIG. 1. AMPK regulation of lipid oxidation in skeletal muscle. External metabolic stresses (exercise, fasting, hypoxia, *etc.*) cause an increase in AMP/ATP ratio. AMPK is activated by AMP binding and a phosphorylation event by upstream kinases. Phospho-AMPK phosphorylates and inhibits ACC activity, thereby inhibiting malonyl-CoA synthesis. This relives 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.

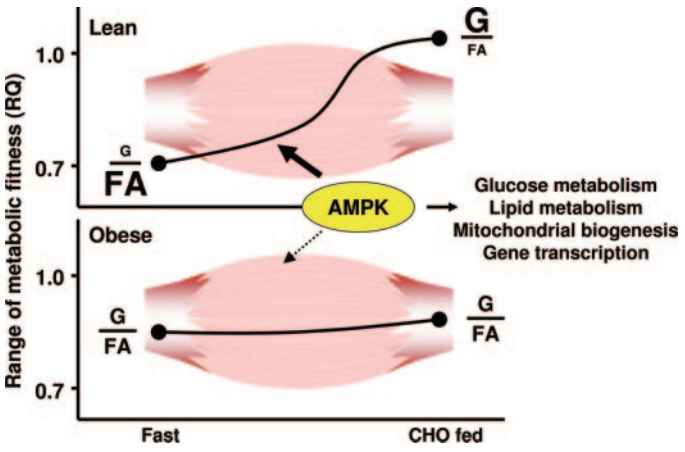

FIG. 2. Opposing states of metabolic fitness impact skeletal muscle metabolism. At rest, a fasted lean muscle relies primarily on fat oxidation (FA) for energy, which is associated with a lower RQ value (~0.7). After a high-carbohydrate (CHO) meal, a lean muscle uses glucose (G) metabolic pathways for 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.

creased ACC phosphorylation, and increased lipid oxidation in these animals after a high-fat diet (37). A human mutation in the γ3-subunit at the homologous site has been identified (56). Subjects carrying the γ3 R225W mutation had a 2-fold increase in AMPK activity, a 90% increase in skeletal muscle glycogen content, and a 30% decrease in im 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 γ3-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 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, α2- rather than α1-containing AMPK complexes appear to mediate metabolic responses to exercise in skeletal muscle (18). However, maximal sprint exercise over 30 sec activates both AMPK α1 and α2 (43), whereas only AMPK α2 activity is coupled with an increase in ACCβ 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 γ1- of γ3-subunit have been associated with enhancements associated with an exercise response and increased energy metabolism (31, 74). Conversely, a decrease in γ3-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)-1α 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-1α activity has naturally been addressed (14, 81, 82). More recently, AMPK has been shown to directly interact with, and phosphorylate, PGC-1α, indicating that many AMPK-induced mitochondrial gene expression changes occur through PGC-1α (83). Further detail regarding the interaction between AMPK and PGC-1α 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, andenhanced 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.

Acknowledgments

Received October 22, 2007. Accepted November 28, 2007.

Address all correspondence and requests for reprints to: Juleen Zierath, Karolinska Institute, Department of Molecular Medicine and Surgery, Integrative Physiology, von Eulers väg 4, 4 SE-171 77 Stockholm, Sweden. E-mail: Juleen.Zierath@ki.se.

The authors are supported by grants from the Swedish Research Council, the Swedish Diabetes Association, the Strategic Research Foundation, Commission of the European Communities (Contract No. LSHM-CT-2004-005272 EXGENESIS and Contract No. LSHM-CT-2004-512013 EUGENE2).

References

1. **Hardie DG, Carling D, Sim ATR** 1989 The AMP-activated protein kinase: a multisubstrate regulator of lipid metabolism. Trends Biochem Sci 14:20–23
2. **Hardie DG, Salt IP, Hawley SA, Davies SP** 1999 AMP-activated protein kinase: an ultrasensitive system for monitoring cellular energy charge. Biochem J 338(Pt 3):717–722
3. **Hawley SA, Davison M, Woods A, Davies SP, Beri RK, Carling D, Hardie DG** 1996 Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. J Biol Chem 271:27879–27887
4. **Beg ZH, Allmann DW, Gibson DM** 1973 Modulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity with cAMP and with protein fractions of rat liver cytosol. Biochem Biophys Res Commun 54:1362–1369
5. **Carlson CA, Kim KH** 1973 Regulation of hepatic acetyl coenzyme A carboxylase by phosphorylation and dephosphorylation. J Biol Chem 248:378–380
6. **Ferrer A, Caelles C, Massot N, Hegardt FG** 1985 Activation of rat liver cytosolic 3-hydroxy-3-methylglutaryl coenzyme A reductase kinase by adenosine 5′-monophosphate. Biochem Biophys Res Commun 132:497–504
7. **Yeh LA, Lee KH, Kim KH** 1980 Regulation of rat liver acetyl-CoA carboxylase. Regulation of phosphorylation and inactivation of acetyl-CoA carboxylase by the adenylate energy charge. J Biol Chem 255:2308–2314
8. **Sim AT, Hardie DG** 1988 The low activity of acetyl-CoA carboxylase in basal and glucagon-stimulated hepatocytes is due to phosphorylation by the AMP-activated protein kinase and not cyclic AMP-dependent protein kinase. FEBS Lett 233:294–298
9. **Carling D, Clarke PR, Zammit VA, Hardie DG** 1989 Purification and characterization of the AMP-activated protein kinase. Copurification of acetyl-CoA carboxylase kinase and 3-hydroxy-3-methylglutaryl-CoA reductase kinase activities. Eur J Biochem 186:129–136
10. **Mitchelhill KI, Stapleton D, Gao G, House C, Michell B, Katsis F, Witters LA, Kemp BE** 1994 Mammalian AMP-activated protein kinase shares structural and functional homology with the catalytic domain of yeast Snf1 protein kinase. J Biol Chem 269:2361–2364
11. **Thompson-Jaeger S, Francois J, Gaughran JP, Tatchell K** 1991 Deletion of SNF1 affects the nutrient response of yeast and resembles mutations which activate the adenylate cyclase pathway. Genetics 129:697–706
12. **Randle PJ, Garland PB, Hales CN, Newsholme EA** 1963 The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785–789
13. **Winder WW, Holmes BF, Rubink DS, Jensen EB, Chen M, Holloszy JO** 2000 Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. J Appl Physiol 88:2219–2226
14. **Zong H, Ren JM, Young LH, Pypaert M, Mu J, Birnbaum MJ, Shulman GI** 2002 AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. Proc Natl Acad Sci USA 99:15983–15987
15. **Hardie DG, Scott JW, Pan DA, Hudson ER** 2003 Management of cellular energy by the AMP-activated protein kinase system. FEBS Lett 546:113–120
16. **Ruderman NB, Saha AK** 2006 Metabolic syndrome: adenosine monophosphate-activated protein kinase and malonyl coenzyme A. Obesity (Silver Spring) 14(Suppl 1):25S–33S
17. **Winder WW, Hardie DG** 1999 AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am J Physiol 277:E1–E10
18. **Fujii N, Hayashi T, Hirshman MF, Smith JT, Habinowski SA, Kaijser L, Mu J, Ljungqvist O, Birnbaum MJ, Witters LA, Thorell A, Goodyear LJ** 2000 Exercise induces isoform-specific increase in 5′AMP-activated protein kinase activity in human skeletal muscle. Biochem Biophys Res Commun 273:1150–1155
19. **Winder WW, Hardie DG** 1996 Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. Am J Physiol 270:E299–E304
20. **Wojtaszewski JF, Nielsen P, Hansen BF, Richter EA, Kiens B** 2000 Isoform-specific and exercise intensity-dependent activation of 5′-AMP-activated protein kinase in human skeletal muscle. J Physiol 528(Pt 1):221–226
21. **Winder WW, Thomson DM** 2007 Cellular energy sensing and signaling by AMP-activated protein kinase. Cell Biochem Biophys 47:332–347
22. **Bateman A** 1997 The structure of a domain common to archaebacteria and the homocystinuria disease protein. Trends Biochem Sci 22:12–13
23. **Hawley SA, Pan DA, Mustard KJ, Ross L, Bain J, Edelman AM, Frenguelli BG, Hardie DG** 2005 Calmodulin-dependent protein kinase kinase-β is an alternative upstream kinase for AMP-activated protein kinase. Cell Metab 2:9–19
24. **Hurley RL, Anderson KA, Franzone JM, Kemp BE, Means AR, Witters LA** 2005 The Ca^{2+}/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. J Biol Chem 280:29060–29066
25. **Shaw RJ, Kosmatka M, Bardeesy N, Hurley RL, Witters LA, DePinho RA, Cantley LC** 2004 The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. Proc Natl Acad Sci USA 101:3329–3335
26. **Amodeo GA, Rudolph MJ, Tong L** 2007 Crystal structure of the heterotrimer core of *Saccharomyces cerevisiae* AMPK homologue SNF1. Nature 449:492–495
27. **Townley R, Shapiro L** 2007 Crystal structures of the adenylate sensor from fission yeast AMP-activated protein kinase. Science 315:1726–1729
28. **Viana R, Towler MC, Pan DA, Carling D, Viollet B, Hardie DG, Sanz P** 2007 A conserved sequence immediately N-terminal to the Bateman domains in AMP-activated protein kinase γ-subunits is required for the interaction with the β-subunits. J Biol Chem 282:16117–16125
29. **Verhoeven AJ, Woods A, Brennan CH, Hawley SA, Hardie DG, Scott J, Beri**

RK, Carling D 1995 The AMP-activated protein kinase gene is highly expressed in rat skeletal muscle. Alternative splicing and tissue distribution of the mRNA. Eur J Biochem 228:236–243
30. **Yu H, Fujii N, Hirshman MF, Pomerleau JM, Goodyear LJ** 2004 Cloning and characterization of mouse 5′-AMP-activated protein kinase γ3 subunit. Am J Physiol Cell Physiol 286:C283–C292
31. **Barnes BR, Marklund S, Steiler TL, Walter M, Hjalm G, Amarger V, Mahlapuu M, Leng Y, Johansson C, Galuska D, Lindgren K, Abrink M, Stapleton D, Zierath JR, Andersson L** 2004 The 5′-AMP-activated protein kinase γ3 isoform has a key role in carbohydrate and lipid metabolism in glycolytic skeletal muscle. J Biol Chem 279:38441–38447
32. **Mahlapuu M, Johansson C, Lindgren K, Hjalm G, Barnes BR, Krook A, Zierath JR, Andersson L, Marklund S** 2004 Expression profiling of the γ-subunit isoforms of AMP-activated protein kinase suggests a major role for γ3 in white skeletal muscle. Am J Physiol Endocrinol Metab 286:E194–E200
33. **Weyrich P, Machicao F, Staiger H, Simon P, Thamer C, Machann J, Schick F, Guirguis A, Fritsche A, Stefan N, Haring HU** 2007 Role of AMP-activated protein kinase γ3 genetic variability in glucose and lipid metabolism in non-diabetic whites. Diabetologia 50:2097–2106
34. **Towler MC, Hardie DG** 2007 AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res 100:328–341
35. **Birk JB, Wojtaszewski JF** 2006 Predominant α2/β2/γ3 AMPK activation during exercise in human skeletal muscle. J Physiol 577:1021–1032
36. **Barnes BR, Glund S, Long YC, Hjalm G, Andersson L, Zierath JR** 2005 5′-AMP-activated protein kinase regulates skeletal muscle glycogen content and ergogenics. FASEB J 19:773–779
37. **Barnes BR, Long YC, Steiler TL, Leng Y, Galuska D, Wojtaszewski JF, Andersson L, Zierath JR** 2005 Changes in exercise-induced gene expression in 5′-AMP-activated protein kinase γ3-null and γ3 R225Q transgenic mice. Diabetes 54:3484–3489
38. **Ponticos M, Lu QL, Morgan JE, Hardie DG, Partridge TA, Carling D** 1998 Dual regulation of the AMP-activated protein kinase provides a novel mechanism for the control of creatine kinase in skeletal muscle. EMBO J 17:1688–1699
39. **Bessman SP, Carpenter CL** 1985 The creatine-creatine phosphate energy shuttle. Annu Rev Biochem 54:831–862
40. **Long YC, Barnes BR, Mahlapuu M, Steiler TL, Martinsson S, Leng Y, Wallberg-Henriksson H, Andersson L, Zierath JR** 2005 Role of AMP-activated protein kinase in the coordinated expression of genes controlling glucose and lipid metabolism in mouse white skeletal muscle. Diabetologia 48:2354–2364
41. **Merrill GF, Kurth EJ, Hardie DG, Winder WW** 1997 AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. Am J Physiol 273:E1107–E1112
42. **Bavenholm PN, Pigon J, Saha AK, Ruderman NB, Efendic S** 2000 Fatty acid oxidation and the regulation of malonyl-CoA in human muscle. Diabetes 49:1078–1083
43. **Chen ZP, McConnell GK, Michell BJ, Snow RJ, Canny BJ, Kemp BE** 2000 AMPK signaling in contracting human skeletal muscle: acetyl-CoA carboxylase and NO synthase phosphorylation. Am J Physiol Endocrinol Metab 279:E1202–E1206
44. **McGarry JD, Brown NF** 1997 The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem 244:1–14
45. **Ruderman NB, Saha AK, Vavvas D, Witters LA** 1999 Malonyl-CoA, fuel sensing, and insulin resistance. Am J Physiol 276:E1–E18
46. **Winder WW, Wilson HA, Hardie DG, Rasmussen BB, Hutber CA, Call GB, Clayton RD, Conley LM, Yoon S, Zhou B** 1997 Phosphorylation of rat muscle acetyl-CoA carboxylase by AMP-activated protein kinase and protein kinase A. J Appl Physiol 82:219–225
47. **Park H, Kaushik VK, Constant S, Prentki M, Przybytkowski E, Ruderman NB, Saha AK** 2002 Coordinate regulation of malonyl-CoA decarboxylase, *sn*-glycerol-3-phosphate acyltransferase, and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in response to exercise. J Biol Chem 277:32571–32577
48. **Saha AK, Schwarsin AJ, Roduit R, Masse F, Kaushik V, Tornheim K, Prentki M, Ruderman NB** 2000 Activation of malonyl-CoA decarboxylase in rat skeletal muscle by contraction and the AMP-activated protein kinase activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside. J Biol Chem 275:24279–24283
49. **Abu-Elheiga L, Matzuk MM, Abo-Hashema KA, Wakil SJ** 2001 Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. Science 291:2613–2616
50. **Long YC, Zierath JR** 2006 AMP-activated protein kinase signaling in metabolic regulation. J Clin Invest 116:1776–1783
51. **Helge JW, Kiens B** 1997 Muscle enzyme activity in humans: role of substrate availability and training. Am J Physiol 272:R1620–R1624
52. **Kiens B** 2006 Skeletal muscle lipid metabolism in exercise and insulin resistance. Physiol Rev 86:205–243
53. **Rubink DS, Winder WW** 2005 Effect of phosphorylation by AMP-activated protein kinase on palmitoyl-CoA inhibition of skeletal muscle acetyl-CoA carboxylase. J Appl Physiol 98:1221–1227
54. **Ruderman NB, Saha AK, Kraegen EW** 2003 Minireview: malonyl CoA, AMP-activated protein kinase, and adiposity. Endocrinology 144:5166–5171
55. **Andersson L** 2003 Identification and characterization of AMPK γ3 mutations in the pig. Biochem Soc Trans 31:232–235
56. **Costford SR, Kavaslar N, Ahituv N, Chaudhry SN, Schackwitz WS, Dent R, Pennacchio LA, McPherson R, Harper ME** 2007 Gain-of-function R225W mutation in human AMPK γ3 causing increased glycogen and decreased triglyceride in skeletal muscle. PLoS ONE 2:e903
57. **Henriksson J** 1995 Muscle fuel selection: effect of exercise and training. Proc Nutr Soc 54:125–138
58. **Storlien L, Oakes ND, Kelley DE** 2004 Metabolic flexibility. Proc Nutr Soc 63:363–368
59. **Kelley DE, Mandarino LJ** 2000 Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes 49:677–683
60. **Holloszy JO, Coyle EF** 1984 Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56:831–838
61. **Hickson RC, Rennie MJ, Conlee RK, Winder WW, Holloszy JO** 1977 Effects of increased plasma fatty acids on glycogen utilization and endurance. J Appl Physiol 43:829–833
62. **Holloszy JO, Booth FW** 1976 Biochemical adaptations to endurance exercise in muscle. Annu Rev Physiol 38:273–291
63. **Martin 3rd WH** 1996 Effects of acute and chronic exercise on fat metabolism. Exerc Sport Sci Rev 24:203–231
64. **Rasmussen BB, Winder WW** 1997 Effect of exercise intensity on skeletal muscle malonyl-CoA and acetyl-CoA carboxylase. J Appl Physiol 83:1104–1109
65. **Brooks GA, Mercier J** 1994 Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. J Appl Physiol 76:2253–2261
66. **Edwards H, Margaria R, Dill D** 1934 Metabolic rate blood sugar and the utilization of carbohydrate. Am J Physiol 108:203–209
67. **Winder WW, Arogyasami J, Barton RJ, Elayan IM, Vehrs PR** 1989 Muscle malonyl-CoA decreases during exercise. J Appl Physiol 67:2230–2233
68. **Winder WW, Arogyasami J, Elayan IM, Cartmill D** 1990 Time course of exercise-induced decline in malonyl-CoA in different muscle types. Am J Physiol 259:E266–E271
69. **Hayashi T, Hirshman MF, Kurth EJ, Winder WW, Goodyear LJ** 1998 Evidence for 5′ AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. Diabetes 47:1369–1373
70. **Vavvas D, Apazidis A, Saha AK, Gamble J, Patel A, Kemp BE, Witters LA, Ruderman NB** 1997 Contraction-induced changes in acetyl-CoA carboxylase and 5′-AMP-activated kinase in skeletal muscle. J Biol Chem 272:13255–13261
71. **Stephens TJ, Chen ZP, Canny BJ, Michell BJ, Kemp BE, McConell GK** 2002 Progressive increase in human skeletal muscle AMPKα2 activity and ACC phosphorylation during exercise. Am J Physiol Endocrinol Metab 282:E688–E694
72. **Durante PE, Mustard KJ, Park SH, Winder WW, Hardie DG** 2002 Effects of endurance training on activity and expression of AMP-activated protein kinase isoforms in rat muscles. Am J Physiol Endocrinol Metab 283:E178–E186
73. **Nielsen JN, Mustard KJ, Graham DA, Yu H, MacDonald CS, Pilegaard H, Goodyear LJ, Hardie DG, Richter EA, Wojtaszewski JF** 2003 5′-AMP-activated protein kinase activity and subunit expression in exercise-trained human skeletal muscle. J Appl Physiol 94:631–641
74. **Rockl KS, Hirshman MF, Brandauer J, Fujii N, Witters LA, Goodyear LJ** 2007 Skeletal muscle adaptation to exercise training: AMP-activated protein kinase mediates muscle fiber type shift. Diabetes 56:2062–2069
75. **Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM** 1998 A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92:829–839
76. **Vega RB, Huss JM, Kelly DP** 2000 The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor α in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. Mol Cell Biol 20:1868–1876
77. **Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM** 1999 Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98:115–124
78. **Wright DC, Han DH, Garcia-Roves PM, Geiger PC, Jones TE, Holloszy JO** 2007 Exercise-induced mitochondrial biogenesis begins before the increase in muscle PGC-1α expression. J Biol Chem 282:194–199
79. **Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, Spiegelman BM** 2002 Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. Nature 418:797–801
80. **Holloszy JO** 1967 Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J Biol Chem 242:2278–2282
81. **Winder WW, Taylor EB, Thomson DM** 2006 Role of AMP-activated protein kinase in the molecular adaptation to endurance exercise. Med Sci Sports Exerc 38:1945–1949
82. **Suwa M, Egashira T, Nakano H, Sasaki H, Kumagai S** 2006 Metformin increases the PGC-1α protein and oxidative enzyme activities possibly via

AMPK phosphorylation in skeletal muscle in vivo. J Appl Physiol 101:1685–1692
83. **Jager S, Handschin C, St-Pierre J, Spiegelman BM** 2007 AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1α. Proc Natl Acad Sci USA 104:12017–12022
84. **Andres R, Cader G, Zierler KL** 1956 The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state: measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J Clin Invest 35:671–682
85. **Kelley DE, Goodpaster B, Wing RR, Simoneau JA** 1999 Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol 277:E1130–E1141
86. **Gaster M** 2007 Metabolic flexibility is conserved in diabetic myotubes. J Lipid Res 48:207–217
87. **Kelley DE, He J, Menshikova EV, Ritov VB** 2002 Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 51:2944–2950
88. **Koistinen HA, Galuska D, Chibalin AV, Yang J, Zierath JR, Holman GD, Wallberg-Henriksson H** 2003 5-Amino-imidazole carboxamide riboside increases glucose transport and cell-surface GLUT4 content in skeletal muscle from subjects with type 2 diabetes. Diabetes 52:1066–1072
89. **Sriwijitkamol A, Coletta DK, Wajcberg E, Balbontin GB, Reyna SM, Barrientes J, Eagan PA, Jenkinson CP, Cersosimo E, DeFronzo RA, Sakamoto K, Musi N** 2007 Effect of acute exercise on AMPK signaling in skeletal muscle of subjects with type 2 diabetes: a time-course and dose-response study. Diabetes 56:836–848
90. **Schimmack G, Defronzo RA, Musi N** 2006 AMP-activated protein kinase: role in metabolism and therapeutic implications. Diabetes Obes Metab 8:591–602
91. **Bergeron R, Previs SF, Cline GW, Perret P, Russell 3rd RR, Young LH, Shulman GI** 2001 Effect of 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside infusion on in vivo glucose and lipid metabolism in lean and obese Zucker rats. Diabetes 50:1076–1082
92. **Buhl ES, Jessen N, Pold R, Ledet T, Flyvbjerg A, Pedersen SB, Pedersen O, Schmitz O, Lund S** 2002 Long-term AICAR administration reduces metabolic disturbances and lowers blood pressure in rats displaying features of the insulin resistance syndrome. Diabetes 51:2199–2206
93. **Yu X, McCorkle S, Wang M, Lee Y, Li J, Saha AK, Unger RH, Ruderman NB** 2004 Leptinomimetic effects of the AMP kinase activator AICAR in leptin-resistant rats: prevention of diabetes and ectopic lipid deposition. Diabetologia 47:2012–2021
94. **Pold R, Jensen LS, Jessen N, Buhl ES, Schmitz O, Flyvbjerg A, Fujii N, Goodyear LJ, Gotfredsen CF, Brand CL, Lund S** 2005 Long-term AICAR administration and exercise prevents diabetes in ZDF rats. Diabetes 54:928–934
95. **Chabowski A, Coort SL, Calles-Escandon J, Tandon NN, Glatz JF, Luiken JJ, Bonen A** 2005 The subcellular compartmentation of fatty acid transporters is regulated differently by insulin and by AICAR. FEBS Lett 579:2428–2432
96. **Luiken JJ, Coort SL, Willems J, Coumans WA, Bonen A, van der Vusse GJ, Glatz JF** 2003 Contraction-induced fatty acid translocase/CD36 translocation in rat cardiac myocytes is mediated through AMP-activated protein kinase signaling. Diabetes 52:1627–1634
97. **Chabowski A, Momken I, Coort SL, Calles-Escandon J, Tandon NN, Glatz JF, Luiken JJ, Bonen A** 2006 Prolonged AMPK activation increases the expression of fatty acid transporters in cardiac myocytes and perfused hearts. Mol Cell Biochem 288:201–212
98. **Campbell SE, Tandon NN, Woldegiorgis G, Luiken JJ, Glatz JF, Bonen A** 2004 A novel function for fatty acid translocase (FAT)/CD36: involvement in long chain fatty acid transfer into the mitochondria. J Biol Chem 279:36235–36241
99. **Holloway GP, Bezaire V, Heigenhauser GJ, Tandon NN, Glatz JF, Luiken JJ, Bonen A, Spriet LL** 2006 Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise. J Physiol 571:201–210
100. **Dobrzyn A, Dobrzyn P** 2006 Stearoyl-CoA desaturase—a new player in skeletal muscle metabolism regulation. J Physiol Pharmacol 57(Suppl 10):31–42

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**