Published in final edited form as:

Acta Physiol (Oxf). 2009 May ; 196(1): 147–154. doi:10.1111/j.1748-1716.2009.01973.x.

AMPK Control of Fat Metabolism in Skeletal Muscle

David M. Thomson and William W. Winder

Department of Physiology and Developmental Biology, Brigham Young University, Provo, Utah 84602

Abstract

AMP-activated protein kinase (AMPK) has emerged as a key regulator of skeletal muscle fat metabolism. Because abnormalities in skeletal muscle metabolism contribute to a variety of clinical diseases and disorders, understanding AMPK's role in the muscle is important. It was originally shown to stimulate fatty acid oxidation decades ago, and since then much research has been accomplished describing this role. In this brief review we summarize much of this data, particularly in relation to changes in fatty acid oxidation that occur during skeletal muscle exercise. Potential roles for AMPK exist in regulating fatty acid transport into the mitochondria via interactions with acetyl-CoA carboxylase, malonyl-CoA decarboxylase, and perhaps fatty acid transporter/CD36 (FAT/CD36). Likewise, AMPK may regulate transport of fatty acids into the cell through FAT/CD36. AMPK may also regulate capacity for fatty acid oxidation by phosphorylation of transcription factors such as CREB or coactivators such as PGC-1 α .

INTRODUCTION

Because skeletal muscle makes up a relatively large percentage of body mass, it accounts for a great portion of the usage of nutritive substrates both at rest and during exercise or increased physical activity. Faulty regulation of fat metabolism in skeletal muscle may be a contributing factor in a number of disease states including obesity (Houmard, 2008) and insulin resistance (Hulver and Dohm, 2004). Therefore, understanding the role AMPK plays in controlling skeletal muscle FA oxidation is of great clinical importance.

AMP-activated protein kinase (AMPK) is recognized as a major regulator of skeletal muscle metabolism. One of the first described functions of AMPK, reported in 1973, was that of a kinase activity leading to the phosphorylation and inactivation of hepatic acetyl CoA carboxylase (ACC) the rate-limiting enzyme in fatty acid (FA) synthesis (Carlson and Kim, 1973). While additional roles for AMPK have subsequently been described in the regulation of glucose and protein metabolism, its role in the regulation of fat metabolism continues to be a topic of great interest. In skeletal muscle, fatty acid synthesis is virtually nonexistent because it does not express the enzyme fatty acid synthase. Therefore, in this tissue, the role of ACC as a regulator of FA synthesis is unimportant. However, ACC is also known to regulate FA oxidation, and thus, the role of AMPK as a potential regulator of this aspect of lipid metabolism has become an area of intense research.

POTENTIAL POINTS OF REGULATION IN FA OXIDATION

The regulation of FA oxidation in skeletal muscle is complex. It may be controlled at a number of levels including the following: 1) the rate of FA transport across the plasma membrane, 2)

the capacity for FA transport within the cytosol, 3) the rate of FA uptake across the mitochondrial membrane, 4) the mitochondrial oxidative capacity in terms of mitochondrial enzyme contents and activities, 5) feedback by intermediates in the fatty acid oxidation pathway (including acetyl-CoA inhibition of thiolase and control of the citric acid cycle and oxidative phosphorylation by ATP and NADH) and 6) the relative availability of carbohydrate as an energy source. The importance of each level of regulation may vary depending upon cellular status. In other words, the rate limiting step under one set of circumstances may not necessarily be rate limiting under other circumstances. As described below, a role for AMPK in contributing to control of FA oxidation has been described in the literature, particularly at the level of FA transport into the cell and mitochondria, and in increasing mitochondrial content by regulation of protein transcription.

ROLE OF AMPK IN CONTROL OF FA UPTAKE ACROSS THE MITOCHONDRIAL MEMBRANE

Regulation of ACC by AMPK

A major point of regulation of FA oxidation in skeletal muscle is at the level of long-chain fatty acyl CoA transport from the cytosol into the mitochondria where it is oxidized (McGarry and Brown, 1997, Ruderman et al., 1999). The rate-limiting enzyme in this process is carnitine palmitoyltransferase-1 (CPT-1). In skeletal muscle, ACC plays an important role in regulating CPT-1 activity. The primary isoform of ACC in skeletal muscle, ACC-2, is similar to its more widely expressed counterpart ACC-1, but has an n-terminal sequence targeting it to the mitochondrial membrane (Ha et al., 1996), in close proximity to CPT-1. There it carboxylates acetyl-CoA, forming malonyl-CoA (MCoA), which allosterically inhibits CPT-1 activity (McGarry and Brown, 1997). Therefore, an increase in ACC activity increases malonyl-CoA levels, decreases CPT-1 activity, and thereby decreases the uptake of fatty-acyl-CoA into the mitochondria for subsequent oxidation. ACC activity is increased allosterically by citrate (Kim et al., 1989) and inhibited by cytosolic MCoA and long-chain fatty acyl-CoAs such as palmitoyl-CoA (Fediuc et al., 2006, Trumble et al., 1995). Increased substrate supply (acetyl-CoA) in the cytosol can also increase ACC production of MCoA. MCoA can be converted back to acetyl-CoA, but this reaction is catalyzed by a separate enzyme, malonyl-CoA decarboxylase (MCD). Two concerns have been raised concerning the role of MCoA in regulating FA oxidation in human skeletal muscle. Firstly, some studies in humans (Odland et al., 1996, Odland et al., 1998) have failed to detect changes in MCoA concentration despite changes in FA oxidation with exercise. Secondly, skeletal muscle CPT-1 is more sensitive to inhibition by MCoA than its liver counterpart, and based upon its IC_{50} the measured concentration of MCoA in muscle is high enough that it would be expected to suppress CPT-1 activity completely (McGarry and Brown, 1997). It has been speculated that most of the MCoA in the cell is bound to carriers or contained within the mitochondria and that only a small fraction, that produced acutely by ACC-2 on the mitochondrial membrane, has access to CPT-1 (McGarry and Brown, 1997). If so, this explanation would effectively address both concerns. In addition, IC_{50} measurements in isolated mitochondria likely do not represent conditions in the intact muscle. In fact, the IC_{50} moves into the physiological range of malonyl-CoA excursions if albumin concentration is increased in the reaction mix (Winder and Holmes, 2000). In any case, this is an area in which further research will be important.

As noted above, MCoA levels decline and FA oxidation increases in rodent and human skeletal muscle during exercise and/or electrical stimulated contractions (Dean et al., 2000, Duan and Winder, 1992, Roepstorff et al., 2005, Winder et al., 1989, Winder and Hardie, 1996). Because AMPK had been shown to phosphorylate and inactivate muscle ACC in vitro, and to be activated by muscle contraction (Winder and Hardie, 1996), it became an attractive candidate for a signaling protein involved in controlling the change in MCoA levels and FA oxidation

with muscle contraction. Subsequent studies confirmed this role. Perfusion of the rat hindlimb with 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), an AMPK activator, led to a 2.8 fold increase in fatty acid oxidation along with increased ACC phosphorylation and decreased malonyl-CoA concentration (Merrill et al., 1997). Similar results were observed for incubated rat soleus muscle (Kaushik et al., 2001, Smith et al., 2005b, Smith et al., 2005a). AICAR also prevented the decline in fatty acid oxidation that normally occurs with insulin treatment as well as the associated increase in glucose uptake (Winder and Holmes, 2000).

Genetic evidence for AMPK's role in AICAR-stimulated fat oxidation comes from muscle-specific knockout LKB1 knockout (MLKB1-KO) mice. While in vitro incubation with AICAR increased fatty acid oxidation by 85% in extensor digitorum longus (EDL) muscles from wild-type (WT) mice, this effect was essentially eliminated in muscles from MLKB1-KO mice (Thomson et al., 2007a). Notably, basal rates of fatty-acid oxidation were not different in that study between genotypes, suggesting that LKB1/AMPK signaling may not play an essential regulatory role in basal rates of fatty acid oxidation. However, MLKB1-KO has been reported to affect only basal and AICAR stimulated AMPK $\alpha 2$, and not AMPK $\alpha 1$ activity (Koh et al., 2006). Thus, it is possible that the contribution of AMPK $\alpha 1$ signaling in MLKB1-KO muscles may be sufficient to maintain basal rates of fatty acid oxidation. The inability of MLKB1-KO muscle fatty acid oxidation to respond to AICAR is likely due in part to a deficiency in ACC phosphorylation, but may also be affected by lower levels of mitochondrial proteins (Koh et al., 2006, Thomson et al., 2007b), and thus a lower potential for increasing fat oxidation in MLKB1-KO muscles, as discussed below.

Regulation of MCD by AMPK

Malonyl-CoA decarboxylation by MCD may also be regulated by AMPK phosphorylation, but this is controversial. Purified AMPK has been reported to phosphorylate immunopurified MCD (Park et al., 2002), and incubation of skeletal muscle with AICAR leads to increased MCD activity that appears to be due to a phosphorylation event (Saha et al., 2000). However, others have found no effect of AICAR or contraction upon MCD activity in several fast-twitch muscles, and observed no in vitro phosphorylation of recombinant or skeletal muscle MCD by AMPK (Habinowski et al., 2001). It should be noted that the AMPK used in the in vitro assay was either an $\alpha 1\beta 1\gamma 1$ complex or a constitutively active truncated preparation, and is not representative of skeletal muscle AMPK, but these data certainly cast a degree of doubt on the direct role of AMPK in acute regulation of MCD activity. Interestingly, exercise training increases MCD gene expression and protein content in human muscle, an effect that was hypothesized to have occurred due to repetitive activation of AMPK with training (Kuhl et al., 2006). Although direct evidence for this hypothesis remains to be demonstrated, overexpression of AMPK in heart muscle cells increased the concentration of MCD in the cytoplasmic and mitochondrial fractions, suggesting that AMPK may be able to regulate MCD expression (Sambandam et al., 2004).

AMPK control of FA-oxidation during exercise

While the AICAR studies indicated that AMPK can control malonyl-CoA levels and FA oxidation, its role during muscle contraction appears to be more complex. As noted above, treadmill running and electrical stimulation of rat hindlimb muscles leads to activation of AMPK, deactivation of ACC, and a decrease in malonyl-CoA concentration in rat quadriceps muscle, suggesting that it plays a role in the associated increase in FA oxidation (Hutber et al., 1997, Vavvas et al., 1997, Winder and Hardie, 1996). Although fatty acid oxidation has not been measured in contracting skeletal muscle from MLKB1-KO mice, the expected contraction-induced increase in ACC phosphorylation (Thomson et al., 2007a, Koh et al., 2006, Sakamoto et al., 2005) and decline in malonyl-CoA levels were attenuated in muscles lacking LKB1 compared to those from wild-type mice (Thomson et al., 2007a), suggesting

that LKB1/AMPK contributes significantly to FA oxidation during muscle contraction. This may be an important mechanism explaining the observation that MLKB1-KO mice run much less than WT mice when given access to voluntary running wheels, and perform very poorly in response to treadmill running (Thomson et al., 2007b). Likewise, while basal levels of fat oxidation do not appear to be affected by the lack of LKB1, as noted above, an attenuated fatty-acid oxidation response to normal activity in the cage might underlie the elevated intramuscular triglyceride concentrations that have been reported in these mice (Koh et al., 2006). However, the fact that ACC phosphorylation and MCoA concentration were changed somewhat in the MLKB1-KO muscles with contraction in the above-mentioned studies, while AMPK phosphorylation was essentially absent, suggests that an additional ACC kinase may exist which can phosphorylate ACC at the AMPK site. Alternatively, ZMP and AMP can allosterically activate AMPK in the absence of a change in phosphorylation (Thomson et al., 2007a), such that the very low level of basal AMPK activity in the knockouts could have been increased allosterically by contraction.

In contrast to the results observed in MLKB1-KO mice, two very recent reports suggest that AMPK may not be necessary for increases in fatty acid oxidation with AICAR or contraction (Dzamko et al., 2008, Miura et al., 2008). Similar to the MLKB1-KO studies, overexpression of a dominant negative kinase-dead AMPK $\alpha 2$ subunit in skeletal muscle (AMPK $\alpha 2$ DN) reduced ACC phosphorylation in the EDL and soleus muscles, but did not prevent an increase in ACC phosphorylation after in vitro AICAR treatment or contraction (Dzamko et al., 2008). However, unlike in MLKB1-KO muscles, ex vivo increases in fatty acid oxidation with AICAR and contraction were not attenuated in muscles from the AMPK $\alpha 2$ DN mice. In situ contractions also increased ACC phosphorylation and tended to decrease malonyl CoA levels similarly between genotypes, and oxygen uptake and respiratory exchange ratio (RER) values were similar for WT and AMPK $\alpha 2$ DN mice, suggesting that fat oxidation was not affected by genotype. Similarly, dominant negative expression of a kinase-dead AMPK $\alpha 1$, which also ablated AMPK $\alpha 2$ activity, failed to affect lipid oxidation in EDL and soleus muscles during low-intensity exercise, or in vitro fatty acid oxidation after low-intensity treadmill running (Miura et al., 2008). While these studies support the idea that AMPK may not be necessary for increasing fatty acid oxidation in skeletal muscle, they do not indicate that AMPK does not play a role in this regulation under normal circumstances. Likewise, in both cases, a degree of residual AMPK activity remained in the dominant negative mice, which, coupled with allosteric activation of the enzyme could explain the increased ACC phosphorylation and subsequent effects on fatty acid oxidation. However, these findings, taken together with the MLKB1-KO studies, suggest that there may be an additional protein besides AMPK, perhaps of the AMPK family, which is regulated by LKB1 and regulates fatty acid metabolism.

It is likewise possible that redundant signaling pathways or mechanisms exist, independent of LKB1 targets including AMPK, that are involved in the control of FA oxidation. In support of this, it has been reported that while AICAR and low intensity muscle contraction both increased fatty acid oxidation and uptake (Raney et al., 2005), AMPK activation and ACC inactivation were not affected by the low intensity contraction bout, suggesting that AMPK may not be involved in the regulation of FA oxidation under some contraction protocols. Although allosteric activation of AMPK (undetectable in measurements of AMPK activity) should be considered, one possible alternative mediator is calcium/calmodulin-dependent protein kinase II (CaMKII). Inhibition of CaMKII activity with KN93 abolished contraction-induced increases in fatty acid oxidation, and decreased contraction-induced increase in FA uptake by 33% (Raney and Turcotte, 2008). However, since KN93 also reduced AMPK $\alpha 2$ activation by 51%, the role of AMPK in the reported change in contraction induced FA oxidation cannot be entirely discounted.

ROLE OF AMPK IN CONTROL OF FA UPTAKE ACROSS THE PLASMA MEMBRANE

Although FA transport across the cell membrane was traditionally thought to occur only by simple diffusion, current evidence suggests that FA transporters in the membrane exist and are involved in the process (Bonen et al., 1998, Chabowski et al., 2007). A role for AMPK in regulating FA uptake into the cell was first suggested by findings in cardiomyocytes in which AMPK activation by AICAR and oligomycin increased FA uptake and stimulated translocation of the FA transporter FAT/CD36 from intracellular stores to the plasma membrane (Luiken et al., 2003), suggesting that AMPK could regulate FAT/CD36 in a manner similar to its well-documented effect upon GLUT-4. Additionally, chronic AICAR treatment increased the gene and protein expressions, as well as the membrane contents of FAT/CD36 as well as another fatty acid transporter, FABP_{pm}, suggesting that chronic AMPK activation increases the capacity for FA uptake in cardiac muscle (Chabowski et al., 2006). Less work has been done in skeletal muscle. Chronic activation of AMPK with beta-guanidinopropionic acid (β -GPA) was associated with an increase in AICAR-stimulated fat oxidation as well as increased total and membrane-bound FAT/CD36 protein levels (Pandke et al., 2008). Nevertheless, direct evidence of AMPK-mediated translocation in skeletal muscle is still lacking. However, it would appear that if AMPK plays a role in FAT/CD36 translocation with muscle contraction, its activation is not sufficient for translocation since ERK1/2 signaling is also required (Turcotte et al., 2005). Interestingly, recent findings show that FAT/CD36 is also present in the mitochondrial membrane and may be involved along with CPT-1 in the transportation of acyl-CoAs into the mitochondria, and that its concentration there increases with chronic muscle contraction (Holloway et al., 2008). However, the role of AMPK in regulating this function of FAT/CD36 has not been determined.

AMPK IN THE CONTROL OF MITOCHONDRIAL CONTENT

Since FA oxidation occurs in the mitochondria, long-term regulation of mitochondrial content also affects that capacity of the cell to oxidize fats. AMPK has been known to promote mitochondrial biogenesis for some time. In the first studies demonstrating this effect, rats were given daily AICAR injections for 4 weeks, which resulted in increased skeletal muscle citrate synthase, succinate dehydrogenase, and malate dehydrogenase activities, as well as cytochrome C, δ -aminolevulinic acid synthase (δ -ALAS), and UCP-3 protein levels (Putman et al., 2003, Winder et al., 2000). Fatty acid oxidizing enzymes were unaffected (Winder et al., 2000). In fact it is likely that stimulation of PPAR by chronic elevation of FFA may be required for full expression of the fatty acid oxidizing enzymes (Garcia-Roves et al., 2007). AMPK activation via chronic β -GPA treatment also increased mitochondrial content in rats (Bergeron et al., 2001), and this effect was completely blocked in muscles from mice with skeletal muscle expression of a dominant-negative AMPK (Zong et al., 2002). MLKB1-KO resulted in decreased mitochondrial protein contents and decreased exercise capacity (Thomson et al., 2007b). Whole-body AMPK α 2 knockout (AMPK α 2-KO) resulted in lower basal contents for cytochrome C and cytochrome C oxidase-1 (COX-1) proteins, as well as lower citrate synthase and 3-hydroxyacyl-CoA dehydrogenase activities (Jorgensen et al., 2007). In this study, AICAR-induced increases in these mitochondrial markers were blocked by the AMPK α 2-KO. The mice were also trained using voluntary activity wheels, but only COX-1 protein content was increased by this training in wild-type (WT) mice, and this increase was not affected by AMPK α 2-KO. Thus, while the necessity of AMPK for exercise-induced mitochondrial adaptations is not yet well-established, taken together, these data suggest that AMPK is an important regulator of mitochondrial protein expression.

The mechanism by which AMPK regulates expression of mitochondrial enzyme genes is not yet well defined. However, AMPK is known to regulate a number of transcription factors that regulate expression of mitochondrial genes.

Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α) co-activates several transcription factors involved in mitochondrial biogenesis. It is thought to mediate exercise effects on mitochondrial enzyme gene expression, and to be a prime AMPK target, either through regulation of its content or phosphorylation. AICAR treatment of incubated muscle and cells increases PGC-1 α mRNA and protein expression (Ojuka, 2004, Terada et al., 2002), and chronic AICAR treatment increases its protein level (Suwa et al., 2003). AICAR's effect upon PGC-1 α mRNA is blocked in AMPK α 2-KO mice (Jorgensen et al., 2005). However, exercise-induced increases in PGC-1 α mRNA are not blocked by AMPK α 2-KO (Jorgensen et al., 2005). Two studies have reported decreased PGC-1 α protein levels in muscle from MLKB1-KO mice (Thomson et al., 2007b, Koh et al., 2006), while PGC-1 α levels were decreased, but insignificantly so in a third study (McGee et al., 2008), which taken together suggest that LKB1/AMPK plays a role in regulating PGC-1 α levels in skeletal muscle. In addition to affecting PGC-1 α expression, AMPK has been reported to phosphorylate PGC-1 α directly, activating PGC-1 α in cultured muscle cells (Jager et al., 2007), though the importance of this effect in vivo still needs to be verified. Other mechanisms are also likely important in the control of PGC-1 α activity under conditions of energy stress, such as the regulation of acetylation status by SIRT1 (Rodgers et al., 2008). Thus, additional roles for AMPK in PGC-1 α control may soon emerge.

Control of PGC-1 α transcription, as well as that of mitochondrial proteins is mediated partially by cyclic-AMP response element binding protein (CREB). AMPK phosphorylates CREB and other CREB family members in vitro, and AICAR treatment stimulates CREB phosphorylation in cells and incubated muscle. AICAR-stimulated CREB phosphorylation in HEK293 cells is associated with increased transcriptional activity and is blocked by the AMPK inhibitor compound C (Thomson et al., 2008). However, greater understanding of the role of CREB phosphorylation by AMPK or other CREB kinases will require additional research.

Peroxisome proliferator-activated receptor- α (PPAR α) is a nuclear hormone receptor and transcription factor. It controls the expression of many genes encoding proteins involved in fatty acid uptake and oxidation (Gulick et al., 1994). Chronic AICAR incubation stimulates fatty acid oxidation but this is attenuated in C₂C₁₂ cells by siRNA depletion of PPAR α and/or PGC-1 (Lee et al., 2006), suggesting that they both lie downstream of AMPK in this effect. Although AMPK has been shown to phosphorylate PPAR γ , which is expressed primarily in adipose and immune cells (Leff, 2003), a direct effect on PPAR α or δ has not been shown.

SUMMARY

Taken as a whole, the available data indicate that AMPK plays an important role in the regulation FA oxidation, as summarized in Figure 1. Much of the acute effect of AMPK activity on fatty acid oxidation appears to be mediated by control of MCoA levels through its target ACC, but control of fatty acid transport across the cell membrane may also be mediated by AMPK through control of CD-36 translocation. In the long term, AMPK activation also appears to contribute to the control of the oxidative capacity of the cell by way of transcriptional regulation. This effect is mediated by an increase in PGC-1 α content, and perhaps via direct phosphorylation of PGC-1 α , CREB, and other transcription factors.

References

- Bergeron R, Ren JM, Cadman KS, Moore IK, Perret P, Pypaert M, et al. Chronic activation of AMP kinase results in NRF-1 activation and mitochondrial biogenesis. *Am J Physiol Endocrinol Metab* 2001;281:E1340–6. [PubMed: 11701451]
- Bonen A, Luiken JJ, Liu S, Dyck DJ, Kiens B, Kristiansen S, et al. Palmitate transport and fatty acid transporters in red and white muscles. *Am J Physiol* 1998;275:E471–8. [PubMed: 9725814]
- Carlson CA, Kim KH. Regulation of hepatic acetyl coenzyme A carboxylase by phosphorylation and dephosphorylation. *J Biol Chem* 1973;248:378–80. [PubMed: 4692841]
- Chabowski A, Gorski J, Luiken JJ, Glatz JF, Bonen A. Evidence for concerted action of FAT/CD36 and FABPpm to increase fatty acid transport across the plasma membrane. *Prostaglandins Leukot Essent Fatty Acids* 2007;77:345–53. [PubMed: 18240411]
- Chabowski A, Momken I, Coort SL, Calles-Escandon J, Tandon NN, Glatz JF, et al. Prolonged AMPK activation increases the expression of fatty acid transporters in cardiac myocytes and perfused hearts. *Mol Cell Biochem* 2006;288:201–12. [PubMed: 16710744]
- Dean D, Dugaard JR, Young ME, Saha A, Vavvas D, Asp S, et al. Exercise diminishes the activity of acetyl-CoA carboxylase in human muscle. *Diabetes* 2000;49:1295–300. [PubMed: 10923628]
- Duan C, Winder WW. Nerve stimulation decreases malonyl-CoA in skeletal muscle. *J Appl Physiol* 1992;72:901–4. [PubMed: 1349012]
- Dzamko NL, Schertzer JD, Ryall J, Steel R, Macaulay SL, Wee S, et al. AMPK independent pathways regulate skeletal muscle fatty acid oxidation. *J Physiol*. 2008
- Fediuc S, Gaidhu MP, Ceddia RB. Regulation of AMP-activated protein kinase and acetyl-CoA carboxylase phosphorylation by palmitate in skeletal muscle cells. *J Lipid Res* 2006;47:412–20. [PubMed: 16304351]
- Garcia-Roves P, Huss JM, Han DH, Hancock CR, Iglesias-Gutierrez E, Chen M, et al. Raising plasma fatty acid concentration induces increased biogenesis of mitochondria in skeletal muscle. *Proc Natl Acad Sci U S A* 2007;104:10709–13. [PubMed: 17548828]
- Gulick T, Cresci S, Caira T, Moore DD, Kelly DP. The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. *Proc Natl Acad Sci U S A* 1994;91:11012–6. [PubMed: 7971999]
- Ha J, Lee JK, Kim KS, Witters LA, Kim KH. Cloning of human acetyl-CoA carboxylase-beta and its unique features. *Proc Natl Acad Sci U S A* 1996;93:11466–70. [PubMed: 8876158]
- Habinowski SA, Hirshman M, Sakamoto K, Kemp BE, Gould SJ, Goodyear LJ, et al. Malonyl-CoA decarboxylase is not a substrate of AMP-activated protein kinase in rat fast-twitch skeletal muscle or an islet cell line. *Arch Biochem Biophys* 2001;396:71–9. [PubMed: 11716464]
- Holloway GP, Luiken JJ, Glatz JF, Spriet LL, Bonen A. Contribution of FAT/CD36 to the regulation of skeletal muscle fatty acid oxidation: an overview. *Acta Physiol (Oxf)*. 2008
- Houmard JA. Intramuscular lipid oxidation and obesity. *Am J Physiol Regul Integr Comp Physiol* 2008;294:R1111–6. [PubMed: 18256136]
- Hulver MW, Dohm GL. The molecular mechanism linking muscle fat accumulation to insulin resistance. *Proc Nutr Soc* 2004;63:375–80. [PubMed: 15294058]
- Hutber CA, Hardie DG, Winder WW. Electrical stimulation inactivates muscle acetyl-CoA carboxylase and increases AMP-activated protein kinase. *Am J Physiol* 1997;272:E262–6. [PubMed: 9124333]
- Jager S, Handschin C, St-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci U S A* 2007;104:12017–22. [PubMed: 17609368]
- Jorgensen SB, Treebak JT, Viollet B, Schjerling P, Vaulont S, Wojtaszewski JF, et al. Role of AMPKalpha2 in basal, training-, and AICAR-induced GLUT4, hexokinase II, and mitochondrial protein expression in mouse muscle. *Am J Physiol Endocrinol Metab* 2007;292:E331–9. [PubMed: 16954334]
- Jorgensen SB, Wojtaszewski JF, Viollet B, Andreelli F, Birk JB, Hellsten Y, et al. Effects of alpha-AMPK knockout on exercise-induced gene activation in mouse skeletal muscle. *Faseb J*. 2005

- Kaushik VK, Young ME, Dean DJ, Kurowski TG, Saha AK, Ruderman NB. Regulation of fatty acid oxidation and glucose metabolism in rat soleus muscle: effects of AICAR. *Am J Physiol Endocrinol Metab* 2001;281:E335–40. [PubMed: 11440910]
- Kim KH, Lopez-Casillas F, Bai DH, Luo X, Pape ME. Role of reversible phosphorylation of acetyl-CoA carboxylase in long-chain fatty acid synthesis. *FASEB J* 1989;3:2250–6. [PubMed: 2570725]
- Koh HJ, Arnolds DE, Fujii N, Tran TT, Rogers MJ, Jessen N, et al. Skeletal muscle-selective knockout of LKB1 increases insulin sensitivity, improves glucose homeostasis, and decreases TRB3. *Mol Cell Biol* 2006;26:8217–27. [PubMed: 16966378]
- Kuhl JE, Ruderman NB, Musi N, Goodyear LJ, Patti ME, Crunkhorn S, et al. Exercise training decreases the concentration of malonyl-CoA and increases the expression and activity of malonyl-CoA decarboxylase in human muscle. *Am J Physiol Endocrinol Metab* 2006;290:E1296–303. [PubMed: 16434556]
- Lee WJ, Kim M, Park HS, Kim HS, Jeon MJ, Oh KS, et al. AMPK activation increases fatty acid oxidation in skeletal muscle by activating PPARalpha and PGC-1. *Biochem Biophys Res Commun* 2006;340:291–5. [PubMed: 16364253]
- Leff T. AMP-activated protein kinase regulates gene expression by direct phosphorylation of nuclear proteins. *Biochem Soc Trans* 2003;31:224–7. [PubMed: 12546690]
- Luiken JJ, Coort SL, Willems J, Coumans WA, Bonen A, van der Vusse GJ, et al. Contraction-induced fatty acid translocase/CD36 translocation in rat cardiac myocytes is mediated through AMP-activated protein kinase signaling. *Diabetes* 2003;52:1627–34. [PubMed: 12829625]
- McGarry JD, Brown NF. The mitochondrial carnitine palmitoyl transferase system. From concept to molecular analysis. *Eur J Biochem* 1997;244:1–14. [PubMed: 9063439]
- McGee SL, Mustard KJ, Hardie DG, Baar K. Normal hypertrophy accompanied by phosphorylation and activation of AMP-activated protein kinase alpha1 following overload in LKB1 knockout mice. *J Physiol* 2008;586:1731–41. [PubMed: 18202101]
- Merrill GF, Kurth EJ, Hardie DG, Winder WW. AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. *Am J Physiol* 1997;273:E1107–12. [PubMed: 9435525]
- Miura S, Kai Y, Kamei Y, Bruce CR, Kubota N, Febbraio MA, et al. α 2-AMPK activity is not essential for an increase in fatty acid oxidation during low-intensity exercise. *Am J Physiol Endocrinol Metab*. 2008
- Odland LM, Heigenhauser GJ, Lopaschuk GD, Spriet LL. Human skeletal muscle malonyl-CoA at rest and during prolonged submaximal exercise. *Am J Physiol* 1996;270:E541–4. [PubMed: 8638703]
- Odland LM, Howlett RA, Heigenhauser GJ, Hultman E, Spriet LL. Skeletal muscle malonyl-CoA content at the onset of exercise at varying power outputs in humans. *Am J Physiol* 1998;274:E1080–5. [PubMed: 9611159]
- Ojuka EO. Role of calcium and AMP kinase in the regulation of mitochondrial biogenesis and GLUT4 levels in muscle. *Proc Nutr Soc* 2004;63:275–8. [PubMed: 15294043]
- Pandke KE, Mullen KL, Snook LA, Bonen A, Dyck DJ. Decreasing intramuscular phosphagen content simultaneously increases plasma membrane FAT/CD36 and GLUT4 transporter abundance. *Am J Physiol Regul Integr Comp Physiol*. 2008
- Park H, Kaushik VK, Constant S, Prentki M, Przybytkowski E, Ruderman NB, et al. 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* 2002;277:32571–7. [PubMed: 12065578]
- Putman CT, Kiricsi M, Pearcey J, MacLean IM, Bamford JA, Murdoch GK, et al. AMPK activation increases uncoupling protein-3 expression and mitochondrial enzyme activities in rat muscle without fibre type transitions. *J Physiol* 2003;551:169–78. [PubMed: 12813156]
- Raney MA, Turcotte LP. Evidence for the involvement of CaMKII and AMPK in Ca²⁺-dependent signaling pathways regulating FA uptake and oxidation in contracting rodent muscle. *J Appl Physiol* 2008;104:1366–73. [PubMed: 18309092]
- Raney MA, Yee AJ, Todd MK, Turcotte LP. AMPK activation is not critical in the regulation of muscle FA uptake and oxidation during low-intensity muscle contraction. *Am J Physiol Endocrinol Metab* 2005;288:E592–8. [PubMed: 15547141]

- Rodgers JT, Lerin C, Gerhart-Hines Z, Puigserver P. Metabolic adaptations through the PGC-1 α and SIRT1 pathways. *FEBS Lett* 2008;582:46–53. [PubMed: 18036349]
- Roepstorff C, Halberg N, Hillig T, Saha AK, Ruderman NB, Wojtaszewski JF, et al. Malonyl-CoA and carnitine in regulation of fat oxidation in human skeletal muscle during exercise. *Am J Physiol Endocrinol Metab* 2005;288:E133–42. [PubMed: 15383373]
- Ruderman NB, Saha AK, Vavvas D, Witters LA. Malonyl-CoA, fuel sensing, and insulin resistance. *Am J Physiol* 1999;276:E1–E18. [PubMed: 9886945]
- Saha AK, Schwarsin AJ, Roduit R, Masse F, Kaushik V, Tornheim K, et al. 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* 2000;275:24279–83. [PubMed: 10854420]
- Sakamoto K, McCarthy A, Smith D, Green KA, Grahame Hardie D, Ashworth A, et al. Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction. *EMBO J* 2005;24:1810–20. [PubMed: 15889149]
- Sambandam N, Steinmetz M, Chu A, Altarejos JY, Dyck JR, Lopaschuk GD. Malonyl-CoA decarboxylase (MCD) is differentially regulated in subcellular compartments by 5'AMP-activated protein kinase (AMPK). Studies using H9c2 cells overexpressing MCD and AMPK by adenoviral gene transfer technique. *Eur J Biochem* 2004;271:2831–40. [PubMed: 15206948]
- Smith AC, Bruce CR, Dyck DJ. AMP kinase activation with AICAR further increases fatty acid oxidation and blunts triacylglycerol hydrolysis in contracting rat soleus muscle. *J Physiol* 2005a;565:547–53. [PubMed: 15774529]
- Smith AC, Bruce CR, Dyck DJ. AMP kinase activation with AICAR simultaneously increases fatty acid and glucose oxidation in resting rat soleus muscle. *J Physiol* 2005b;565:537–46. [PubMed: 15774530]
- Suwa M, Nakano H, Kumagai S. Effects of chronic AICAR treatment on fiber composition, enzyme activity, UCP3, and PGC-1 in rat muscles. *J Appl Physiol* 2003;95:960–8. [PubMed: 12777406]
- Terada S, Goto M, Kato M, Kawanaka K, Shimokawa T, Tabata I. Effects of low-intensity prolonged exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. *Biochem Biophys Res Commun* 2002;296:350–4. [PubMed: 12163024]
- Thomson DM, Brown JD, Fillmore N, Condon BM, Kim HJ, Barrow JR, et al. LKB1 and the regulation of malonyl-CoA and fatty acid oxidation in muscle. *Am J Physiol Endocrinol Metab* 2007a;293:E1572–9. [PubMed: 17925454]
- Thomson DM, Herway ST, Fillmore N, Kim H, Brown JD, Barrow JR, et al. AMP-activated protein kinase phosphorylates transcription factors of the CREB family. *J Appl Physiol* 2008;104:429–38. [PubMed: 18063805]
- Thomson DM, Porter BB, Tall JH, Kim HJ, Barrow JR, Winder WW. Skeletal muscle and heart LKB1 deficiency causes decreased voluntary running and reduced muscle mitochondrial marker enzyme expression in mice. *Am J Physiol Endocrinol Metab* 2007b;292:E196–202. [PubMed: 16926377]
- Trumble GE, Smith MA, Winder WW. Purification and characterization of rat skeletal muscle acetyl-CoA carboxylase. *Eur J Biochem* 1995;231:192–8. [PubMed: 7628470]
- Turcotte LP, Raney MA, Todd MK. ERK1/2 inhibition prevents contraction-induced increase in plasma membrane FAT/CD36 content and FA uptake in rodent muscle. *Acta Physiol Scand* 2005;184:131–9. [PubMed: 15916673]
- Vavvas D, Apazidis A, Saha AK, Gamble J, Patel A, Kemp BE, et al. Contraction-induced changes in acetyl-CoA carboxylase and 5'-AMP-activated kinase in skeletal muscle. *J Biol Chem* 1997;272:13255–61. [PubMed: 9148944]
- Winder WW, Arogyasami J, Barton RJ, Elayan IM, Vehrs PR. Muscle malonyl-CoA decreases during exercise. *J Appl Physiol* 1989;67:2230–3. [PubMed: 2558099]
- Winder WW, Hardie DG. Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. *Am J Physiol* 1996;270:E299–304. [PubMed: 8779952]
- Winder WW, Holmes BF. Insulin stimulation of glucose uptake fails to decrease palmitate oxidation in muscle if AMPK is activated. *J Appl Physiol* 2000;89:2430–7. [PubMed: 11090599]

- Winder WW, Holmes BF, Rubink DS, Jensen EB, Chen M, Holloszy JO. Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J Appl Physiol* 2000;88:2219–26. [PubMed: 10846039]
- Zong H, Ren JM, Young LH, Pypaert M, Mu J, Birnbaum MJ, et al. AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. *Proc Natl Acad Sci U S A* 2002;99:15983–7. [PubMed: 12444247]

Figure 1. Regulation of skeletal muscle fatty acid oxidation by AMPK

ACC: acetyl CoA carboxylase-2; AMPK: 5' AMP-activated protein kinase; CD36: fatty acid translocase/CD36; CPT1: carnitine palmitoyl transferase-1; CREB: cyclic amp response element binding protein; ETC: electron transport chain; IMTG: intramuscular triglyceride; LCFA: long-chain fatty acid; LCFA-CoA: long-chain fattyacyl-CoA; MDC: malonyl-CoA decarboxylase; PGC1: peroxisome proliferator-activated receptor- γ co-activator-1; PPAR: peroxisome proliferator-activated receptor. Arrows indicate movement, reactions or a stimulatory influence. Cross-bars indicate inhibitory influence. Question mark indicates an effect that is not well established.