

AMPK: A cellular metabolic and redox sensor. A minireview

Najeeb A Shirwany¹, Ming-Hui Zou¹

¹Section of Molecular Medicine, Department of Medicine, and Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA

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1. ABSTRACT

AMPK is a serine/threonine kinase that is found in all eukaryotes and is ubiquitously expressed in all organ systems. Once activated, AMPK stimulates hepatic fatty acid oxidation and ketogenesis, inhibits cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibits adipocyte lipolysis and lipogenesis, stimulates skeletal muscle fatty acid oxidation and muscle glucose uptake, and modulates insulin secretion by the pancreas. Thus its importance in many critical cellular processes is well established. For cells it is critical that energy supply and demand are closely matched. AMPK is recognized as a critical integrator of this balance. It is known to be allosterically activated by an increased AMP:ATP ratio. Activation of the kinase switches on catabolic pathways while switching off anabolic ones. It also acts as a redox sensor in endothelial cells where oxidative stress can disturb NO signaling. Abnormal NO signaling leads to disturbed vasodilatory responses. By inhibiting the formation of reactive oxygen species in the endothelium, AMPK can optimize the redox balance in the vasculature. Here, we review the role of AMPK in the cell.

2. SENSORS IN BIOLOGY

Sensors in an engineering sense are devices or components that are designed to measure a physical quantity (such as temperature or pressure) and convert that measurement into a signal that can be detected or observed by an observer or an instrument. In biology, sensors (biological sensors) operate in a manner analogous to the one described for engineered devices and different in that the input and output are biological signals. Most biological sensors are specialized cells of various types and some are intracellular molecules. Some of the many specialized cells include (a) Light: Retinal neurothelium, (b) Sound: Organ of Corti hair cells, (c) Gravity: Inner ear semi-circular canals and (d) Stretch: Carotid sinus, atrial stretch receptors, proprioceptive receptors. In addition to these larger macroscopic systems, sub-cellular sensors also operate to provide cellular systems a way of detecting changes in cellular dynamics and homeostasis and providing set points toward which dynamic corrections can be made by the cellular machinery. Some of the better studied cellular sensors include (a) pH: Regulation of a variety of protein function by changes in cell pH. Examples

include alterations to substrate binding of glycinamide ribonucleotide (1), modulated protein-protein interaction of neonatal Fc receptor (2) and aggregation of P0rP octapeptide repeats (3). (b) Hypoxia: For example, the neuroepithelial bodies (NEB) found in the lungs of all vertebrates that are sensitive to changes in oxygen tension and respond by releasing bioactive substances (such as serotonin and CGRP) (4). And (c) Cell volume sensors: There are a large number of molecules that respond to an increase or decrease in cell volume and include PIP5K/PIP2, PLA2/Arachidonic acid, PI3K/PIP3, several tyrosine kinases, several ser/thr kinases and Rho GTPases (5). One of these sensor systems is the AMP-activated kinase AMPK, which is the putative metabolic or energy sensor of the cell. In recent years, another role for AMPK appears to be emerging. Endothelial cell physiology and pathology is intimately linked to the causation of many human diseases and homeostasis in these cells appears to be a central element in these disorders. Endothelial dysfunction is characterized by decreased bioavailability of endothelium-derived NO and this is paradoxically accompanied by increased expression of eNOS, together with increased ROS generation leading to the appearance and toxic effects of damaging free radical species such as peroxynitrite (6, 7). Intriguingly, it is now emerging from work done by many investigators (including our own group), that there is an intricate balance between AMPK and the cellular signaling systems that control endothelial redox balance (7). Therefore, this review will focus on AMPK's emerging role as an ROS modulator or ROS sensor in the cell, with a particular emphasis on the endothelium.

3. DISCOVERY

In relative terms, the discovery of AMPK is not recent. Its existence was uncovered by two independent observations reported in 1973 with the discovery that the same kinase inactivates 3-hydroxy-3-methylglutaryl coenzyme A (CoA) reductase and acetyl-CoA carboxylase (ACC) in hepatic fat metabolism (8, 9). Several years after these seminal reports, Munday *et al*, in the process of studying the V_{max} of ACC, proposed the name AMP-activated protein kinase as the primary enzyme responsible for attenuating this parameter (10). Finally, with Carling *et al* reporting that the HMG-CoA reductase (HMGR) and ACC kinases were one and the same enzyme, the name was formally adopted in 1989 (11, 12). Subsequently, AMPK was purified and its subunit structure was analyzed by Grahame Hardie's group at the University of Dundee (13) with detailed analysis of its all important catalytic subunit published by Bruce Kemp's laboratory at the St. Vincent's Medical Research Institute in Victoria, Australia (14). Based on these ground breaking studies and work by various other investigators, it has been revealed that AMPK is a heterotrimer with alpha, beta and gamma. The alpha subunit has catalytic activity while the other two have a regulatory role. Overall, multiple AMPK subunit isoform combinations have been identified and these subunits are encoded by distinct genes. Thus far, two alpha subunits, two beta subunits and three gamma subunits have been identified (15-17).

4. PHYSIOLOGICAL ASPECTS OF AMPK SIGNALLING

4.1. AMPK subunits

The standardized nomenclature of AMPK subunit genes utilizes a prefix PRKA followed by the subunit identifier A1, A2, B1, B2, G1, G2 and G3 (e.g., PRKAG3) (12). The gene loci for the subunits are located on 5 different chromosomes: alpha1 (5p12), alpha2 (1q31), beta1 (12q24.1), beta2 (1q21.1), gamma1 (12q12-14), gamma2 (7q35-36) and gamma3 (2q35). With this plethora of subunits it is not surprising that gene expression and variant splicing can give rise to twelve possible heterotrimeric combinations of AMPK (18) (Figure 1).

Both α subunits are similar in that they both have about 550 residues (Figure 1) and both have conserved NH₂-terminal catalytic domains. The beta subunits differ in the first 65 residues but in all other respects are highly conserved. The gamma subunits on the other hand (and in contrast to the other two), differ in length (gamma1 being the shortest at 331, gamma3 intermediate at 489 and gamma2 the longest at 569) (12). However, all three share a COOH-terminal having about 300 residues. Significant differences exist in AMPK subunit structure and genetic sequence between mammals and yeast, for example (multiple alpha and gamma subunits in mammals and 2 rather than three beta subunits in contrast to yeast).

Evidence suggests that variance of the alpha subunits determines subcellular localization of the molecule with the alpha1 isoform being largely cytosolic as well as being associated with the plasma membrane in carotid body type 1 cells and airway epithelial cells (19, 20). In contrast alpha2 appears to be concentrated in the nuclei of several cell types such as pancreatic beta cells, neurons and skeletal muscle (21-23).

The beta subunits feature the glycogen binding domain (GBD) which occupies a position on central conserved region of the subunit. The crystal structure of the GBD was reported in 2005 (24, 25). Another conserved region on this subunit is in the C-terminal region and there is compelling evidence that the C-terminal domain is all that is needed for a functional alpha/beta/gamma unit that can be regulated by AMP (26).

The three gamma subunits have variable N-terminal regions followed by four tandem repeats of a 60-aa sequence named as a CBS (cystathionine beta-synthase) motif by Bateman *et al* (27). It has since been discovered that these are actually two domains on the subunit (Figure 1; Bateman 1 and 2 domains), each with the capacity to bind AMP with a 1:1 stoichiometry (28). The critical nature of these domains was revealed when investigators reported attenuated AMP binding and activation when mutations were induced in these regions (28). The Bateman domains also bind ATP antagonistically, but with a lower affinity than that for AMP (28) and this is consistent with the fact that ATP antagonizes activation of AMPK by AMP (29). Interestingly, the two Bateman domains also act cooperatively in that the second site remains inaccessible to

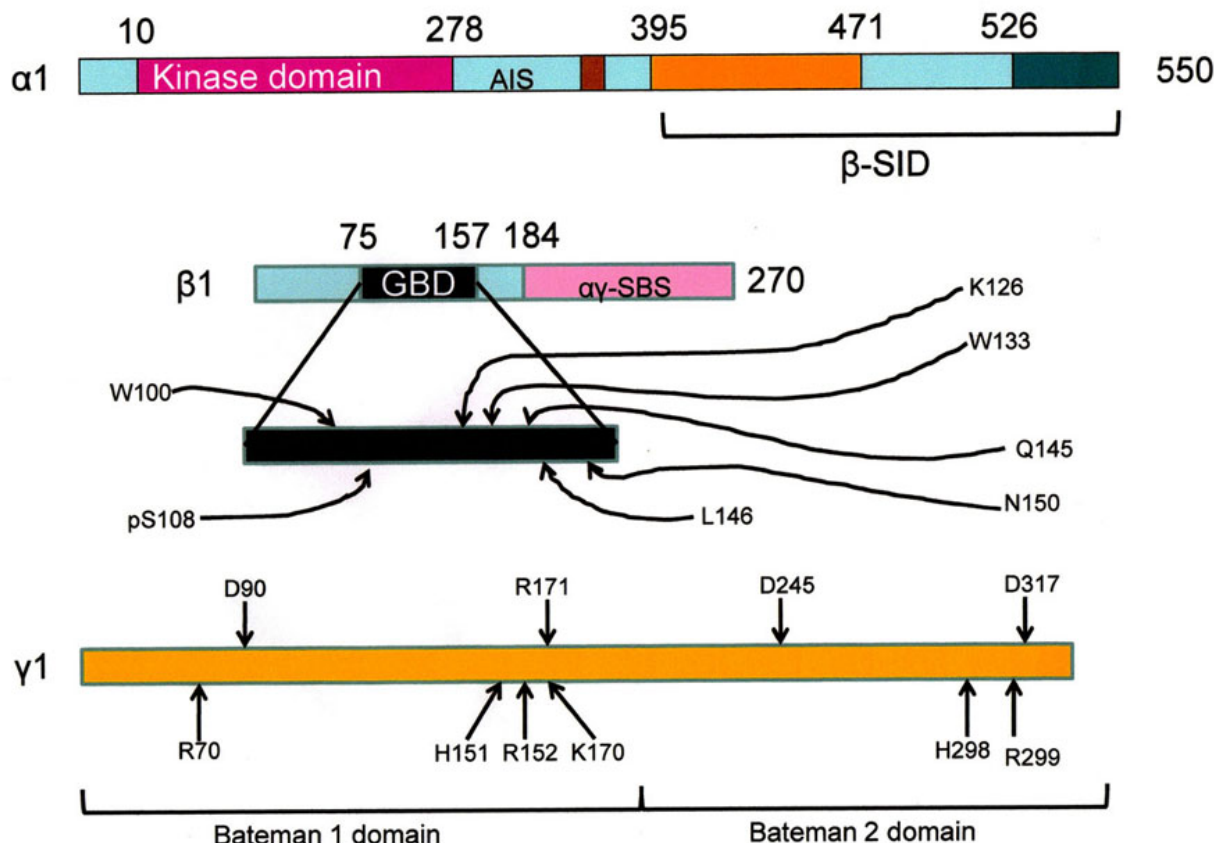


Figure 1. Features of the AMPK subunits. Mammalian AMPK. Colored regions are ones whose structure is known. Numbers associated with α and β subunits are N- and C- terminal residues from the crystal structure. AIS: Autoinhibitory sequence. β -SID: β -subunit interacting domain. GBD: Glycogen binding domain. $\alpha\gamma$ -SBS: α and γ subunit interacting sequence. In the expanded glycogen binding domain schematic of the β subunits, letters with numbers are sites of sugar binding (pS108 is the site at Serine 108 where phosphorylation occurs). In the γ subunit, D90, R171, D245 and D317 are residues that form H-bonds with 2' 3'-ribose hydroxyl groups, while R70, H151, R152, K170, H298 and R299 represent basic residues that occupy the solvent accessible core of the subunit which makes contact with the nucleotide phosphates. Modified with permission from Steinberg and Kemp *Physiol Rev* 89:1025-1078.

AMP until the nucleotide has bound to the first (28). This synergy between the two domains is a potential mechanism by which AMPK activation can respond to even small changes in cellular AMP levels (18).

4.2. AMPK activation

Mammalian AMPK is sensitive to the AMP:ATP ratio. It is therefore activated as a consequence of any cellular process, normal or anomalous, that either decreases ATP levels, or increase AMP concentrations (Figure 2). For example, mechanisms such as hypoxia, glucose deprivation or metabolic inhibition of ATP synthesis, will all activate AMPK (30). If ATP production remains unaltered but consumption is increased, the same result will ensue. Examples of increased ATP synthesis include activation of motor proteins, activity of ion channels/pumps and utilization by biosynthetic pathways. In addition, less well understood yet empirically established modulators of AMPK activity have appeared in literature and the list is ever growing. Such modulators include cytokines

{leptin, adiponectin, ghrelin, cannabinoids, IL-6 (31), ciliary neurotrophic factor [CNTF; (32)]}, certain drugs [metformin (33), thiazolidinediones (34)] and some plant derived compounds [berberine (35), resveratrol (36)], to name a few (Figure 3).

Activation of AMPK complexes that contain the $\alpha 1$ subunit isoform are reported to be localized in the cytosol. In contrast, AMPK $\alpha 2$ activation results in translocation to the nucleus and one assumes that this occurs to facilitate modulation of gene expression (7, 37). The beta subunit also appears to be involved in determining the localization fate of the molecule in that myristoylation of the beta subunit targets the complex to the Golgi while phosphorylation on various residues promotes nuclear translocation (38).

Once AMPK is activated (Figure 3-4), it switches on (the concept of the "metabolic master switch"; most likely first used in the context of AMPK by Prof.

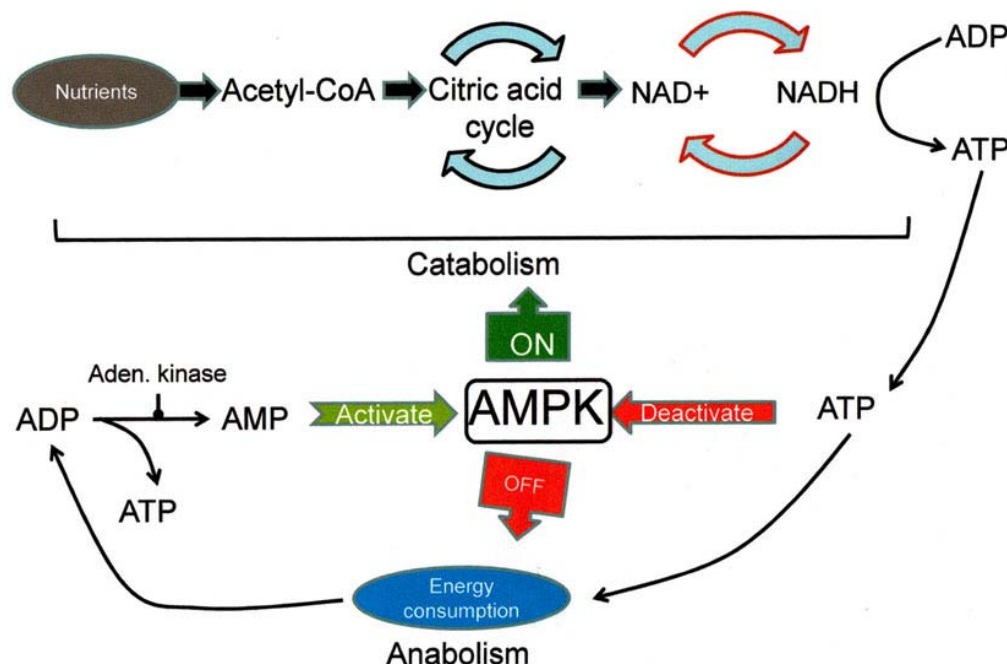


Figure 2. Activation of AMPK by AMP:ATP ratio and influence of activated AMPK on anabolic and catabolic cellular processes.

Hardie of the University of Dundee) catabolic pathways that can generate ATP (e.g., cellular uptake and utilization of glucose; Figure . 2) while at the same time, terminates processes that consume ATP (e.g., cellular synthesis pathways; Figure . 2). The rapid “switching” required to closely and quickly regulate and balance cellular energy resources is achieved by brisk phosphorylation of metabolic enzymes as well as that of various transcription factors and co-activators which control gene expression (30, 39). The activation sequence of AMPK possesses fascinating details. It is now known that the nucleotide AMP, allosterically binds to and activates the γ subunit of AMPK which triggers phosphorylation of the α subunit at Thr172 (Figure 4).

4.3. Upstream AMPK kinases

Up until the early years of the 2000s decade, it had been clear that a critical phosphorylation event took place on the α subunit (on Thr172) in the process of AMPK activation (Figure 4). However, the identities of upstream phosphotransferases that were responsible for this had remained elusive. In 2003, breakthrough discoveries in the yeast system (*Schizosaccharomyces pombe*) identified Sak1 (Snf1-activating kinase-1), Elm-1 (elongated morphology-1) and Tos3 (Target of Sbf3) as kinases upstream of the Snf1 complex (homolog of the serine/threonine protein kinase found in *S cerevisiae*) (30, 40, 41). Although unequivocal human orthologs of these three kinases have not been found in the human genome, the two protein kinases closest in sequence to these are LKB1 (a serine/threonine tumor suppressor kinase) and the Ca^{2+} /calmodulin-dependent protein kinase kinase beta (CaMKKbeta) (Fisslthaler and Fleming 2009). Evidence now demonstrates phosphorylation of the AMPK α

subunit can either be dependent on, or independent of, its LKB1 activity. Specifically, LKB1 appears to be critically involved in the activation of AMPK α 2 but not AKPK α 1 (7, 41). AICAR (5-Aminoimidazole-4-carboxamide ribonucleoside; an important activator of AMPK) is an adenosine analog taken up by muscle and phosphorylated to form 5-aminoimidazole-4-carboxamide-1-D-ribofuranosyl-5'-monophosphate (ZMP), which stimulates AMPK activity and glucose transport in skeletal muscle. LKB1 is essential for AICAR induced activation of AMPK. Empirically, it has been reported that deletion of LKB1 will prevent activation of AMPK α 2 in cardiac and skeletal muscle cells (42).

Unlike LKB1, CaMKKbeta is regulated within the cell and its levels increase in response to elevations in intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$). Therefore, stimuli that amplify ($[\text{Ca}^{2+}]_i$) (such as bradykinin and thrombin), also activate AMPK α 1 consequent upon increased CaMKKbeta activity (43, 44).

4.4. Downstream targets of AMPK

Once activated, AMPK can influence several downstream targets in the cell. Many of these targets are currently recognized [~20; (18)], however more are being discovered and it has been speculated that this number may eventually rise into the hundreds. These downstream effectors of AMPK influence diverse cellular processes and include lipid metabolism [e.g., acetyl-CoA carboxylase (ACC); HMG-CoA reductase], carbohydrate metabolism (e.g., glycogen synthase; 6-phosphofructo-2-kinase); cell signaling [e.g., endothelial NO synthase (eNOS); insulin receptor substrate-1 (IRS-1)], ion transport [cystic fibrosis transmembrane conductance regulator (CFTR)] and

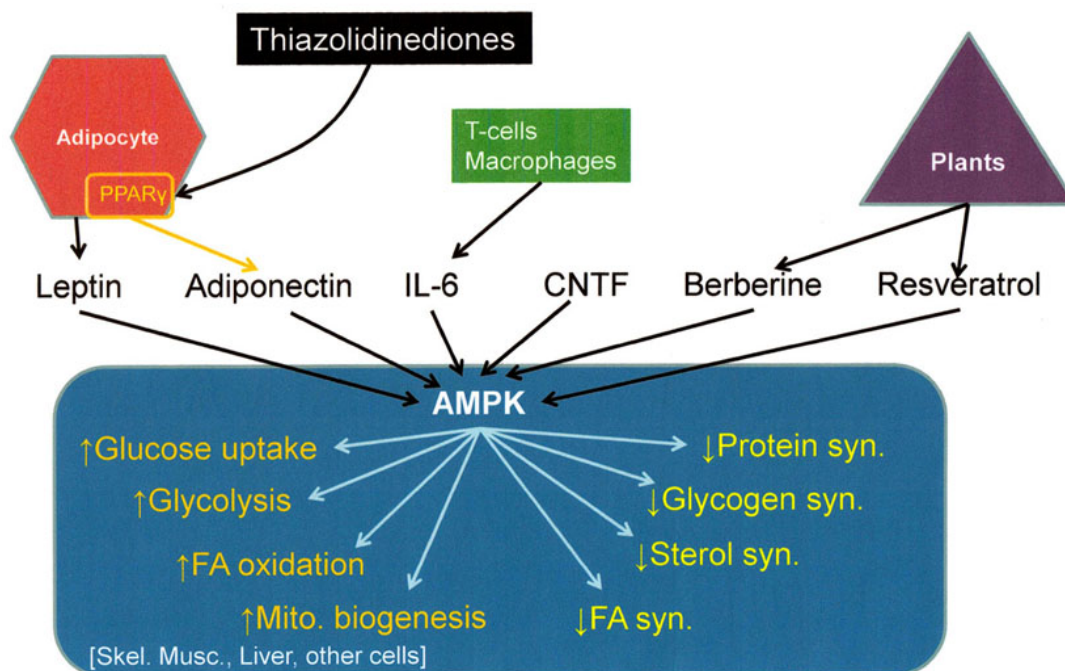


Figure 3. Cellular effects of AMPK activation. A variety of triggers can activate AMPK that include adipocyte derived hormones like adiponectin, cytokines like IL-6, CNTF (ciliary neurotrophic factor) and plant derived modulators like resveratrol. Downstream effects are schematically outlined in typical target organs. Modified with permission from Hardie DG *Nat Rev* 2007.

transcription [e.g., p300; hepatocyte nuclear factor-4 α (HNF4- α); transducer of regulated CREB activity 2 (TORC2)] (16, 19, 45-52). One of the most intriguing questions has been: How does AMPK recognize its downstream targets? AMPK has been found to phosphorylate a serine residue in these targets. Further, phosphorylation sites appear to have conserved motifs where hydrophobic residues are found 5 residues from the N terminal and 4 from the C terminal (P-5 and P+4). This motif is designated phi-[beta.X]-X-X-S/T-X-X-X-phi, where phi is hydrophobic and beta is basic. This motif has been confirmed using variant synthetic peptide substrates (53, 54).

5. METABOLIC EFFECTS OF AMPK ACTIVATION

The primary forms of carbohydrate storage in eukaryotes are glycogen and starch. AMPK plays a key role in the interconversion of glucose (the primary cellular energy substrate) and its storage forms, by affecting the transcription and translocation of the GLUT4 glucose transporter, glycogen synthesis, glycolysis and gluconeogenesis.

The uptake of glucose across the plasma membrane via GLUT4 is regarded as the rate limiting step in glucose uptake by cells. Translocation of the transporter to the membrane is therefore a critical element in glucose utilization. The fact that AMPK activation leads to GLUT4 translocation is generally irrefutable. The signaling sequence that is involved in this translocation secondary to AMPK activation is an intensely studied topic. AMPK is

now thought to directly phosphorylate AS160 [a Rab GTPase-activating protein (GAP)], which then binds to 14-3-3 proteins, an event that controls the recycling of GLUT4 vesicles (55). In addition, in skeletal muscle, the chronic elevation of AMPK by AICAR has been shown to increase GLUT4 (56). The process through which AMPK increases GLUT4 expression appears to involve the transcription factors MEF2A and 2D (myocyte enhancer factor) as well (57).

Glycogen (a short-term energy store with structural analogy to starch and amylopectin) is an important substrate for muscle tissue during exercise and for the liver during fasting (12). AMPK phosphorylates glycogen synthase (GS) at Ser7 which is a known inhibitory site for the enzyme. Young *et al* have also shown that AICAR-induced activation of AMPK in rat muscle cells also provoked heightened glycogen phosphorylase (GP) activity (58). Overall AMPK will therefore promote breakdown of glycogen (thereby elevating blood glucose) and suppress glycogen formation. Consistent with this idea is the fact that AMPK signaling is attenuated and muscle glycogen levels are decreased in AMPK α 2 null mice (59).

Bergeron *et al* were the first to report that AMPK could inhibit hepatic glucose production in rodents (60). More convincing evidence to this effect was forthcoming from studies in AMPK α 2 knockout mice which display hyperglycemia, glucose intolerance and increased hepatic glucose production (61). In addition, isolated hepatocytes have also shown AMPK suppression of reduced

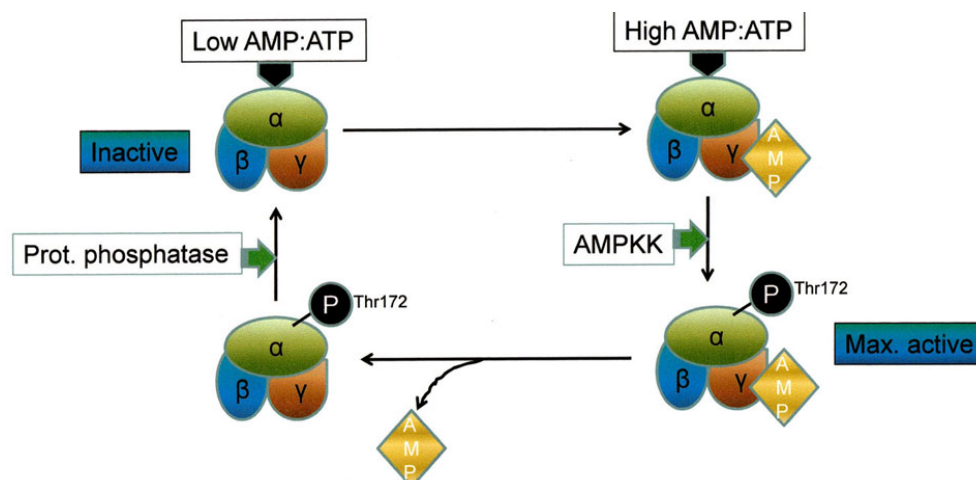


Figure 4. Activation-deactivation cycle of AMPK. Activation of AMPK is initiated by a high AMP:ATP ratio and is triggered by AMP binding to the γ subunit and phosphorylation of Thr172 of the α subunit. This is catalyzed by AMPK kinase. The reverse (deactivation) occurs when protein phosphatase dephosphorylates the α subunit, returning AMPK to a basal, inactive state.

gluconeogenic gene expression when treated with metformin and adiponectin (both known activators of AMPK) (33, 62). Activation of AMPK appears to negatively regulate the transcription of gluconeogenic enzymes like L-type pyruvate kinase (L-PK), phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-P) (63-65). CREB (cAMP response element binding)-regulated transcription coactivator 2 (CRTC2) is a critical regulator of gluconeogenesis (52). It has been reported that a mutation in CRTC2 (at Ser171Ala) attenuates SIK1 (salt-induced kinase 1) and AICAR mediated suppression of G-6-P, PEPCK and PGC-1 α (Peroxisome proliferator-activated receptor gamma coactivator-1- α ; an important regulator of mitochondrial biogenesis) (12) in primary hepatocytes. These findings suggest that CRTC2 is likely a downstream target of AMPK (as well as SIK1). In fact, it has also been found that hyperglycemia in LKB1 knockout mice is much more marked than that observed in mice with hepatic deletion of both AMPK α 1 and α 2 (61, 66).

When a ready supply of nutrients is not available (as might occur during fasting), lipids become the primary substrate for energy needs (67). AMPK plays a critical role in determining the fate of fatty acids through the regulation of specific substrates. Acetyl-CoA Carboxylase (ACC) is a biotin-dependent enzyme that catalyzes the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA through its two catalytic enzymes, biotin carboxylase (BC) and carboxyltransferase (CT). The most important function of ACC is to provide the malonyl-CoA substrate for the biosynthesis of fatty acids (68). It was reported several years ago that AMPK phosphorylated ACC at three sites: Ser79, Ser1200 and Ser1215 (10, 69). Subsequently, using site directed mutagenesis it was confirmed that Ser79 is the physiologically relevant phosphorylation site involved in inhibition of ACC by AMPK (70). Indeed, experiments with AICAR have also confirmed the importance of Ser79 in this context (29).

Fatty acid synthase (FAS) is the enzyme responsible for de novo synthesis of fatty acids (71). It is thought to catalyze the formation of long-chain fatty acids from acetyl-CoA, malonyl-CoA and NADPH. Foretz *et al* have demonstrated that AMPK inhibits the glucose-stimulated transcription of FAS (72). FAS expression is more directly controlled by the transcription factor SREBP1c (sterol regulatory element binding protein 1c). Although it has been observed that when the constitutively active, truncated form of AMPK, CA-AMPK is overexpressed in the liver, SREBP1c expression is down-regulated (73), the exact mechanism behind this phenomenon is not understood. Studies using ^{32}P labeling have also shown that both AICAR and metformin increase the incorporation of the label into FAS, further strengthening the idea that AMPK can modulate the expression of this important enzyme in the context of lipid metabolism (74).

Glycerol-3-Phosphate Acyl-Transferase (GPAT) catalyzes the initial and rate-limiting step of glycerolipid synthesis. Two distinct GPAT isoenzymes had been identified in mammalian tissues, an *N*-ethylmaleimide (NEM)-sensitive isoform in the endoplasmic reticulum membrane (microsomal GPAT), and an NEM-resistant form in the outer mitochondrial membrane (mtGPAT) (75). The activation of AMPK by AICAR has been reported to reduce GPAT activity in the liver (76). In addition, AMPK activation in the liver and adipose tissue in endurance athletes has also been shown to reduce the activity of mtGPAT (77). It is also intriguing to note that AMPK activation in the liver in the fasting state inhibits both GPAT and DGAT (an enzyme that catalyzes the formation of triglycerides from diacylglycerol and Acyl-CoA), and these effects are reversed by breaking the fast which effectively terminates AMPK signaling (78, 79). Therefore, the overall evidence suggests that AMPK regulation of these enzymes is likely an additional control point in this important lipid metabolism pathway.

Hormone sensitive lipase (HSL) functions to hydrolyze the first fatty acid from a triacylglycerol molecule, freeing a fatty acid and diglyceride (a process termed lipolysis). It is also known as triglyceride lipase, while the enzyme that cleaves the second fatty acid in the triglyceride is known as diglyceride lipase, and the third enzyme that cleaves the final fatty acid is called monoglyceride lipase. In the context of a role of AMPK in lipolysis, it is interesting to realize that experiments using 3T3-L1 adipocytes expressing dominant negative as well as CA-AMPK have demonstrated a role for AMPK inhibition of this process. Indeed, data from adipocytes derived from AMPK α 1 knockout mice have also supported this idea (80). Furthermore, AMPK also appears to down regulate HSL activity during muscle contraction. When skeletal muscle is in the relaxed state, AICAR-induced AMPK activation also suppresses triglyceride hydrolysis (76, 81-83). Thus, AMPK activation also appears to be a negative regulator of hydrolysis of fatty acid residues.

Against the background of literature that increasingly supports the role of AMPK as “lipid status” sensor as well as a metabolic switch, several facts are known. In the heart and the liver, AMPK activation may be influenced by fatty acid availability independent of cellular AMP levels (84, 85). Clinically, reduced fat oxidative capacity has been reported in type 2 diabetics (86). In rodents, a high fat diet has been reported to significantly decrease phospho-AMPK in the liver and skeletal muscle (87, 88). It has also been observed that AMPK activity is reduced in aortic endothelium and muscle in obese rats compared with lean controls (89, 90). Overall, these studies suggest that chronic exposure to fatty acids can act to inhibit AMPK but the underlying mechanism has not been fully explained. Our group has previously examined a possible mechanistic framework previously. For example, we have evaluated the effect of the saturated fatty acid palmitate (which contributes 30-40% to the circulating plasma free fatty acid (FFA) levels) on AMPK both *in vitro* and *in vivo*. In summary, we found that in bovine aortic endothelial cells (BAEC), high concentrations of palmitate significantly suppressed AMPK and ACC phosphorylation and that the inhibition of AMPK by this fatty acid was independent of the upstream AMPK kinase, LKB1. Further, we found that PP2A (an important serine/threonine kinase that is critically involved in cell signaling systems such as those involving RAF, MEK and AKT) mediated the inhibition of AMPK by palmitate. Since ceramide is known to be a potent activator of PP2A, we tested to see if this sphingolipid was also involved in mediating palmitate-induced inhibition of AMPK. In fact we found that palmitate appeared to inhibit AMPK by increasing the synthesis of ceramide. Finally, we also discovered that mice fed with a palmitate-based high fat diet were not only prone to obese in comparison with controls, but also developed insulin at 3 months of age (91). Our laboratory was also able to demonstrate another intriguing and rather more indirect relation between AMPK activity and fatty acid metabolism. In trying to uncover mechanism at work behind the phenomenon of weight loss in chronic smokers and subsequent weight gain in these individuals if they quit smoking, we observed that nicotine (a principal addictive

constituent of tobacco smoke), could increase the phosphorylation of AMPK, ACC and LKB1 in adipocytes and that the activation of AMPK was dependent on oxidative stress provoked by nicotine. Interestingly, we also found that nicotine inhibited fatty acid lipase (FAS) activity in this cell type. In fact, AMPK activation (or constitutive activation) inhibited FAS activity in adipocytes and this inhibition was affected by phosphorylation of the enzyme. We concluded that the anti-lipogenic effects of nicotine are manifest, in part, from an induction of AMPK phosphorylation (at Thr172) and LKB1 (at Ser428) (74).

6. AMPK AND MITOCHONDRIAL BIOGENESIS

Mitochondrial biogenesis is the process by which new mitochondria are formed in the cell. This complex process is activated by numerous signaling elements, typically triggered in cellular stress or in response to environmental stimuli. It is thought that higher mitochondrial copy number (or higher mitochondrial mass) is protective for the cell. Thus, it has been reported that mitochondrial density is critically reduced in the face of insulin resistance and lipid accumulation in muscle tissue (92). Historically, Winder *et al* were the first to report a connection between AMPK and mitochondrial biogenesis when they reported that chronic AICAR treatment stimulated this process in skeletal muscle (93). Several different transcription factors and coactivators appear to be involved in controlling mitochondrial biogenesis. NRF-1 and -2 (nuclear respiratory factor 1 and 2) transcriptionally control the genes that encode for all the five electron chain complexes in the mitochondrion (94). Therefore it is interesting to note that NRF expression is increased by chronic AMPK activation, such as that occurs with beta-GPA (beta-guanidopropionic, a creatine analogue) (60). Another important mitochondrial biogenesis regulator is the inducible coactivator of nuclear receptors, PGC1 α (PPAR-gamma-coactivator 1 α). This coactivator is increased in response to activation by AMPK and is reduced in AMPK α 2 null mice as well as AMPK α 2 dominant negative animals (77, 95, 96). More recently, it has also been reported that PGC1 α coimmunoprecipitates with AMPK α 2 and that AMPK can directly phosphorylate PGC1 α on Thr177. Kukidome *et al* and others have also shown that AICAR increases mRNA expression of NRF-1 and mitochondrial DNA transcription factor A (mtTFA) and stimulates mitochondrial proliferation (97) (Figure 5). Thus AMPK also lives up to its reputation as a “master switch” in that it can potentially control organellar biogenesis in response to cell stress or high metabolic demand.

7. ROLE OF AMPK IN CELLULAR SYNTHETIC FUNCTION

HMG-CoA reductase (or 3-hydroxy-3-methyl-glutaryl-CoA reductase or HMGR) is the rate-controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids. The catalytic activity of HMGR is inhibited by phosphorylation on Ser872 by AMPK (12, 98, 99). Further, HMGR is also inhibitable by ATP depletion. In transfected

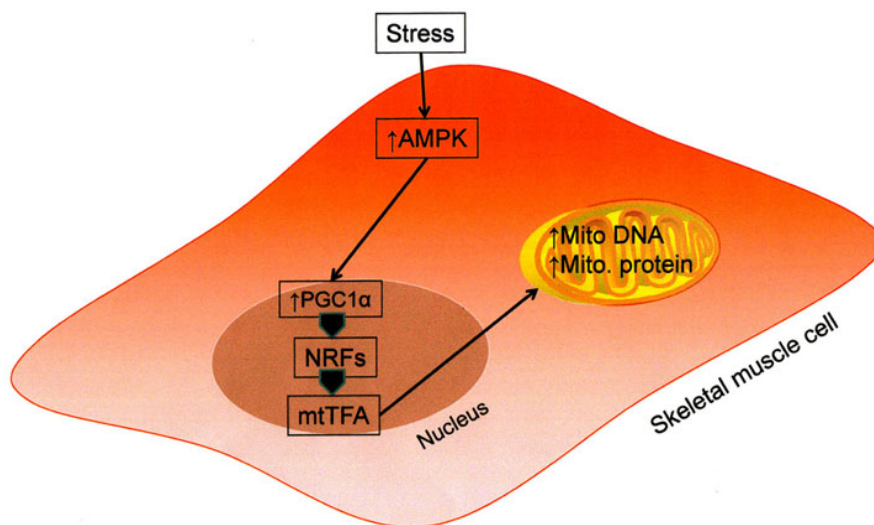


Figure 5. AMPK activation signaling that leads to mitochondrial biogenesis. Activated AMPK signals via Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1 α), nuclear respiratory factors (NRFs) and mitochondrial transcription factors (mtTFA) to express genes encoding for mitochondrial DNA and proteins in stressed conditions. This leads to increase in mitochondrial density.

cells, mutation in HMGR Ser871Ala creates insensitivity to AMPK inhibition(12). Finally, AMPK can also be activated by adiponectin and this can reduce cholesterol synthesis and consequent atherosclerotic lesions in ApoE-deficient mice (100, 101). These data compellingly suggest that HMGR regulation by AMPK is critically involved in cholesterol metabolism.

Given that protein synthesis by the cellular machinery is such an energy intensive process, it is not surprising that AMPK is intrinsically involved in this area of cell function as well. It effectively inhibits protein synthesis at multiple points (Figure 6). First, it phosphorylates and activates the eukaryote elongation factor 2 kinase (eEF-2-kinase). This phosphorylation causes phosphorylation of eEF2 (eukaryotic elongation factor 2) which in turn leads to inhibition of protein synthesis (102). Second, AMPK also inhibits the mammalian target of rapamycin complex (mTORC). While insulin and IGF-1 can promote protein synthesis by activating mTORC, AMPK counteracts this effect in various tissues. It appears to do this by: (i) Phosphorylation of TSC2 (tuberous sclerosis gene 2) at Ser1387. This amplifies the activity of Rheb (Ras homolog enriched in brain) and in turn leads to inhibition of mTORC1 signaling, and (ii) AMPK also inhibits mTORC by directly phosphorylating Raptor (a binding partner on mTORC) which causes Raptor binding to 14-3-3 proteins and ultimately, to inhibition of mTORC as well (103-105).

8. ROLE OF AMPK IN CELL GROWTH AND CELL DEATH

AMPK also has a far reaching impact on cell growth and proliferation and this manifests via several known mechanisms. Overall, the molecule tends to inhibit cell growth. AMPK induces G1/S phase cell cycle

arrest (Steinberg 2009). Several investigators have shown that the tumor suppressor p53 and cyclin-dependent kinase inhibitors, p21 and p27, are involved in this process. The primary event is the phosphorylation of p53 at Ser15 by AMPK which occurs upstream of p21 and p27 (106-108). Furthermore, AMPK appears to be involved in reducing the cytoplasmic-to-nuclear ratio of the RNA-binding protein HuR (Hu family of RNA-binding proteins; from the rare neurological disorder, anti-Hu syndrome discovered in a patient of that name) which tends to reduce the mRNA stability of critical cell cycle regulators like Cyclin A and Cyclin B1 (109). Thus, in a complex and interactive manner, AMPK can also function to negatively regulate cell cycle progression with the final consequence of inhibiting cell growth and proliferation (Figure 7).

9. ROLE OF AMPK IN MEMBRANE EXCITABILITY AND ELECTROCHEMICAL GRADIENTS

All cells exist and function in an exquisitely regulated ionic environment where the extracellular milieu and the cellular interior are distinct in their ionic makeup. This difference has to be maintained if normal signaling between the extracellular space and the subcellular machinery can occur and, indeed for normal metabolic processes to proceed nominally. This is largely achieved through a system of ionic channels and pumps that are distributed on the plasma membrane as well as organellar surfaces inside the cell. These channels and pumps have specific properties, electrodynamic properties and kinetics. AMPK plays an important role in influencing the function of these cellular components as well. For example, AMPK has been shown to inhibit the function of the CFTR Cl⁻ channel (cystic fibrosis transmembrane conductance regulator) as well as that of the endothelial Na⁺ channel, ENaC (110, 111). In the kidney, when

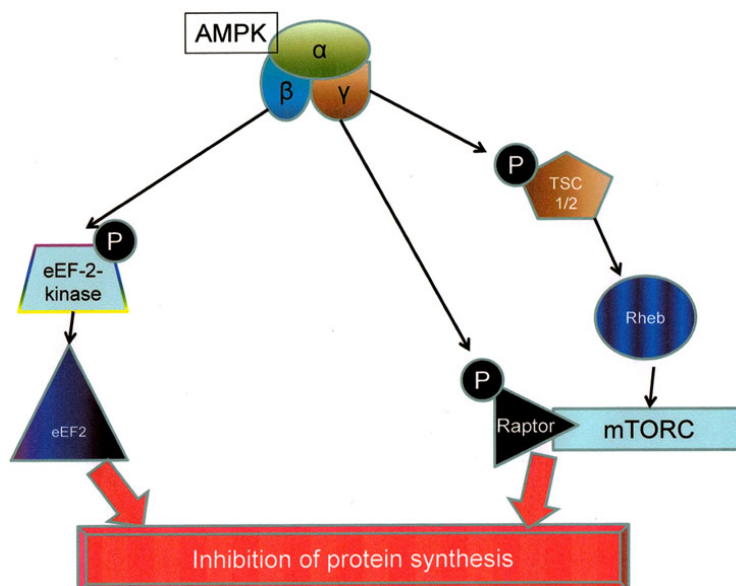


Figure 6. Inhibition of protein synthesis by AMPK. eEF2 kinase: Eukaryotic elongation factor 2 kinase. TSC1/2: Tuberous sclerosis complex 1-2. Rheb: Ras homolog enriched in brain. mTORC: Mammalian target of rapamycin complex.

osmotic stress is increased, AMPK is activated in the distal nephron. This activation increases the activity of NKCC2 ($\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter 2) thereby tending to return the osmotic environment to nominal levels (112). More recently, it has also been reported that AMPK inhibits the activity of the KCa3.1 K^+ channel (a Ca^{2+} activated K^+ channel expressed at the basal lateral membrane of many epithelial cells) (113). The caveat here is that despite these disparate effects, contrary to expectation, no evidence has been uncovered that would suggest that AMPK is involved in regulating or controlling the single most energy intensive cellular function in regard to ionic homeostasis: maintaining the resting membrane potential. However, it is possible that a more indirect impact of the molecule may be revealed with subsequent study (114).

10. INTEGRATIVE PHYSIOLOGICAL IMPACT OF AMPK

At the supracellular or whole organism level, studying the impact of AMPK on human physiology is perhaps more relevant than simply elucidating its subcellular role. In this context, AMPK has diverse influences and is also influenced itself by a variety of factors.

A conundrum linked to lipid metabolism is that extracellular fatty acid availability somehow triggers cellular fatty acid metabolism (114). The trigger or switch that allows this to happen has not been clearly defined. Tantalizingly, reports suggest that AMPK may be sensitive to the lipid status of the cell in a variety of tissues (84, 115) and this may be independent of the AMP-induced activation of AMPK. It further appears that fatty acid activation of AMPK is only possible when AMPK α 1-312 (a gamma binding site on the alpha subunit) is expressed, suggesting that activation by fatty acids requires

interaction with the gamma subunits of the AMPK complex (32). Further evidence for a role of AMPK as a sensor of fatty acids *in vivo* comes from experiments on knockout mice that lack steroyl-CoA desaturase 1 (SCD-1; which catalyzes the biosynthesis of monounsaturated fatty acids) or those that are null for the fatty acid binding protein, AP2/MAL, demonstrating that these gene deletions provoke increased levels of AMPK in the liver and skeletal muscle which affords protection to these animals from diet-induced obesity (116-118).

By phosphorylation and activation of elongation factor 2 kinase (eEF2K) and inhibition of the mTOR signaling pathway (above), AMPK can regulate protein translation. Since both eEF2K function as well as the integrity of mTOR signaling is dependent on amino acid availability, it is reasonable to suspect that AMPK might also be regulated by amino acids. In fact, it is known that amino acids can inhibit AMPK phosphorylation and this appears to be independent of mTOR (treatment with rapamycin does not alter this inhibition) (12). Recent studies compellingly demonstrate that while amino acid inhibition of AMPK does occur in the cell, it is likely an indirect phenomenon because amino acids are known to stimulate mitochondrial glutamate dehydrogenase which leads to enhanced tricarboxylic acid cycle flux and attenuation of cellular AMP levels (119).

Appetite is controlled through a complex and multi-loop feedback system that integrates in the arcuate nucleus of the mediobasal hypothalamus. Inputs into the nucleus come from circulating nutrients (such as glucose and fatty acids) and hormonal signals (from the pancreas, adipose tissue and the gastrointestinal tract). Many studies have established that AMPK is a critical link in integrating these signals. There are several lines of evidence to support this idea. AMPK subunits co-localize with neuropeptide-Y

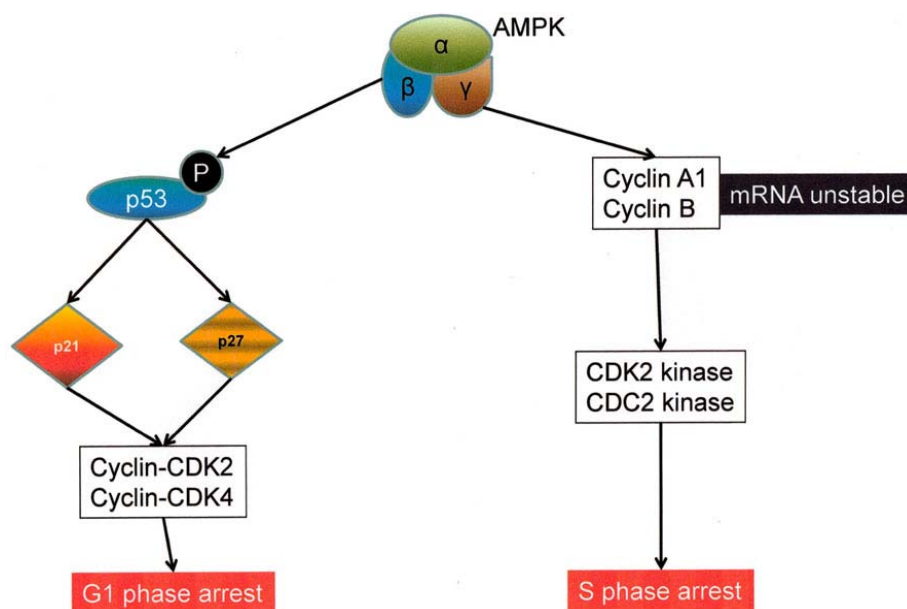


Figure 7. Putative mechanism of cell cycle control by AMPK activation.

(NPY; an important orexigenic neurotransmitter)-expressing neurons. Hypothalamic AMPK is activated by fasting and inhibited by refeeding (12, 120). If AICAR is injected into the cerebral ventricles, AMPK levels rise in the hypothalamus as does the desire to feed (121). Other anorexigenic signals (such as insulin and glucose, etc.) also suppress AMPK activity in the brain.

A very intense area of medical research has been the effort to understand the role of exercise in health and disease. It has been known for a while that lack of exercise (and obesity, perhaps as an independent variable), is a risk factor for several human pathologies ranging from heart disease and stroke to diabetes and malignancy. However, the exact mechanism that might explain these very strong associations are ill understood. One of the more remarkable discoveries in biomedical research was that AMPK was activated in exercise (most likely by skeletal muscle contraction) in humans as well as rodents (122, 123). Studies in LKB1 mice have now shown that the activation of AMPK α 2 is dependent on LKB1 phosphorylation (124, 125). Further, both AMPK α 1 and α 2 expression is upregulated in the heart in proportion to intensity of exercise (126).

During exercise, AMPK can regulate increased glucose uptake by skeletal muscle (12). In fact its possible role in enhancing glucose uptake via mechanisms that are independent of insulin signaling has attracted considerable interest, especially because this is likely an important mechanism *in situations* where muscle is insulin-resistant (as might occur in type II diabetes). It was therefore intriguing when reports surfaced that muscle contraction, glucose uptake and AMPK activity appeared to be positively associated. However, more detailed experiments such as ones using dominant negative AMPK mutant mice and AMPK α 2 knockouts, have revealed

that glucose uptake by contracted skeletal muscle was either normal or slightly decreased (59, 127-129), the latter during tetanic muscle contraction. Overall, these reports point to the possibility of more than one signaling system being responsible for dynamic changes linking exercise associated glucose uptake, and AMPK activity.

During exercise and/or muscle contraction, evidence suggests that AMPK activity is associated with deactivation of ACC2 and malonyl CoA decarboxylase (MCD)(122, 130, 131). However, specific links between fatty acid oxidation rate and AMPK activation have not clearly been established. For example, in LKB1-deficient mice, although ACC phosphorylation in both resting and contracted conditions is reduced, the level of malonyl-CoA remain unaltered and the influence of muscle contraction on this coenzyme is somewhat weak (132). Furthermore, studies also suggest that contraction-induced increases in fatty acid oxidation are independent of AMPK activity (133). Muscle blood flow is also an important compensatory mechanism wherein increased metabolic demands of active muscle groups is readily met by increases in local blood flow which serves to enhance muscle perfusion and performance. It has been observed that AMPK-induced stimulation of muscle uptake of glucose is dependent on nitric oxide (NO; a critical vasodilatory endothelium derived signaling molecule) (134). In addition, it has also been established that AMPK can phosphorylate eNOS (endothelium derived NO synthase) at Ser1177 in contracting human skeletal muscle (135). These finding suggest that NOS phosphorylation by AMPK in the endothelium serves to connect metabolic demand with muscle perfusion.

AMPK has many disparate influences on eukaryotic endocrine physiology which include effects on specific hormonal signaling molecules and target tissues:

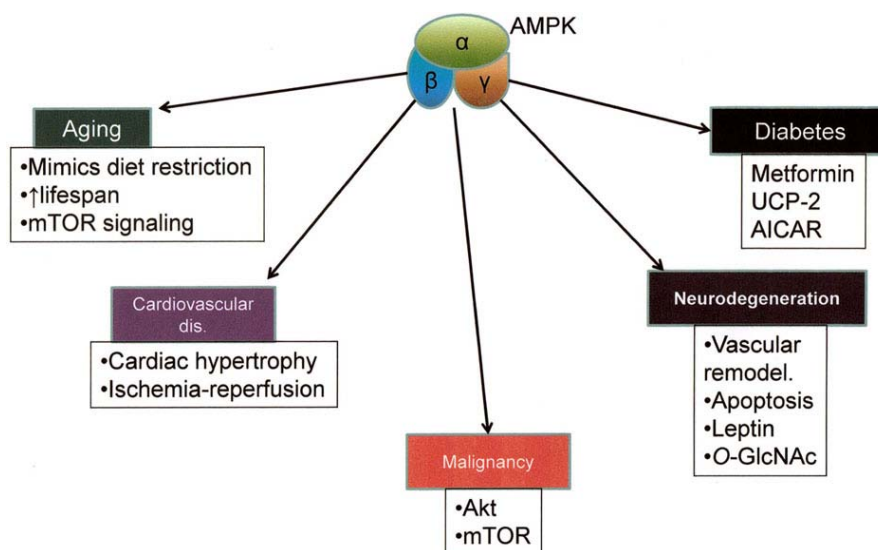


Figure 8. Role of AMPK in pathogenesis of various human disorders.

(1) Leptin (16 kDa protein hormone) plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. Leptin is one of the most important adipose derived hormones. It is known to increase AMPK activity in skeletal muscle (136). Furthermore, rodents who lack leptin or leptin-receptors, show decreased AMPK activity in the liver (137). Brabant *et al* have also demonstrated that in lean animals, this hormone causes raised AMPK activity (138). In addition, it has been observed to inhibit triacylglycerol storage and increase fatty acid oxidation in the heart in an AMPK-dependent and AMPK-independent manner (139, 140). In contrast, in the brain (hypothalamus), leptin has been shown to decrease AMPK activity thus inhibiting appetite and increasing peripheral fatty acid consumption (121, 136). (2) Adiponectin (a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism) activates and stimulates liver and muscle AMPK activity *in vivo* and *in vitro*, thus leading to increased glucose uptake and enhanced fatty acid oxidation (141). Adiponectin has also been shown to activate AMPK in primary rat adipocytes (101, 142). Shibata *et al* have reported that adiponectin can protect the heart from ischemic injury via AMPK-mediated mechanisms (143). In fact, impaired AMPK signaling in adiponectin deficient mice provokes a heart failure phenotype (144). (3) Resistin (is a cysteine-rich protein hormone/signaling molecule) secreted by adipose tissue in rodents. In other mammals (primates, pigs and dogs), resistin is secreted by immune and epithelial cells. It is known to induce insulin resistance and stimulate hepatic gluconeogenesis (141). This hormone has been shown to phosphorylate AMPK in the hypothalamus, an interesting fact that does not quite match the overall anorexigenic effect of resistin (141). Vazquez *et al* have further opined that ACC inactivation (secondary to AMPK activation) might represent a physiological compensatory mechanism that serves to block the deleterious effect of

high malonyl-CoA in the hypothalamus from resistin-induced FAS (apoptosis-mediating surface antigen) inhibition (145). (4) Ghrelin is a hormone produced mainly by P/D1 cells lining the fundus of the human stomach and ϵ cells of the pancreas that stimulates appetite. This hormone has also been shown to regulate AMPK activity in the hypothalamus as well as peripheral tissues (141). Kola *et al* have reported that ghrelin stimulates cardiac and hypothalamic AMPK activity on one hand, while inhibits it in adipose tissue and the liver on the other (146-148). (5) Perhaps the greatest impact relevant to human disease that is attributable to AMPK, is its association with insulin signaling. Centrally, insulin manifests anorexigenic effects by inhibiting AMPK activity in hypothalamus (120). AMPK activation and subsequent increase in appetite has been noted in streptozocin-induced diabetic rats (149). In peripheral tissues AMPK also has interesting associations with insulin signaling. In adipose tissue, for example, insulin will inhibit AMPK by activating the B/Akt protein kinase complex. This leads to AMPK α phosphorylation at Ser485 and Ser491 and reduced phosphorylation at Thr172 (150). Both insulin and AMPK can upregulate glucose uptake by skeletal muscle via enhance GLUT1 and GLUT4 translocation to the cell membrane as well as an increase in GLUT4 transcription (56, 151). AMPK is also known to upregulate IRS1 (insulin receptor substrate 1) via an inhibition of the insulin-mTOR pathway (152, 153). Thus, in several different ways, insulin and AMPK actions are interlocked to modulate hormonal control of cellular metabolism.

11. ROLE OF AMPK IN HUMAN DISEASES

Given how far reaching the effects of AMPK are on cell function, it is not surprising that this molecule plays a central role in a variety of human pathologies and with growing interest and continued research, an understanding and appreciation of this role is expanding at a high rate (Figure 8).

11.1. Obesity

It is probably appropriate to begin with a description of AMPK's role in obesity because its human health implications are so significant. Obesity is now strongly associated with diseases or disease syndromes ranging from cardiovascular diseases, diabetes mellitus type 2, obstructive sleep apnea, certain types of cancer, and osteoarthritis. Obesity is also one of the leading preventable causes of death worldwide (154). Winder *et al* were the first to report that AMPK activation could reduce adiposity (93). In their studies, these investigators also reported a decrease in hepatic and muscle triglyceride content. Buhl *et al* observed then when obese rats were treated with AICAR, intra-abdominal adipose tissue was reduced by 15% compared to pair-fed controls (155). In mice with part of the ACC2 (the dominant isoform observed in heart and skeletal muscle) gene deleted, adipose tissue was markedly reduced as was the lipid content of the liver (156). Furthermore mGPAT (mitochondrial Glycerol-3-phosphate acyltransferase; an enzyme that catalyzes the first committed step in glycerolipid synthesis and one which is inhibited by AMPK) deletion in mice gives rise to a lean body weight and reduced liver triglyceride levels (157). There is also a close link between the important anorexigenic hormone, leptin and AMPK signaling. For example, Kahn and colleagues first reported that leptin administration increased AMPK activity in skeletal muscle (136). Another important cell-derived hormone that has anorexigenic activity and is derived from adipocytes, is adiponectin. Studies a few years ago have shown that this hormone activates AMPK and attenuates ACC activity in muscle and liver, and g-adiponectin (adiponectin globular domain only) activates AMPK in adipose tissue (62, 101, 158).

11.2. Aging

The term "aging" is somewhat ambiguous. Distinctions may be made between "universal ageing" (age changes that all people share) and "probabilistic ageing" (age changes that may happen to some, but not all people as they grow older, such as the onset of type II diabetes). Thus, many investigators also make a distinction between chronological aging (age of an organism) and biological aging (changes in an organism, some of whom may be pathological). The role of AMPK in biological aging is also a hotly investigated area of research. Most fascinating is the finding in *C. elegans*, that AMPK overexpression can increase lifespan by as much as 15% (159). Dietary or caloric restriction (DR) has long been known to extend lifespan in a variety of species across the phylogenetic scale. Anisimov *et al* and Ingram *et al* and others have opined, that AMPK activation can increase rodent lifespans by mimicking DR (160-162). AMPK signals to influence protein synthesis via the mTOR signaling system (above). Thus, TOR mutant worms also have extended lifespans as do those bearing Raptor mutations (above) (163, 164). Furthermore, modulation of translation by a dominant negative form of TOR in *Drosophila*, also extends lifespan (165).

11.3. Malignant disease

Malignant pathologies are characterized by anaplasia, invasiveness and metastases. The National Cancer Institute (NIH; Bethesda MD) have published

several reviews that identify and delineate various mechanism-based targets for preventive interventions in malignant diseases. These include Akt, mTOR and epigenetic modulators (such as those affecting cell differentiation) (166). As discussed above, two of these (Akt and mTOR) are associated with AMPK activity (Akt activates mTOR signaling by inhibiting AMPK activity). Therefore, it is provocative to consider a role of AMPK in cancer biology as well.

In the simplest and most global sense, decreased AMPK activity is observed in those human metabolic disorders that are associated with high cancer risk and these include obesity, diabetes and the metabolic syndrome (166, 167). Intriguingly, even though it is not fully understood how AMPK activity contributes to these complex, multifactorial diseases/syndromes, pharmacological intervention that activates AMPK appear to have positive therapeutic impact (168). LKB1 is an important upstream activator of AMPK. Germ-line mutations in LKB1 are linked to Peutz-Jegher syndrome (hereditary intestinal polyposis syndrome; an autosomal dominant genetic disease characterized by the development of benign hamartomatous polyps in the gastrointestinal tract and hyperpigmented macules on the lips and oral mucosa). Further, about 50% of non-small cell malignant lung tumors also show inactivating LKB1 mutations (169). Loss of LKB1 expression has also been noted in recent reports of high grade *in situ* ductal breast cancer (170). Evidence also demonstrates that mice lacking PTEN (phosphatase and tensin homolog; a tumor suppressor) have reduced levels of LKB1 and high AMPK activity and are protected from tumorigenesis (166). Mice that are hypomorphic to LKB1, also resist tumor formation. Indeed, pharmacologic AMPK activation can significantly delay tumor formation in PTEN^{+/−} mice (171). p53 (a critical cell cycle regulator and tumor suppressor) expression has been observed to increase when pharmacologic AMPK activation is used in a variety of experimental settings as well as increased expression of cyclin-dependent kinase inhibitors, p21^{CIP} and p27^{KIP1} (108, 172, 173). Several enzymes that are regulated by AMPK and are closely involved in cellular metabolic control have also been linked to malignant pathogenesis. These include FAS, ACC and HMGCoAR (18, 30). AICAR (widely used to activate AMPK) has been shown to inhibit growth of established tumor cell lines *in vitro* and include colon, breast, prostate, gastric, and glioma (108, 174). This phenomenon has also been seen *in vivo* in rat gliomas, human breast cancer cells and colon xenografts (108, 174, 175). In addition, AICAR has also demonstrated the ability to arrest tumor cells in S phase, increase expression of p21^{CIP}, p27^{KIP1} and p53 along with inhibition of PI3K/Akt/mTOR signaling. All of these effects of AICAR have been variously confirmed as being dependent on AMPK activity (108, 174). Metformin (a biguanide activator of AMPK) has also shown some positive effects in tumor biology. For example, it has shown suppression of colon tumor xenografts in p53^{−/−} and inhibition of estrogen receptor alpha positive breast cancer cells.

Overall, it is likely that AMPK in the activated state has a beneficial impact on solid tumor biology.

However, more extensive investigation of the underlying mechanism is warranted as well as an extension of AMPK's role in other cancerous conditions such as hemopoietic malignant pathologies and lymphomas, etc..

11.4. Neurodegeneration

Neurodegenerative processes underpin a diverse array of neurological pathologies. These include those where inflammation and necrosis supervene (such as neurotrauma and stroke), to those where apoptotic programmed cell death are predominant (such as Parkinson's disease and Alzheimer's dementia).

It is well known that AMPK α 1 and α 2 are highly expressed in the brain (176). AMPK in astrocytes is involved in ketogenesis and in prevention of apoptosis (177). In Alzheimer's disease (AD), abnormal vascular remodeling has been reported and AMPK has been implicated as a contributing factor in this phenomenon (178). Interestingly, AICAR treatment has also been shown to protect neurons from injury by agents such as glucose deprivation, hypoxia, excitotoxicity and amyloid β peptide (the protein implicated in neuronal apoptosis in AD) (179). AMPK α 2 deletion in mice has been shown to be protective in induced stroke with reduced size of infarcts (180). The adipocyte derived hormone, leptin is decreased in AD patients, and serum levels are inversely correlated to severity of dementia. Leptin has also been observed to reduce the amyloid β protein load both *in vitro* and *in vivo*, and suppress tau phosphorylation *in vitro* (181). In a rodent model of Huntington's disease, treatment with metformin significantly prolongs survival time (182). In contrast to these potential benefits of AMPK activation in neurodegeneration, it has also been reported that in some conditions AMPK activity might have contrasting effects. For example, metformin, at doses that lead to activation of the AMP-activated protein kinase (AMPK), significantly increases the generation of both intracellular and extracellular A β species. Furthermore, the effect of metformin on A β generation is mediated by transcriptional up-regulation of beta-secretase [BACE1; one of the two proteolytic complexes that cleave amyloid precursor protein (APP) sequentially with gamma secretase to produce the amyloidogenic amyloid peptide], which results in an elevated protein level and increased enzymatic activity (183). A meta-analysis has also revealed that obesity and diabetes significantly and independently increase risk for AD (184). Finally, Har and colleagues have shown that a wide array of stress signals induces O-GlcNAc transferase (OGT) expression and increases O-GlcNAcylation of many intracellular proteins, a response that is critical for cell survival. His group has also described a mechanism by which glucose deprivation induces OGT expression and activity in Neuro-2a neuroblastoma cells where glucose deprivation increases OGT mRNA and protein expression in an AMP-activated protein kinase-dependent manner, whereas OGT enzymatic activity is regulated in a p38 MAPK-dependent manner (185).

11.5. Diabetes

Beginning with Hardie's pioneering paradigm of a possible role of disturbed AMPK signaling in diabetes, a growing body of evidence has begun to validate this idea

(186). In the decade of the 2000s, both clinical and basic science data has accumulated pointing to a role of AMPK signaling in type II diabetes. For example, Calvert *et al* have shown clinical data that suggests metformin reduces cardiovascular end points of type 2 diabetic subjects by actions that cannot solely be attributed to glucose-lowering effects. These therapeutic effects of metformin have been reported to be mediated by its activation of AMP-activated protein kinase AMPK (187). In high-fat and high-sucrose diet (HFHSD) induced diabetes in mice, Bonnard *et al* reported tissue-specific defects of adiponectin-receptor expression and AMPK activity (188). Several studies from our own laboratory have defined a specific role of AMPK signaling in diabetes. For example, we have shown that in human umbilical vein endothelial cells and aortas isolated from streptozocin-injected diabetic mice, AMPK activation normalizes vascular endothelial function by suppressing 26S proteasome-mediated GTPCH I degradation (189). Furthermore, when confluent HUVECs or mice were treated with AICAR for the detection of AMPK phosphorylation and the expression of mitochondrial uncoupling protein (UCP)-2, UCP-2 expression was increased, resulting in the inhibition of both O $_2^{\cdot -}$ and prostacyclin synthase nitration in diabetes (190). In addition, Ren and colleagues have shown that the protease inhibitor 5-[5-(2-nitrophenyl) furfuryliodine]-1,3-diphenyl-2-thiobarbituric acid (UCF-101; demonstrated to protect the heart against ischemic injury), in streptozotocin (STZ)-induced diabetes, can normalize hyperglycemia and alleviates STZ-induced anomalies in cardiomyocyte contractile mechanics (191). Activation of AMPK by AICAR is rat or in muscle cells that overexpress constitutively active AMPK (CA-AMPK) can increase glucose uptake and induces translocation of GLUT1 and GLUT4 transporters (192). In AMPK α 2 knockouts, AICAR stimulated glucose transport is blocked in muscle but insulin stimulated glucose transport is normal (127). Transgenic mice overexpressing muscle specific DN-AMPK α 2 fail to respond to AICAR treatment in terms of muscle glucose uptake (193). Insulin resistant Zucker *fa/fa* rats have improved insulin sensitivity with just one dose of AICAR (194). Overall, these reports suggest that in both *in vivo* and *ex vivo* systems, AMPK mediated glucose uptake is complimentary to insulin as well as possibly independent of this hormone, thereby implicating the kinase in diabetes pathophysiology.

12. AMPK AND OXIDATIVE STRESS

The role of AMPK in vascular pathology is so intimately linked to endothelial function and its relationship with oxidative stress that is reasonable to study the two in a somewhat integrated manner.

12.1. Cardiac disease

The effect of AMPK activity on cardiac pathology can be considered under two broad topics. First, AMPK appears to have a role in disorders that manifest as hypertrophy of the cardiac muscle architecture. Dyck and colleagues have reported that activation of the Akt pathway is critically involved in the genesis of cardiac hypertrophy (195). Furthermore, mice lacking adiponectin that have

reduced AMPK signaling are more susceptible to afterload-induced hypertrophy (196). Second, myocardial ischemia-reperfusion injury (inflammatory and oxidative damage to the cardiac muscle caused by reperfusion following a relief of ischemia), is an important cardiopathic mechanism where AMPK is also thought to play a role. It is known for example, that AMPK α 1 and α 2 activity is elevated during cardiac ischemia (114). Increased AMPK activity likely compensates for the ischemic condition by upregulating GLUT4 translocation and enhancing fatty acid oxidation (197, 198). However, it remains unclear if AMPK activity contributes positively or negatively to reperfusion in that reports have suggested both scenarios being dominant (199, 200).

12.2. Vascular endothelial function

Oxidative stress and a shift in the cellular redox balance are critical underpinnings of endothelial dysfunction. In turn, endothelial disturbances underlie cardiovascular pathology (7). One important hallmark of endothelial dysfunction is a decreased bio-availability of NO, the central vasodilatory molecule that helps regulate vascular tone. The decreased availability of NO is also associated with the generation of reactive oxidative species in the vessel wall such as peroxynitrite (6). Many studies have now established that there is an intricate balance between AMPK signaling and the redox balance in the vascular milieu. For example, AMPK has been shown to inhibit the formation of reactive oxygen species (ROS) by NADPH oxidase and stimulate NO production by eNOS (endothelial nitric oxide synthase) (7). Furthermore, AMPK has also been implicated in JNK activation, NF- κ B-mediated transcription, E-selectin expression and VCAM-1 expression, in endothelial cells that have been exposed to H₂O₂, TNF- α or fatty acids. As a consequence, these signaling events lead to attenuated monocyte adhesion onto the endothelial surface (201-204). Silencing AMPK α 1 has also been reported to decrease the expression of MnSOD, catalase, gamma-glutamylcysteine synthase and thioredoxin, in endothelial cells (205). AMPK also appears to have direct links to NADPH oxidase, a membrane bound enzyme complex which is normally latent in neutrophils and is activated to assemble in the membranes during the respiratory burst. It generates O₂⁻ transferring electrons from NADPH inside the cell across the membrane and coupling these to molecular oxygen to produce O₂⁻. It has been shown that AICAR or AMP can suppress the production of superoxide anion stimulated by phorbol esters or fMLP (Formyl-Methionyl-Leucyl-Phenylalanine) (206). In neutrophils, AICAR also has the ability to reduce PMA-dependent (phorbol 12-myristate 13-acetate; a potent activator of PKC) H₂O₂ release and induction of phosphorylation of JNK, p38, MAPK and ERK1/2 (7). The exposure of cultured human endothelial cells (HEC) to high glucose concentrations (10 mM/L) can generate ROS and this can effectively be attenuated by AMPK-activating drugs, such as rosiglitazone (207). AMPK can also influence the cellular redox state via prevention of tyrosine nitration and inhibition of prostacyclin synthase in endothelial cells that have been exposed to high glucose. This has been reported recently by our group when we demonstrated that AICAR could inhibit high glucose-

induced nitration and inactivation of prostacyclin synthase in HUVECs, that AMPK activation was necessary for AICAR to reduce oxidative stress, that prostacyclin synthase nitration by AMPK was mediated through an upregulation of UCP-2 (mitochondrial uncoupling protein-2) and that this was dependent on activation of p38 kinase as well (190). These findings from our laboratory are especially of note given that the basic role of uncoupling proteins is to prevent oxidative injury or minimize oxidative stress. In fact, such a mechanism can also be validated in *in vivo* experiments where AICAR-mediated AMPK activation markedly increases UCP-2 expression and reduction of O₂⁻ and prostacyclin synthase nitration in diabetic wild-type but not in AMPK α -deleted mice (also demonstrated in our report cited above) (190).

12.3. Vascular smooth muscle dysfunction

AMPK activation also appears to play a role in vascular pathology that centers on hyperplasia and hypertrophy of vascular tissues as well as angiotensin-II mediated vascular smooth muscle and cardiomyocyte proliferation (208, 209). Moreover, AICAR has been shown to decrease neointimal hyperplasia in the rat femoral artery denudation model linked to ERK1/2 inhibition (in part) (208).

To date, AMPK is the only known regulator that can potentially phosphorylate eNOS on more than one site (Ser117 and Ser633) (7, 48, 210). These are both activating sites in the reductase domain, as well as on Thr495 which is an inhibitory site in the CaM-binding domain (16, 211). A large body of evidence has accumulated that demonstrates that hypoxia, shear stress and thrombin, are all powerful AMPK activators in the endothelial tissues (202, 212-215). In addition, somewhat weaker yet significant stimulation of AMPK is also associated with agents such as VEGF, PPAR agonists, AICAR, metformin and adiponectin in the vascular endothelium (216-219). In 2004, our senior author (M.Z.) and colleagues had reported the biguanide drug, metformin activated AMPK mediated by mitochondrial RNS (reactive nitrogen species) and the PI3K pathway (220). Specifically, this report demonstrated that metformin activated AMPK and increased the phosphorylation of ACC (its downstream effector) at Ser79 in cultured BAEC (bovine aortic endothelial cells), that this was mediated through c-Src and was PI3K-dependent, was ONOO⁻-dependent, that the peroxynitrite oxidant was sourced to the mitochondrion and that inhibition of mitochondrial complex I activated AMPK. Further, the paper also validated these findings *in vivo*. Studies from our laboratory have also established that metformin-induced AMPK activation is beneficial to endothelial function via Heat Shock Protein 90 (hsp90) mediated activation of eNOS (221). In this report our group demonstrated that metformin could increase the conversion of arginine into citrulline in BAECs in a dose-dependent fashion, suggesting that NO synthesis was occurring via activation of eNOS. This study also revealed that eNOS activation by metformin was PI3K-dependent, was clearly mediated through the activation of AMPK and that it enhanced the association of hsp90 (an important stress response marker) with eNOS. Recently, our group has also provided evidence

that NO itself might act as an endogenous activator of AMPK (222). In this report, we have shown that NO activates AMPK in endothelial cells through a Ca^{2+} -dependent mechanism involving CaMKK β and that AMPK activation can itself increase NO release through AMPK-dependent phosphorylation of eNOS at Ser1177. These data imply that a positive feedback relationship might exist between eNOS and AMPK activation. However, further investigation of this possibility is warranted.

In terms of the consequences of endothelial activation of AMPK, several aspects need to be borne in mind. Overall, the activation of this molecule by FGF (fibroblast growth factor), adiponectin, hypoxia and VEGF, seems to be critical for angiogenesis (7, 100, 212, 223). For example, dominant negative AMPK (DN-AMPK) is able to suppress both endothelial cell migration in response to VEGF as well as *in vitro* differentiation into tube-like structures under hypoxic conditions (212). Similarly, increased VEGF expression has been shown in muscle cells treated with AICAR leading to enhanced angiogenic repair in response to ischemic injury in the mouse hind limb. This phenomenon reportedly involves AMPK-dependent activation of p38 MAPK (224). Quite recently, Leick and colleagues have reported that an AMPK/PGC-1 α -dependent mechanism is likely responsible for exercise-induced VEGF expression in skeletal muscle as well as exercise-training-induced prevention of senescent decline in VEGF protein content (225).

AMPK activation in the endothelium is also linked to endothelial control of vascular smooth muscle function, particularly vasorelaxation by NO which appears to be regulated, at least in part by AMPK activity. Vasorelaxation is a critical arm of vascular tone and tone is a central determinant of blood pressure regulation. Sustained high blood pressure is responsible for a variety of serious disease states such as hypertrophic cardiomyopathy, coronary artery insufficiency and myocardial infarction, hypertensive encephalopathy and cerebrovascular disease, hypertensive retinopathy and hypertensive nephropathy being important examples. It has been established that metformin therapy is beneficial for the cardiovascular system by virtue of its ability to improve vasodilatory function (226, 227). It is possible that these effects of metformin are dependent on eNOS activation as demonstrated by our group and others. Another possibility that merits consideration is the fact that endothelium-dependent vasorelaxation is not exclusively regulated by NO. It is provocative to speculate that AMPK might be linked to vasorelaxation via the generation of epoxyeicosatrienoic acids by the cytochrome P450 epoxygenases (7). In fact LKB1 and AMPK can be activated by stimulation of the constitutive androstane receptor and pregnane X receptor (both of the nuclear receptor superfamily that are thought to be involved in the detoxification of xenobiotics), using Phenobarbital (a classic P450 inducer) (228). In mice that lack hepatic $\alpha 1$ and $\alpha 2$ isoforms of AMPK, this response is not detectable (229). In keeping with the vasorelaxative role of AMPK, it is now fairly well established that AICAR and

metformin can both relax arterial preparations and the evidence for this comes from others as well as our group and senior author (220, 221, 230). Interestingly, there seems to be a species- and vessel-dependent difference in published literature in terms of chemical sensitivity of AMPK activation. For example, unlike rat and mouse aortae, porcine carotid artery smooth muscle is reportedly insensitive to AICAR and metformin, even though this tissue can have AMPK activation by other means, such as by hypoxia and 2-deoxyglucose (which causes a metabolic block in the glycolytic pathway) (231).

AMPK and its link with redox balance as well as NF- κ B activity provide another putative link between endothelial dysfunction and downregulated AMPK signaling. For instance, hyperglycemia induced endothelial abnormalities can be corrected by AMPK activation, as can the lipotoxicity provoked by substances such as palmitate (232). Indirectly, AMPK activity in skeletal muscle in exercise can also be shown to have beneficial attributes (7).

The role of AMPK activation in anti-atherosclerotic processes in the vasculature is also currently under intense study and several fascinating insights have been revealed. For example, the activation of mTOR by oxidized LDL has been shown to be involved in smooth muscle cell proliferation, and AMPK activation by resveratrol can block the activation of the PI3K/Akt/mTOR/p70S6K pathways with consequent inhibition of DNA synthesis and proliferation of smooth muscle cells (233).

Our group has provided several intriguing lines of empirical information that underscore the beneficial role of AMPK in endothelial cell function. Some have been highlighted above. Others include the novel demonstration that hypoxia-reperfusion via ONOO $^-$ can activate AMPK in a c-Src mediated, PI3K-dependent manner (234), that the thromboxane receptor (TP α) once stimulated can trigger ROS-mediated LKB1-dependent AMPK activation in vascular smooth muscle resulting in inhibition of cellular protein synthesis (235), that PKC ζ can regulate AMPK activity by increasing phosphorylation at Ser428 of LKB1 (resulting in association of LKB1 with AMPK and consequent phosphorylation of LKB1 itself at Thr172) (236), that the PKC ζ phosphorylation of LKB1 results in nuclear export of the kinase and prompts AMPK activation in endothelial cells (236), and most recently, ROS are required in the process of AMPK activation by statin drugs as well as PKC ζ (237). More recent work (in the process of being prepared for publication) in our laboratory, has revealed a novel mechanism through which H $_2$ O $_2$ can mediate AMPK activation independent of changes in the AMP:ATP ratio in endothelial cells. This has been observed in this cell type under nutritional stress and the implicate free radical species appear to be derived from the mitochondria. The most interesting aspect of this recent study is that under nutritive challenge (in our case by utilizing 2-deoxyglucose to induce a biochemical block by preventing glycolytic breakdown at the hexokinase step), AMPK activation led to autophagy rather than cell death. Further, under conditions of hypoxia, endothelial cell death

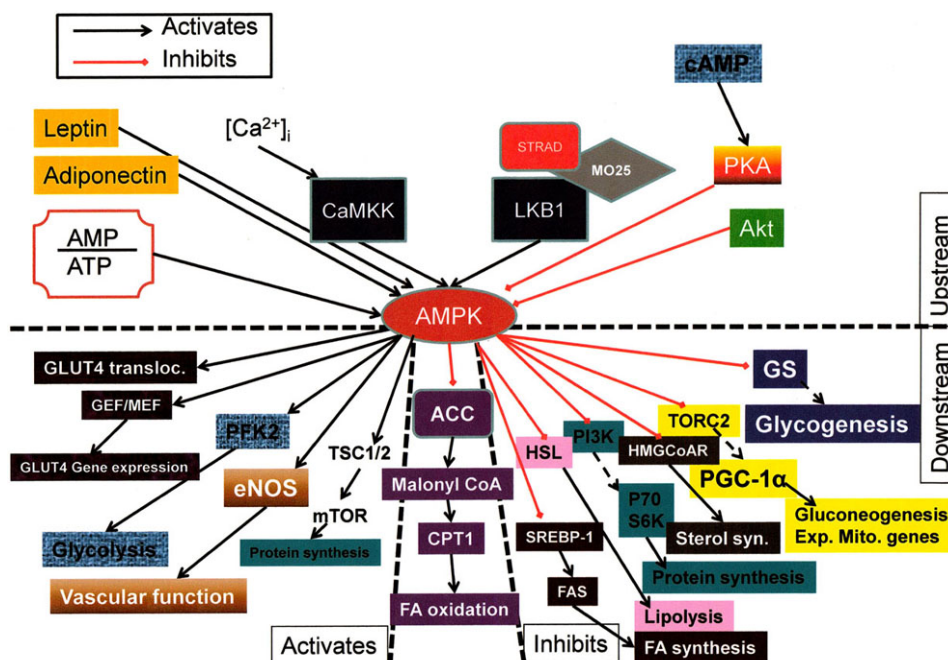


Figure 9. Integrated AMPK signaling pathways. Top half of schematic represents upstream events. Lower half represents downstream processes. In the lower half, left side are the events that are activated by AMPK while on the right are those that are inhibited by the kinase. In the center of the lower half is the main downstream effector, ACC and the consequence of its inhibition.

was prevented in an AMPK and ROS dependent manner. Overall, studies from our group reinforce the idea that AMPK activation is acutely sensitive to cellular redox balance and this relationship appears to be independent of its putative function of responding to ATP depletion (or more precisely elevated AMP:ATP) and triggering energy conserving pathways in the cell. AMPK's response to the redox balance also appears to favor cell survival pathways as evidenced by data from our laboratory as well as from other investigators.

As has been alluded to a previous section, AMPK dysregulation has been linked to obesity and the vascular morbidity observed in the metabolic syndrome and diabetes. There is a buildup of consensus that these vascular consequences may be circumvented, at least theoretically, by pharmaceutical strategies aimed at AMPK activation (238). In clinical use, a number of compounds have modes of action involving AMPK activation and these include popular and effective drugs such as cilostazol, fenofibrate and rosiglitazone (238). Interestingly, polyphenols like Resveratrol also increase phosphorylation of AMPK and have been shown to improve survival in mice who have been fed a high-fat, pro-atherogenic diet (36, 239). In addition, it has been shown that the treatment of type 1 diabetic LDL-receptor deficient mice with a synthetic polyphenols can prevent diabetes-induced decrease in AMPK and ACC phosphorylation and lipid accumulation in the liver and accretion of aortic atherosclerotic lesions (240). Metformin has also been shown to attenuate hyperglycemia and improve insulin sensitivity as well as harboring vasculoprotective effects in

many studies including those from our laboratory. For example, it has shown to reduce atherosclerotic lesions in animal models as well as in clinical studies in which the carotid intima-media thickness was measured via ultrasound (241). The endothelial effects of AMPK can largely be mimicked by its commonly used activator, AICAR. For example, in mesenteric vessels isolated from a rat model of type 2 diabetes, the NO and prostacyclin independent relaxation was impaired and endothelium-dependent contraction was enhanced. Metformin and AICAR were both able to correct this imbalance and decrease oxidative stress in this model (242). Overall, from both the bench top and the bedside, the emerging mechanistic picture provides ample encouragement to fine tune a therapeutic approach for AMPK activation in the vasculature that will not only enable better glycemic control but also augment the clinical phenotype of many human diseases by improving vascular endothelial function through this important kinase.

13 SUMMARY AND CONCLUSIONS

It has become evident with continuing research that AMPK plays a pivotal role in the complex dynamics of bioenergetics, in both health and disease. The signaling system that has AMPK as its centerpiece, is fairly complex (Figure 9). A fact that speaks to how far reaching are the effects of activation or inhibition of this kinase. In a physiologic sense, this molecule has layers of influence that it exerts on cellular processes. These include switching between anabolic and catabolic states and altering cellular dynamics by directly influencing genetic controls and

protein expression. In conditions where cellular systems are under metabolic strain, AMPK's ability to quickly correct dynamic processes appears to be an evolutionarily conserved mechanism seen across a wide phylogenetic scale, indeed in all organisms studied thus far. Even when the cellular homeostatic machinery has been overrun by pathological forces, AMPK acquires a central position in buffering these fluxes, frequently pointing them in a correcting direction and occasionally contributing to the mounting damage that leads to cell death. Some drugs and compounds that have become centerpieces of therapeutics as well as public health dogma, in disorders like diabetes (e.g., metformin), cardiovascular disease (e.g., polyphenols a resveratrol) and infectious diseases (e.g., berberine), appear to deliver their protective/therapeutic effects via modulation of AMPK signaling. However, many important details of underlying mechanisms remain somewhat obscure. There is also the belief among many pioneering and experienced investigators who have studied AMPK for many years (e.g., D.G. Hardie of the University of Dundee), that closer scrutiny of this molecule has also diluted its importance as a regulator, simply because more investigation has revealed many more molecular actors that control bioenergetics. However, at this point AMPK's dominance as the quintessential metabolic regulator remains relatively unchallenged.

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15. REFERENCES

1. E. G. Alexov and M. R. Gunner: Incorporating protein conformational flexibility into the calculation of pH-dependent protein properties. *Biophys J*, 72(5), 2075-93 (1997)
2. O. Aruksakunwong, K. Wittayanarakul, P. Sompornpisut, V. Sanghiran, V. Parasuk and S. Hannongbua: Structural and dynamical properties of different protonated states of mutant HIV-1 protease complexed with the saquinavir inhibitor studied by molecular dynamics simulations. *J Mol Graph Model*, 25(3), 324-32 (2006)
3. D. A. Begg and L. I. Rebhun: pH regulates the polymerization of actin in the sea urchin egg cortex. *J Cell Biol*, 83(1), 241-8 (1979)

4. W. L. Neuhuber: Lung sensors: complex functions require complex structures. *Am J Respir Cell Mol Biol*, 28(3), 265-6 (2003)
5. M. Koivusalo, A. Kapus and S. Grinstein: Sensors, transducers, and effectors that regulate cell size and shape. *J Biol Chem*, 284(11), 6595-9 (2009)
6. A. Bouloumie, J. Bauersachs, W. Linz, B. A. Scholkens, G. Wiemer, I. Fleming and R. Busse: Endothelial dysfunction coincides with an enhanced nitric oxide synthase expression and superoxide anion production. *Hypertension*, 30(4), 934-41 (1997)
7. B. Fisslthaler and I. Fleming: Activation and signaling by the AMP-activated protein kinase in endothelial cells. *Circ Res*, 105(2), 114-27 (2009)
8. Z. H. Beg, D. W. Allmann and D. M. Gibson: 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(4), 1362-9 (1973)
9. C. A. Carlson and K. H. Kim: Regulation of hepatic acetyl coenzyme A carboxylase by phosphorylation and dephosphorylation. *J Biol Chem*, 248(1), 378-80 (1973)
10. M. R. Munday, D. G. Campbell, D. Carling and D. G. Hardie: Identification by amino acid sequencing of three major regulatory phosphorylation sites on rat acetyl-CoA carboxylase. *Eur J Biochem*, 175(2), 331-8 (1988)
11. D. Carling, P. R. Clarke, V. A. Zammit and D. G. Hardie: 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(1-2), 129-36 (1989)
12. G. R. Steinberg and B. E. Kemp: AMPK in Health and Disease. *Physiol Rev*, 89(3), 1025-78 (2009)
13. S. P. Davies, S. A. Hawley, A. Woods, D. Carling, T. A. Haystead and D. G. Hardie: Purification of the AMP-activated protein kinase on ATP-gamma-sepharose and analysis of its subunit structure. *Eur J Biochem*, 223(2), 351-7 (1994)
14. K. I. Mitchelhill, D. Stapleton, G. Gao, C. House, B. Michell, F. Katsis, L. A. Witters and B. E. Kemp: Mammalian AMP-activated protein kinase shares structural and functional homology with the catalytic domain of yeast Snf1 protein kinase. *J Biol Chem*, 269(4), 2361-4 (1994)
15. D. Stapleton, G. Gao, B. J. Michell, J. Widmer, K. Mitchelhill, T. Teh, C. M. House, L. A. Witters and B. E. Kemp: Mammalian 5'-AMP-activated protein kinase non-catalytic subunits are homologs of proteins that interact with yeast Snf1 protein kinase. *J Biol Chem*, 269(47), 29343-6 (1994)

16. Z. Chen, J. Heierhorst, R. J. Mann, K. I. Mitchelhill, B. J. Mitchell, L. A. Witters, G. S. Lynch, B. E. Kemp and D. Stapleton: Expression of the AMP-activated protein kinase beta1 and beta2 subunits in skeletal muscle. *FEBS Lett*, 460(2), 343-8 (1999)
17. P. C. Cheung, I. P. Salt, S. P. Davies, D. G. Hardie and D. Carling: Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. *Biochem J*, 346 Pt 3, 659-69 (2000)
18. M. C. Towler and D. G. Hardie: AMP-activated protein kinase in metabolic control and insulin signaling. *Circ Res*, 100(3), 328-41 (2007)
19. K. R. Hallows, G. P. Kobinger, J. M. Wilson, L. A. Witters and J. K. Fosskett: Physiological modulation of CFTR activity by AMP-activated protein kinase in polarized T84 cells. *Am J Physiol Cell Physiol*, 284(5), C1297-308 (2003)
20. A. M. Evans, K. J. Mustard, C. N. Wyatt, C. Peers, M. Dipp, P. Kumar, N. P. Kinnear and D. G. Hardie: Does AMP-activated protein kinase couple inhibition of mitochondrial oxidative phosphorylation by hypoxia to calcium signaling in O₂-sensing cells? *J Biol Chem*, 280(50), 41504-11 (2005)
21. I. P. Salt, G. Johnson, S. J. Ashcroft and D. G. Hardie: AMP-activated protein kinase is activated by low glucose in cell lines derived from pancreatic beta cells, and may regulate insulin release. *Biochem J*, 335 (Pt 3), 533-9 (1998)
22. A. M. Turnley, D. Stapleton, R. J. Mann, L. A. Witters, B. E. Kemp and P. F. Bartlett: Cellular distribution and developmental expression of AMP-activated protein kinase isoforms in mouse central nervous system. *J Neurochem*, 72(4), 1707-16 (1999)
23. H. Ai, J. Ihlemann, Y. Hellsten, H. P. Lauritzen, D. G. Hardie, H. Galbo and T. Ploug: Effect of fiber type and nutritional state on AICAR- and contraction-stimulated glucose transport in rat muscle. *Am J Physiol Endocrinol Metab*, 282(6), E1291-300 (2002)
24. G. Polekhina, S. C. Feil, A. Gupta, P. O'Donnell, D. Stapleton and M. W. Parker: Crystallization of the glycogen-binding domain of the AMP-activated protein kinase beta subunit and preliminary X-ray analysis. *Acta Crystallogr Sect F Struct Biol Cryst Commun*, 61(Pt 1), 39-42 (2005)
25. G. Polekhina, A. Gupta, B. J. van Denderen, S. C. Feil, B. E. Kemp, D. Stapleton and M. W. Parker: Structural basis for glycogen recognition by AMP-activated protein kinase. *Structure*, 13(10), 1453-62 (2005)
26. E. R. Hudson, D. A. Pan, J. James, J. M. Lucocq, S. A. Hawley, K. A. Green, O. Baba, T. Terashima and D. G. Hardie: A novel domain in AMP-activated protein kinase causes glycogen storage bodies similar to those seen in hereditary cardiac arrhythmias. *Curr Biol*, 13(10), 861-6 (2003)
27. A. Bateman: The structure of a domain common to archaeobacteria and the homocystinuria disease protein. *Trends Biochem Sci*, 22(1), 12-3 (1997)
28. J. W. Scott, S. A. Hawley, K. A. Green, M. Anis, G. Stewart, G. A. Scullion, D. G. Norman and D. G. Hardie: CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations. *J Clin Invest*, 113(2), 274-84 (2004)
29. J. M. Corton, J. G. Gillespie, S. A. Hawley and D. G. Hardie: 5-aminoimidazole-4-carboxamide ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells? *Eur J Biochem*, 229(2), 558-65 (1995)
30. D. G. Hardie: AMPK and SNF1: Snuffing Out Stress. *Cell Metab*, 6(5), 339-40 (2007)
31. N. B. Ruderman, C. Keller, A. M. Richard, A. K. Saha, Z. Luo, X. Xiang, M. Giralt, V. B. Ritov, E. V. Menshikova, D. E. Kelley, J. Hidalgo, B. K. Pedersen and M. Kelly: Interleukin-6 regulation of AMP-activated protein kinase. Potential role in the systemic response to exercise and prevention of the metabolic syndrome. *Diabetes*, 55 Suppl 2, S48-54 (2006)
32. M. J. Watt, N. Dzamko, W. G. Thomas, S. Rose-John, M. Ernst, D. Carling, B. E. Kemp, M. A. Febbraio and G. R. Steinberg: CNTF reverses obesity-induced insulin resistance by activating skeletal muscle AMPK. *Nat Med*, 12(5), 541-8 (2006)
33. G. Zhou, R. Myers, Y. Li, Y. Chen, X. Shen, J. Fenyk-Melody, M. Wu, J. Ventre, T. Doebber, N. Fujii, N. Musi, M. F. Hirshman, L. J. Goodyear and D. E. Moller: Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest*, 108(8), 1167-74 (2001)
34. L. G. Fryer, A. Parbu-Patel and D. Carling: Protein kinase inhibitors block the stimulation of the AMP-activated protein kinase by 5-amino-4-imidazolecarboxamide riboside. *FEBS Lett*, 531(2), 189-92 (2002)
35. Y. S. Lee, W. S. Kim, K. H. Kim, M. J. Yoon, H. J. Cho, Y. Shen, J. M. Ye, C. H. Lee, W. K. Oh, C. T. Kim, C. Hohnen-Behrens, A. Gosby, E. W. Kraegen, D. E. James and J. B. Kim: Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. *Diabetes*, 55(8), 2256-64 (2006)
36. J. A. Baur, K. J. Pearson, N. L. Price, H. A. Jamieson, C. Lerin, A. Kalra, V. V. Prabhu, J. S. Allard, G. Lopez-Lluch, K. Lewis, P. J. Pistell, S. Poosala, K. G. Becker, O. Boss, D. Gwinn, M. Wang, S. Ramaswamy, K. W. Fishbein, R. G. Spencer, E. G. Lakatta, D. Le Couteur, R. J. Shaw, P. Navas, P. Puigserver, D. K. Ingram, R. de Cabo

and D. A. Sinclair: Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*, 444(7117), 337-42 (2006)

37. J. F. Wojtaszewski, P. Nielsen, B. F. Hansen, E. A. Richter and B. Kiens: Isoform-specific and exercise intensity-dependent activation of 5'-AMP-activated protein kinase in human skeletal muscle. *J Physiol*, 528 Pt 1, 221-6 (2000)

38. S. M. Warden, C. Richardson, J. O'Donnell, Jr., D. Stapleton, B. E. Kemp and L. A. Witters: Post-translational modifications of the beta-1 subunit of AMP-activated protein kinase affect enzyme activity and cellular localization. *Biochem J*, 354(Pt 2), 275-83 (2001)

39. D. G. Hardie and B. G. Frenguelli: A neural protection racket: AMPK and the GABA(B) receptor. *Neuron*, 53(2), 159-62 (2007)

40. S. P. Hong, F. C. Leiper, A. Woods, D. Carling and M. Carlson: Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. *Proc Natl Acad Sci U S A*, 100(15), 8839-43 (2003)

41. C. M. Sutherland, S. A. Hawley, R. R. McCartney, A. Leech, M. J. Stark, M. C. Schmidt and D. G. Hardie: Elm1p is one of three upstream kinases for the *Saccharomyces cerevisiae* SNF1 complex. *Curr Biol*, 13(15), 1299-305 (2003)

42. K. Sakamoto, E. Zarrinpashneh, G. R. Budas, A. C. Pouleur, A. Dutta, A. R. Prescott, J. L. Vanoverschelde, A. Ashworth, A. Jovanovic, D. R. Alessi and L. Bertrand: Deficiency of LKB1 in heart prevents ischemia-mediated activation of AMPKalpha2 but not AMPKalpha1. *Am J Physiol Endocrinol Metab*, 290(5), E780-8 (2006)

43. S. A. Hawley, D. A. Pan, K. J. Mustard, L. Ross, J. Bain, A. M. Edelman, B. G. Frenguelli and D. G. Hardie: Calmodulin-dependent protein kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab*, 2(1), 9-19 (2005)

44. N. Stahmann, A. Woods, D. Carling and R. Heller: Thrombin activates AMP-activated protein kinase in endothelial cells via a pathway involving Ca²⁺/calmodulin-dependent protein kinase beta. *Mol Cell Biol*, 26(16), 5933-45 (2006)

45. D. Carling and D. G. Hardie: The substrate and sequence specificity of the AMP-activated protein kinase. Phosphorylation of glycogen synthase and phosphorylase kinase. *Biochim Biophys Acta*, 1012(1), 81-6 (1989)

46. P. R. Clarke and D. G. Hardie: Calmodulin-dependent multiprotein kinase and protein kinase C phosphorylate the same site on HMG-CoA reductase as the AMP-activated protein kinase. *FEBS Lett*, 269(1), 213-7 (1990)

47. P. R. Clarke and D. G. Hardie: Regulation of HMG-CoA reductase: identification of the site phosphorylated by

the AMP-activated protein kinase *in vitro* and in intact rat liver. *EMBO J*, 9(8), 2439-46 (1990)

48. Z. P. Chen, K. I. Mitchelhill, B. J. Michell, D. Stapleton, I. Rodriguez-Crespo, L. A. Witters, D. A. Power, P. R. Ortiz de Montellano and B. E. Kemp: AMP-activated protein kinase phosphorylation of endothelial NO synthase. *FEBS Lett*, 443(3), 285-9 (1999)

49. G. Hardie, N. Morrice and H. E. Tornqvist: 5'-AMP-activated protein kinase phosphorylates IRS-1 on Ser-789 in mouse C2C12 myotubes in response to 5-aminoimidazole-4-carboxamide riboside. *J Biol Chem*, 276(50), 46912-6 (2001)

50. X. P. Jiang, D. C. Yang, R. L. Elliott and J. F. Head: Down-regulation of expression of interleukin-6 and its receptor results in growth inhibition of MCF-7 breast cancer cells. *Anticancer Res*, 31(9), 2899-906 (2011)

51. Y. H. Hong, U. S. Varanasi, W. Yang and T. Leff: AMP-activated protein kinase regulates HNF4alpha transcriptional activity by inhibiting dimer formation and decreasing protein stability. *J Biol Chem*, 278(30), 27495-501 (2003)

52. S. H. Koo, L. Flechner, L. Qi, X. Zhang, R. A. Screaton, S. Jeffries, S. Hedrick, W. Xu, F. Boussouar, P. Brindle, H. Takemori and M. Montminy: The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. *Nature*, 437(7062), 1109-11 (2005)

53. J. Weekes, K. L. Ball, F. B. Caudwell and D. G. Hardie: Specificity determinants for the AMP-activated protein kinase and its plant homologue analysed using synthetic peptides. *FEBS Lett*, 334(3), 335-9 (1993)

54. S. Dale, W. A. Wilson, A. M. Edelman and D. G. Hardie: Similar substrate recognition motifs for mammalian AMP-activated protein kinase, higher plant HMG-CoA reductase kinase-A, yeast SNF1, and mammalian calmodulin-dependent protein kinase I. *FEBS Lett*, 361(2-3), 191-5 (1995)

55. K. M. Geraghty, S. Chen, J. E. Harthill, A. F. Ibrahim, R. Toth, N. A. Morrice, F. Vandermoere, G. B. Moorhead, D. G. Hardie and C. MacKintosh: Regulation of multisite phosphorylation and 14-3-3 binding of AS160 in response to IGF-1, EGF, PMA and AICAR. *Biochem J*, 407(2), 231-41 (2007)

56. D. Zheng, P. S. MacLean, S. C. Pohnert, J. B. Knight, A. L. Olson, W. W. Winder and G. L. Dohm: Regulation of muscle GLUT-4 transcription by AMP-activated protein kinase. *J Appl Physiol*, 91(3), 1073-83 (2001)

57. S. S. Lin, J. K. Manchester and J. I. Gordon: Enhanced gluconeogenesis and increased energy storage as hallmarks of aging in *Saccharomyces cerevisiae*. *J Biol Chem*, 276(38), 36000-7 (2001)

58. M. E. Young, G. K. Radda and B. Leighton: Activation of glycogen phosphorylase and glycogenolysis in rat skeletal muscle by AICAR--an activator of AMP-activated protein kinase. *FEBS Lett*, 382(1-2), 43-7 (1996)

59. S. B. Jorgensen, J. N. Nielsen, J. B. Birk, G. S. Olsen, B. Viollet, F. Andreelli, P. Schjerling, S. Vaultont, D. G. Hardie, B. F. Hansen, E. A. Richter and J. F. Wojtaszewski: The $\alpha 2$ -5'AMP-activated protein kinase is a site 2 glycogen synthase kinase in skeletal muscle and is responsive to glucose loading. *Diabetes*, 53(12), 3074-81 (2004)
60. R. Bergeron, J. M. Ren, K. S. Cadman, I. K. Moore, P. Perret, M. Pypaert, L. H. Young, C. F. Semenkovich and G. I. Shulman: Chronic activation of AMP kinase results in NRF-1 activation and mitochondrial biogenesis. *Am J Physiol Endocrinol Metab*, 281(6), E1340-6 (2001)
61. F. Andreelli, M. Foretz, C. Knauf, P. D. Cani, C. Perrin, M. A. Iglesias, B. Pillot, A. Bado, F. Tronche, G. Mithieux, S. Vaultont, R. Burcelin and B. Viollet: Liver adenosine monophosphate-activated kinase- $\alpha 2$ catalytic subunit is a key target for the control of hepatic glucose production by adiponectin and leptin but not insulin. *Endocrinology*, 147(5), 2432-41 (2006)
62. T. Yamauchi, J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, S. Yamashita, M. Noda, S. Kita, K. Ueki, K. Eto, Y. Akanuma, P. Froguel, F. Foufelle, P. Ferre, D. Carling, S. Kimura, R. Nagai, B. B. Kahn and T. Kadowaki: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med*, 8(11), 1288-95 (2002)
63. G. da Silva Xavier, I. Leclerc, A. Varadi, T. Tsuboi, S. K. Moule and G. A. Rutter: Role for AMP-activated protein kinase in glucose-stimulated insulin secretion and preproinsulin gene expression. *Biochem J*, 371(Pt 3), 761-74 (2003)
64. P. A. Lochhead, I. P. Salt, K. S. Walker, D. G. Hardie and C. Sutherland: 5-aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6-phosphatase. *Diabetes*, 49(6), 896-903 (2000)
65. A. Woods, D. Azzout-Marniche, M. Foretz, S. C. Stein, P. Lemarchand, P. Ferre, F. Foufelle and D. Carling: Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. *Mol Cell Biol*, 20(18), 6704-11 (2000)
66. R. J. Shaw, K. A. Lamia, D. Vasquez, S. H. Koo, N. Bardeesy, R. A. Depinho, M. Montminy and L. C. Cantley: The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science*, 310(5754), 1642-6 (2005)
67. B. Kiens: Skeletal muscle lipid metabolism in exercise and insulin resistance. *Physiol Rev*, 86(1), 205-43 (2006)
68. J. G. Voet DV: Biochemistry. Wiley, (2004)
69. S. P. Davies, A. T. Sim and D. G. Hardie: Location and function of three sites phosphorylated on rat acetyl-CoA carboxylase by the AMP-activated protein kinase. *Eur J Biochem*, 187(1), 183-90 (1990)
70. J. Ha, S. Daniel, S. S. Broyles and K. H. Kim: Critical phosphorylation sites for acetyl-CoA carboxylase activity. *J Biol Chem*, 269(35), 22162-8 (1994)
71. A. Baron, T. Migita, D. Tang and M. Loda: Fatty acid synthase: a metabolic oncogene in prostate cancer? *J Cell Biochem*, 91(1), 47-53 (2004)
72. M. Foretz, D. Carling, C. Guichard, P. Ferre and F. Foufelle: AMP-activated protein kinase inhibits the glucose-activated expression of fatty acid synthase gene in rat hepatocytes. *J Biol Chem*, 273(24), 14767-71 (1998)
73. M. Foretz, N. Ancellin, F. Andreelli, Y. Saintillan, P. Grondin, A. Kahn, B. Thorens, S. Vaultont and B. Viollet: Short-term overexpression of a constitutively active form of AMP-activated protein kinase in the liver leads to mild hypoglycemia and fatty liver. *Diabetes*, 54(5), 1331-9 (2005)
74. Z. An, H. Wang, P. Song, M. Zhang, X. Geng and M. H. Zou: Nicotine-induced activation of AMP-activated protein kinase inhibits fatty acid synthase in 3T3L1 adipocytes: a role for oxidant stress. *J Biol Chem*, 282(37), 26793-801 (2007)
75. T. M. Lewin, N. M. Schwerbrock, D. P. Lee and R. A. Coleman: Identification of a new glycerol-3-phosphate acyltransferase isoenzyme, mtGPAT2, in mitochondria. *J Biol Chem*, 279(14), 13488-95 (2004)
76. D. M. Muoio, K. Seefeld, L. A. Witters and R. A. Coleman: AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-phosphate acyltransferase is a novel target. *Biochem J*, 338 (Pt 3), 783-91 (1999)
77. M. J. Watt, A. G. Holmes, G. R. Steinberg, J. L. Mesa, B. E. Kemp and M. A. Febbraio: Reduced plasma FFA availability increases net triacylglycerol degradation, but not GPAT or HSL activity, in human skeletal muscle. *Am J Physiol Endocrinol Metab*, 287(1), E120-7 (2004)
78. L. A. Witters, G. Gao, B. E. Kemp and B. Quistorff: Hepatic 5'-AMP-activated protein kinase: zonal distribution and relationship to acetyl-CoA carboxylase activity in varying nutritional states. *Arch Biochem Biophys*, 308(2), 413-9 (1994)
79. M. M. Assifi, G. Suchankova, S. Constant, M. Prentki, A. K. Saha and N. B. Ruderman: AMP-activated protein kinase and coordination of hepatic fatty acid metabolism of starved/carbohydrate-refed rats. *Am J Physiol Endocrinol Metab*, 289(5), E794-800 (2005)

80. M. Daval, F. Diot-Dupuy, R. Bazin, I. Hainault, B. Viollet, S. Vaulont, E. Hajdouch, P. Ferre and F. Foufelle: Anti-lipolytic action of AMP-activated protein kinase in rodent adipocytes. *J Biol Chem*, 280(26), 25250-7 (2005)
81. J. S. Fisher, J. S. Ju, P. J. Oppelt, J. L. Smith, A. Suzuki and H. Esumi: Muscle contractions, AICAR, and insulin cause phosphorylation of an AMPK-related kinase. *Am J Physiol Endocrinol Metab*, 289(6), E986-92 (2005)
82. A. C. Smith, C. R. Bruce and D. J. Dyck: AMP kinase activation with AICAR simultaneously increases fatty acid and glucose oxidation in resting rat soleus muscle. *J Physiol*, 565(Pt 2), 537-46 (2005)
83. J. L. Smith, P. B. Patil and J. S. Fisher: AICAR and hyperosmotic stress increase insulin-stimulated glucose transport. *J Appl Physiol*, 99(3), 877-83 (2005)
84. H. Clark, D. Carling and D. Saggerson: Covalent activation of heart AMP-activated protein kinase in response to physiological concentrations of long-chain fatty acids. *Eur J Biochem*, 271(11), 2215-24 (2004)
85. G. Suchankova, M. Tekle, A. K. Saha, N. B. Ruderman, S. D. Clarke and T. W. Gettys: Dietary polyunsaturated fatty acids enhance hepatic AMP-activated protein kinase activity in rats. *Biochem Biophys Res Commun*, 326(4), 851-8 (2005)
86. D. E. Kelley and J. A. Simoneau: Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. *J Clin Invest*, 94(6), 2349-56 (1994)
87. E. D. Muse, S. Obici, S. Bhanot, B. P. Monia, R. A. McKay, M. W. Rajala, P. E. Scherer and L. Rossetti: Role of resistin in diet-induced hepatic insulin resistance. *J Clin Invest*, 114(2), 232-9 (2004)
88. J. J. Wilkes, M. T. Nguyen, G. K. Bandyopadhyay, E. Nelson and J. M. Olefsky: Topiramate treatment causes skeletal muscle insulin sensitization and increased Acrp30 secretion in high-fat-fed male Wistar rats. *Am J Physiol Endocrinol Metab*, 289(6), E1015-22 (2005)
89. W. J. Lee, I. K. Lee, H. S. Kim, Y. M. Kim, E. H. Koh, J. C. Won, S. M. Han, M. S. Kim, I. Jo, G. T. Oh, I. S. Park, J. H. Youn, S. W. Park, K. U. Lee and J. Y. Park: Alpha-lipoic acid prevents endothelial dysfunction in obese rats via activation of AMP-activated protein kinase. *Arterioscler Thromb Vasc Biol*, 25(12), 2488-94 (2005)
90. S. J. Lessard, Z. P. Chen, M. J. Watt, M. Hashem, J. J. Reid, M. A. Febbraio, B. E. Kemp and J. A. Hawley: Chronic rosiglitazone treatment restores AMPKalpha2 activity in insulin-resistant rat skeletal muscle. *Am J Physiol Endocrinol Metab*, 290(2), E251-7 (2006)
91. Y. Wu, P. Song, J. Xu, M. Zhang and M. H. Zou: Activation of protein phosphatase 2A by palmitate inhibits AMP-activated protein kinase. *J Biol Chem*, 282(13), 9777-88 (2007)
92. K. F. Petersen, S. Dufour, D. Befroy, R. Garcia and G. I. Shulman: Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med*, 350(7), 664-71 (2004)
93. W. W. Winder, B. F. Holmes, D. S. Rubink, E. B. Jensen, M. Chen and J. O. Holloszy: Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J Appl Physiol*, 88(6), 2219-26 (2000)
94. S. L. McGee and M. Hargreaves: AMPK and transcriptional regulation. *Front Biosci*, 13, 3022-33 (2008)
95. M. A. Iglesias, S. M. Furler, G. J. Cooney, E. W. Kraegen and J. M. Ye: AMP-activated protein kinase activation by AICAR increases both muscle fatty acid and glucose uptake in white muscle of insulin-resistant rats *in vivo*. *Diabetes*, 53(7), 1649-54 (2004)
96. M. J. Watt, G. R. Steinberg, S. Chan, A. Garnham, B. E. Kemp and M. A. Febbraio: Beta-adrenergic stimulation of skeletal muscle HSL can be overridden by AMPK signaling. *FASEB J*, 18(12), 1445-6 (2004)
97. D. Kukidome, T. Nishikawa, K. Sonoda, K. Imoto, K. Fujisawa, M. Yano, H. Motoshima, T. Taguchi, T. Matsumura and E. Araki: Activation of AMP-activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells. *Diabetes*, 55(1), 120-7 (2006)
98. Z. H. Beg, J. A. Stonik and H. B. Brewer, Jr.: 3-Hydroxy-3-methylglutaryl coenzyme A reductase: regulation of enzymatic activity by phosphorylation and dephosphorylation. *Proc Natl Acad Sci U S A*, 75(8), 3678-82 (1978)
99. Z. H. Beg, J. A. Stonik and H. B. Brewer, Jr.: Solubilization of 3-hydroxy-3-methylglutaryl coenzyme A reductase from rat and chicken liver microsomes. *Anal Biochem*, 86(2), 531-5 (1978)
100. N. Ouchi, S. Kihara, Y. Arita, M. Nishida, A. Matsuyama, Y. Okamoto, M. Ishigami, H. Kuriyama, K. Kishida, H. Nishizawa, K. Hotta, M. Muraguchi, Y. Ohmoto, S. Yamashita, T. Funahashi and Y. Matsuzawa: Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation*, 103(8), 1057-63 (2001)
101. E. Tomas, T. S. Tsao, A. K. Saha, H. E. Murrey, C. Zhang Cc, S. I. Itani, H. F. Lodish and N. B. Ruderman: Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. *Proc Natl Acad Sci U S A*, 99(25), 16309-13 (2002)
102. G. J. Browne, S. G. Finn and C. G. Proud: Stimulation of the AMP-activated protein kinase leads to activation of eukaryotic elongation factor 2 kinase and to its

- phosphorylation at a novel site, serine 398. *J Biol Chem*, 279(13), 12220-31 (2004)
103. K. Inoki, Y. Li, T. Xu and K. L. Guan: Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev*, 17(15), 1829-34 (2003)
104. K. Inoki, T. Zhu and K. L. Guan: TSC2 mediates cellular energy response to control cell growth and survival. *Cell*, 115(5), 577-90 (2003)
105. D. M. Gwinn, D. B. Shackelford, D. F. Egan, M. M. Mihaylova, A. Mery, D. S. Vasquez, B. E. Turk and R. J. Shaw: AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell*, 30(2), 214-26 (2008)
106. E. Mizukoshi, M. Suzuki, T. Misono, A. Loupatov, E. Munekata, S. C. Kaul, R. Wadhwa and T. Imamura: Cell-cycle dependent tyrosine phosphorylation on mortalin regulates its interaction with fibroblast growth factor-1. *Biochem Biophys Res Commun*, 280(4), 1203-9 (2001)
107. R. G. Jones, D. R. Plas, S. Kubek, M. Buzzai, J. Mu, Y. Xu, M. J. Birnbaum and C. B. Thompson: AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. *Mol Cell*, 18(3), 283-93 (2005)
108. R. Rattan, S. Giri, A. K. Singh and I. Singh: 5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside inhibits cancer cell proliferation *in vitro* and *in vivo* via AMP-activated protein kinase. *J Biol Chem*, 280(47), 39582-93 (2005)
109. W. Wang, J. Fan, X. Yang, S. Furer-Galban, I. Lopez de Silanes, C. von Kobbe, J. Guo, S. N. Georas, F. Foufelle, D. G. Hardie, D. Carling and M. Gorospe: AMP-activated kinase regulates cytoplasmic HuR. *Mol Cell Biol*, 22(10), 3425-36 (2002)
110. K. R. Hallows, J. E. McCane, B. E. Kemp, L. A. Witters and J. K. Foskett: Regulation of channel gating by AMP-activated protein kinase modulates cystic fibrosis transmembrane conductance regulator activity in lung submucosal cells. *J Biol Chem*, 278(2), 998-1004 (2003)
111. M. D. Carattino, R. S. Edinger, H. J. Grieser, R. Wise, D. Neumann, U. Schlattner, J. P. Johnson, T. R. Kleyman and K. R. Hallows: Epithelial sodium channel inhibition by AMP-activated protein kinase in oocytes and polarized renal epithelial cells. *J Biol Chem*, 280(18), 17608-16 (2005)
112. S. A. Fraser, I. Gimenez, N. Cook, I. Jennings, M. Katerelos, F. Katsis, V. Levidiotis, B. E. Kemp and D. A. Power: Regulation of the renal-specific Na⁺-K⁺-2Cl⁻ co-transporter NKCC2 by AMP-activated protein kinase (AMPK). *Biochem J*, 405(1), 85-93 (2007)
113. H. Klein, L. Garneau, N. T. Trinh, A. Prive, F. Dionne, E. Goupil, D. Thuringer, L. Parent, E. Brochiero and R. Sauve: Inhibition of the KCa3.1 channels by AMP-activated protein kinase in human airway epithelial cells. *Am J Physiol Cell Physiol*, 296(2), C285-95 (2009)
114. G. R. Steinberg, M. J. Watt, M. Ernst, M. J. Birnbaum, B. E. Kemp and S. B. Jorgensen: Ciliary neurotrophic factor stimulates muscle glucose uptake by a PI3-kinase-dependent pathway that is impaired with obesity. *Diabetes*, 58(4), 829-39 (2009)
115. S. Fediuc, M. P. Gaidhu and R. B. Ceddia: Regulation of AMP-activated protein kinase and acetyl-CoA carboxylase phosphorylation by palmitate in skeletal muscle cells. *J Lipid Res*, 47(2), 412-20 (2006)
116. A. Dobrzyn, P. Dobrzyn, S. H. Lee, M. Miyazaki, P. Cohen, E. Asilmaz, D. G. Hardie, J. M. Friedman and J. M. Ntambi: Stearoyl-CoA desaturase-1 deficiency reduces ceramide synthesis by downregulating serine palmitoyltransferase and increasing beta-oxidation in skeletal muscle. *Am J Physiol Endocrinol Metab*, 288(3), E599-607 (2005)
117. K. Maeda, H. Cao, K. Kono, C. Z. Gorgun, M. Furuhashi, K. T. Uysal, Q. Cao, G. Atsumi, H. Malone, B. Krishnan, Y. Minokoshi, B. B. Kahn, R. A. Parker and G. S. Hotamisligil: Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metab*, 1(2), 107-19 (2005)
118. H. Cao, K. Maeda, C. Z. Gorgun, H. J. Kim, S. Y. Park, G. I. Shulman, J. K. Kim and G. S. Hotamisligil: Regulation of metabolic responses by adipocyte/macrophage Fatty Acid-binding proteins in leptin-deficient mice. *Diabetes*, 55(7), 1915-22 (2006)
119. C. E. Gleason, D. Lu, L. A. Witters, C. B. Newgard and M. J. Birnbaum: The role of AMPK and mTOR in nutrient sensing in pancreatic beta-cells. *J Biol Chem*, 282(14), 10341-51 (2007)
120. Y. Minokoshi, T. Alquier, N. Furukawa, Y. B. Kim, A. Lee, B. Xue, J. Mu, F. Foufelle, P. Ferre, M. J. Birnbaum, B. J. Stuck and B. B. Kahn: AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature*, 428(6982), 569-74 (2004)
121. U. Andersson, K. Filipsson, C. R. Abbott, A. Woods, K. Smith, S. R. Bloom, D. Carling and C. J. Small: AMP-activated protein kinase plays a role in the control of food intake. *J Biol Chem*, 279(13), 12005-8 (2004)
122. C. A. Hutber, D. G. Hardie and W. W. Winder: Electrical stimulation inactivates muscle acetyl-CoA carboxylase and increases AMP-activated protein kinase. *Am J Physiol*, 272(2 Pt 1), E262-6 (1997)
123. D. Vavvas, A. Apazidis, A. K. Saha, J. Gamble, A. Patel, B. E. Kemp, L. A. Witters and N. B. Ruderman: Contraction-induced changes in acetyl-CoA carboxylase and 5'-AMP-activated kinase in skeletal muscle. *J Biol Chem*, 272(20), 13255-61 (1997)
124. K. Sakamoto, A. McCarthy, D. Smith, K. A. Green, D. Grahame Hardie, A. Ashworth and D. R. Alessi:

Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction. *EMBO J*, 24(10), 1810-20 (2005)

125. H. J. Koh, D. E. Arnolds, N. Fujii, T. T. Tran, M. J. Rogers, N. Jessen, Y. Li, C. W. Liew, R. C. Ho, M. F. Hirshman, R. N. Kulkarni, C. R. Kahn and L. J. Goodyear: Skeletal muscle-selective knockout of LKB1 increases insulin sensitivity, improves glucose homeostasis, and decreases TRB3. *Mol Cell Biol*, 26(22), 8217-27 (2006)

126. D. L. Coven, X. Hu, L. Cong, R. Bergeron, G. I. Shulman, D. G. Hardie and L. H. Young: Physiological role of AMP-activated protein kinase in the heart: graded activation during exercise. *Am J Physiol Endocrinol Metab*, 285(3), E629-36 (2003)

127. S. B. Jorgensen, B. Viollet, F. Andreelli, C. Frosig, J. B. Birk, P. Schjerling, S. Vaulont, E. A. Richter and J. F. Wojtaszewski: Knockout of the alpha2 but not alpha1 5'-AMP-activated protein kinase isoform abolishes 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranosidebut not contraction-induced glucose uptake in skeletal muscle. *J Biol Chem*, 279(2), 1070-9 (2004)

128. N. Fujii, M. F. Hirshman, E. M. Kane, R. C. Ho, L. E. Peter, M. M. Seifert and L. J. Goodyear: AMP-activated protein kinase alpha2 activity is not essential for contraction- and hyperosmolarity-induced glucose transport in skeletal muscle. *J Biol Chem*, 280(47), 39033-41 (2005)

129. T. E. Jensen, A. J. Rose, S. B. Jorgensen, N. Brandt, P. Schjerling, J. F. Wojtaszewski and E. A. Richter: Possible CaMKK-dependent regulation of AMPK phosphorylation and glucose uptake at the onset of mild tetanic skeletal muscle contraction. *Am J Physiol Endocrinol Metab*, 292(5), E1308-17 (2007)

130. G. F. Merrill, E. J. Kurth, D. G. Hardie and W. W. Winder: AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. *Am J Physiol*, 273(6 Pt 1), E1107-12 (1997)

131. V. K. Kaushik, M. E. Young, D. J. Dean, T. G. Kurowski, A. K. Saha and N. B. Ruderman: Regulation of fatty acid oxidation and glucose metabolism in rat soleus muscle: effects of AICAR. *Am J Physiol Endocrinol Metab*, 281(2), E335-40 (2001)

132. D. M. Thomson, J. D. Brown, N. Fillmore, B. M. Condon, H. J. Kim, J. R. Barrow and W. W. Winder: LKB1 and the regulation of malonyl-CoA and fatty acid oxidation in muscle. *Am J Physiol Endocrinol Metab*, 293(6), E1572-9 (2007)

133. M. A. Raney and L. P. Turcotte: Regulation of contraction-induced FA uptake and oxidation by AMPK and ERK1/2 is intensity dependent in rodent muscle. *Am J Physiol Endocrinol Metab*, 291(6), E1220-7 (2006)

134. J. Shearer, P. T. Fueger, B. Vorndick, D. P. Bracy, J. N. Rottman, J. A. Clanton and D. H. Wasserman: AMP

kinase-induced skeletal muscle glucose but not long-chain fatty acid uptake is dependent on nitric oxide. *Diabetes*, 53(6), 1429-35 (2004)

135. Z. P. Chen, G. K. McConell, B. J. Michell, R. J. Snow, B. J. Canny and B. E. Kemp: AMPK signaling in contracting human skeletal muscle: acetyl-CoA carboxylase and NO synthase phosphorylation. *Am J Physiol Endocrinol Metab*, 279(5), E1202-6 (2000)

136. Y. Minokoshi, Y. B. Kim, O. D. Peroni, L. G. Fryer, C. Muller, D. Carling and B. B. Kahn: Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature*, 415(6869), 339-43 (2002)

137. X. Yu, S. McCorkle, M. Wang, Y. Lee, J. Li, A. K. Saha, R. H. Unger and N. B. Ruderman: Leptinomimetic effects of the AMP kinase activator AICAR in leptin-resistant rats: prevention of diabetes and ectopic lipid deposition. *Diabetologia*, 47(11), 2012-21 (2004)

138. G. Brabant, G. Muller, R. Horn, C. Anderwald, M. Roden and H. Nave: Hepatic leptin signaling in obesity. *FASEB J*, 19(8), 1048-50 (2005)

139. L. L. Atkinson, M. A. Fischer and G. D. Lopaschuk: Leptin activates cardiac fatty acid oxidation independent of changes in the AMP-activated protein kinase-acetyl-CoA carboxylase-malonyl-CoA axis. *J Biol Chem*, 277(33), 29424-30 (2002)

140. Y. Lee, R. H. Naseem, L. Duplomb, B. H. Park, D. J. Garry, J. A. Richardson, J. E. Schaffer and R. H. Unger: Hyperleptinemia prevents lipotoxic cardiomyopathy in acyl CoA synthase transgenic mice. *Proc Natl Acad Sci U S A*, 101(37), 13624-9 (2004)

141. C. T. Lim, B. Kola and M. Korbonsits: AMPK as a mediator of hormonal signalling. *J Mol Endocrinol*, 44(2), 87-97

142. P. Huypens, K. Moens, H. Heimberg, Z. Ling, D. Pipeleers and M. Van de Castele: Adiponectin-mediated stimulation of AMP-activated protein kinase (AMPK) in pancreatic beta cells. *Life Sci*, 77(11), 1273-82 (2005)

143. R. Shibata, K. Sato, D. R. Pimentel, Y. Takemura, S. Kihara, K. Ohashi, T. Funahashi, N. Ouchi and K. Walsh: Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. *Nat Med*, 11(10), 1096-103 (2005)

144. Y. Liao, S. Takashima, N. Maeda, N. Ouchi, K. Komamura, I. Shimomura, M. Hori, Y. Matsuzawa, T. Funahashi and M. Kitakaze: Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism. *Cardiovasc Res*, 67(4), 705-13 (2005)

145. M. J. Vazquez, C. R. Gonzalez, L. Varela, R. Lage, S. Tovar, S. Sangiao-Alvarellos, L. M. Williams, A. Vidal-Puig, R. Nogueiras, M. Lopez and C. Dieguez: Central

resistin regulates hypothalamic and peripheral lipid metabolism in a nutritional-dependent fashion. *Endocrinology*, 149(9), 4534-43 (2008)

146. M. Christ-Crain, B. Kola, F. Lolli, C. Fekete, D. Seboek, G. Wittmann, D. Feltrin, S. C. Igreja, S. Ajodha, J. Harvey-White, G. Kunos, B. Muller, F. Pralong, G. Aubert, G. Arnaldi, G. Giacchetti, M. Boscaro, A. B. Grossman and M. Korbonits: AMP-activated protein kinase mediates glucocorticoid-induced metabolic changes: a novel mechanism in Cushing's syndrome. *FASEB J*, 22(6), 1672-83 (2008)

147. B. Kola: Role of AMP-activated protein kinase in the control of appetite. *J Neuroendocrinol*, 20(7), 942-51 (2008)

148. B. Kola, M. Christ-Crain, F. Lolli, G. Arnaldi, G. Giacchetti, M. Boscaro, A. B. Grossman and M. Korbonits: Changes in adenosine 5'-monophosphate-activated protein kinase as a mechanism of visceral obesity in Cushing's syndrome. *J Clin Endocrinol Metab*, 93(12), 4969-73 (2008)

149. C. Namkoong, M. S. Kim, P. G. Jang, S. M. Han, H. S. Park, E. H. Koh, W. J. Lee, J. Y. Kim, I. S. Park, J. Y. Park and K. U. Lee: Enhanced hypothalamic AMP-activated protein kinase activity contributes to hyperphagia in diabetic rats. *Diabetes*, 54(1), 63-8 (2005)

150. S. Horman, D. Vertommen, R. Heath, D. Neumann, V. Mouton, A. Woods, U. Schlattner, T. Wallimann, D. Carling, L. Hue and M. H. Rider: Insulin antagonizes ischemia-induced Thr172 phosphorylation of AMP-activated protein kinase alpha-subunits in heart via hierarchical phosphorylation of Ser485/491. *J Biol Chem*, 281(9), 5335-40 (2006)

151. K. Barnes, J. C. Ingram, O. H. Porras, L. F. Barros, E. R. Hudson, L. G. Fryer, F. Foufelle, D. Carling, D. G. Hardie and S. A. Baldwin: Activation of GLUT1 by metabolic and osmotic stress: potential involvement of AMP-activated protein kinase (AMPK). *J Cell Sci*, 115(Pt 11), 2433-42 (2002)

152. J. S. Fisher, J. Gao, D. H. Han, J. O. Holloszy and L. A. Nolte: Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. *Am J Physiol Endocrinol Metab*, 282(1), E18-23 (2002)

153. L. S. Harrington, G. M. Findlay, A. Gray, T. Tolkacheva, S. Wigfield, H. Rebholz, J. Barnett, N. R. Leslie, S. Cheng, P. R. Shepherd, I. Gout, C. P. Downes and R. F. Lamb: The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. *J Cell Biol*, 166(2), 213-23 (2004)

154. L. A. Barness, J. M. Opitz and E. Gilbert-Barness: Obesity: genetic, molecular, and environmental aspects. *Am J Med Genet A*, 143A(24), 3016-34 (2007)

E. S. Buhl, N. Jessen, R. Pold, T. Ledet, A. Flyvbjerg, S. B. Pedersen, O. Pedersen, O. Schmitz and S. Lund: Long-term AICAR administration reduces metabolic disturbances and lowers blood pressure in rats displaying features of the insulin resistance syndrome. *Diabetes*, 51(7), 2199-206 (2002)

155. L. Abu-Elheiga, M. M. Matzuk, K. A. Abo-Hashema and S. J. Wakil: Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. *Science*, 291(5513), 2613-6 (2001)

156. L. E. Hammond, P. A. Gallagher, S. Wang, S. Hiller, K. D. Kluckman, E. L. Posey-Marcos, N. Maeda and R. A. Coleman: Mitochondrial glycerol-3-phosphate acyltransferase-deficient mice have reduced weight and liver triacylglycerol content and altered glycerolipid fatty acid composition. *Mol Cell Biol*, 22(23), 8204-14 (2002)

157. X. Wu, H. Motoshima, K. Mahadev, T. J. Stalker, R. Scalia and B. J. Goldstein: Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. *Diabetes*, 52(6), 1355-63 (2003)

158. E. L. Greer, D. Dowlatshahi, M. R. Banko, J. Villen, K. Hoang, D. Blanchard, S. P. Gygi and A. Brunet: An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C. elegans*. *Curr Biol*, 17(19), 1646-56 (2007)

159. D. K. Ingram, R. M. Anson, R. de Cabo, J. Mamczarz, M. Zhu, J. Mattison, M. A. Lane and G. S. Roth: Development of calorie restriction mimetics as a prolongevity strategy. *Ann N Y Acad Sci*, 1019, 412-23 (2004)

160. V. N. Anisimov, L. M. Berstein, P. A. Egormin, T. S. Piskunova, I. G. Popovich, M. A. Zabezhinski, I. G. Kovalenko, T. E. Poroshina, A. V. Semchenko, M. Provinciali, F. Re and C. Franceschi: Effect of metformin on life span and on the development of spontaneous mammary tumors in HER-2/neu transgenic mice. *Exp Gerontol*, 40(8-9), 685-93 (2005)

161. V. N. Anisimov, P. A. Egormin, L. M. Bershtein, M. A. Zabezhinskii, T. S. Piskunova, I. G. Popovich and A. V. Semchenko: Metformin decelerates aging and development of mammary tumors in HER-2/neu transgenic mice. *Bull Exp Biol Med*, 139(6), 721-3 (2005)

162. S. T. Henderson, M. Bonafe and T. E. Johnson: daf-16 protects the nematode *Caenorhabditis elegans* during food deprivation. *J Gerontol A Biol Sci Med Sci*, 61(5), 444-60 (2006)

163. M. Hansen, S. Taubert, D. Crawford, N. Libina, S. J. Lee and C. Kenyon: Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. *Aging Cell*, 6(1), 95-110 (2007)

164. P. Kapahi, B. M. Zid, T. Harper, D. Koslover, V. Sapin and S. Benzer: Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. *Curr Biol*, 14(10), 885-90 (2004)
165. J. R. Fay, V. Steele and J. A. Crowell: Energy homeostasis and cancer prevention: the AMP-activated protein kinase. *Cancer Prev Res (Phila)*, 2(4), 301-9 (2009)
166. Z. Luo, A. K. Saha, X. Xiang and N. B. Ruderman: AMPK, the metabolic syndrome and cancer. *Trends Pharmacol Sci*, 26(2), 69-76 (2005)
167. D. G. Hardie: Role of AMP-activated protein kinase in the metabolic syndrome and in heart disease. *FEBS Lett*, 582(1), 81-9 (2008)
168. M. Sanchez-Cespedes: A role for LKB1 gene in human cancer beyond the Peutz-Jeghers syndrome. *Oncogene*, 26(57), 7825-32 (2007)
169. H. Fenton, B. Carlile, E. A. Montgomery, H. Carraway, J. Herman, F. Sahin, G. H. Su and P. Argani: LKB1 protein expression in human breast cancer. *Appl Immunohistochem Mol Morphol*, 14(2), 146-53 (2006)
170. X. Huang, S. Wullschlegel, N. Shpiro, V. A. McGuire, K. Sakamoto, Y. L. Woods, W. McBurnie, S. Fleming and D. R. Alessi: Important role of the LKB1-AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice. *Biochem J*, 412(2), 211-21 (2008)
171. K. Imamura, T. Ogura, A. Kishimoto, M. Kaminishi and H. Esumi: Cell cycle regulation via p53 phosphorylation by a 5'-AMP activated protein kinase activator, 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside, in a human hepatocellular carcinoma cell line. *Biochem Biophys Res Commun*, 287(2), 562-7 (2001)
172. X. Xiang, A. K. Saha, R. Wen, N. B. Ruderman and Z. Luo: AMP-activated protein kinase activators can inhibit the growth of prostate cancer cells by multiple mechanisms. *Biochem Biophys Res Commun*, 321(1), 161-7 (2004)
173. J. V. Swinnen, A. Beckers, K. Brusselmans, S. Organe, J. Segers, L. Timmermans, F. Vanderhoydonc, L. Deboel, R. Derua, E. Waelkens, E. De Schrijver, T. Van de Sande, A. Noel, F. Foulfelle and G. Verhoeven: Mimicry of a cellular low energy status blocks tumor cell anabolism and suppresses the malignant phenotype. *Cancer Res*, 65(6), 2441-8 (2005)
175. M. Buzzai, R. G. Jones, R. K. Amaravadi, J. J. Lum, R. J. DeBerardinis, F. Zhao, B. Viollet and C. B. Thompson: Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res*, 67(14), 6745-52 (2007)
176. D. Stapleton, K. I. Mitchelhill, G. Gao, J. Widmer, B. J. Michell, T. Teh, C. M. House, C. S. Fernandez, T. Cox, L. A. Witters and B. E. Kemp: Mammalian AMP-activated protein kinase subfamily. *J Biol Chem*, 271(2), 611-4 (1996)
177. C. Blazquez, M. J. Geelen, G. Velasco and M. Guzman: The AMP-activated protein kinase prevents ceramide synthesis de novo and apoptosis in astrocytes. *FEBS Lett*, 489(2-3), 149-53 (2001)
178. C. Lopez-Lopez, M. O. Dietrich, F. Metzger, H. Loetscher and I. Torres-Aleman: Disturbed cross talk between insulin-like growth factor I and AMP-activated protein kinase as a possible cause of vascular dysfunction in the amyloid precursor protein/presenilin 2 mouse model of Alzheimer's disease. *J Neurosci*, 27(4), 824-31 (2007)
179. C. Culmsee, J. Monnig, B. E. Kemp and M. P. Mattson: AMP-activated protein kinase is highly expressed in neurons in the developing rat brain and promotes neuronal survival following glucose deprivation. *J Mol Neurosci*, 17(1), 45-58 (2001)
180. J. Li, Z. Zeng, B. Viollet, G. V. Ronnett and L. D. McCullough: Neuroprotective effects of adenosine monophosphate-activated protein kinase inhibition and gene deletion in stroke. *Stroke*, 38(11), 2992-9 (2007)
181. S. J. Greco, S. Sarkar, G. Casadesus, X. Zhu, M. A. Smith, J. W. Ashford, J. M. Johnston and N. Tezapsidis: Leptin inhibits glycogen synthase kinase-3beta to prevent tau phosphorylation in neuronal cells. *Neurosci Lett*, 455(3), 191-4 (2009)
182. T. C. Ma, J. L. Buescher, B. Oatis, J. A. Funk, A. J. Nash, R. L. Carrier and K. R. Hoyt: Metformin therapy in a transgenic mouse model of Huntington's disease. *Neurosci Lett*, 411(2), 98-103 (2007)
183. Y. Chen, K. Zhou, R. Wang, Y. Liu, Y. D. Kwak, T. Ma, R. C. Thompson, Y. Zhao, L. Smith, L. Gasparini, Z. Luo, H. Xu and F. F. Liao: Antidiabetic drug metformin (GlucophageR) increases biogenesis of Alzheimer's amyloid peptides via up-regulating BACE1 transcription. *Proc Natl Acad Sci U S A*, 106(10), 3907-12 (2009)
184. L. A. Profenno, A. P. Porsteinsson and S. V. Faraone: Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders. *Biol Psychiatry*, 67(6), 505-12 (2010)
185. W. D. Cheung and G. W. Hart: AMP-activated protein kinase and p38 MAPK activate O-GlcNAcylation of neuronal proteins during glucose deprivation. *J Biol Chem*, 283(19), 13009-20 (2008)
186. W. W. Winder and D. G. Hardie: AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. *Am J Physiol*, 277(1 Pt 1), E1-10 (1999)
187. J. W. Calvert, S. Gundewar, S. Jha, J. J. Greer, W. H. Besterman, R. Tian and D. J. Lefer: Acute metformin therapy confers cardioprotection against myocardial

infarction via AMPK-eNOS-mediated signaling. *Diabetes*, 57(3), 696-705 (2008)

188. C. Bonnard, A. Durand, H. Vidal and J. Rieusset: Changes in adiponectin, its receptors and AMPK activity in tissues of diet-induced diabetic mice. *Diabetes Metab*, 34(1), 52-61 (2008)

189. S. Wang, J. Xu, P. Song, B. Viollet and M. H. Zou: *In vivo* activation of AMP-activated protein kinase attenuates diabetes-enhanced degradation of GTP cyclohydrolase I. *Diabetes*, 58(8), 1893-901 (2009)

190. Z. Xie, J. Zhang, J. Wu, B. Viollet and M. H. Zou: Upregulation of mitochondrial uncoupling protein-2 by the AMP-activated protein kinase in endothelial cells attenuates oxidative stress in diabetes. *Diabetes*, 57(12), 3222-30 (2008)

191. Q. Li, J. Li and J. Ren: UCF-101 mitigates streptozotocin-induced cardiomyocyte dysfunction: role of AMPK. *Am J Physiol Endocrinol Metab*, 297(4), E965-73 (2009)

192. R. Bergeron, R. R. Russell, 3rd, L. H. Young, J. M. Ren, M. Marcucci, A. Lee and G. I. Shulman: Effect of AMPK activation on muscle glucose metabolism in conscious rats. *Am J Physiol*, 276(5 Pt 1), E938-44 (1999)

193. J. Mu, J. T. Brozinick, Jr., O. Valladares, M. Bucan and M. J. Birnbaum: A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. *Mol Cell*, 7(5), 1085-94 (2001)

194. M. A. Iglesias, J. M. Ye, G. Frangioudakis, A. K. Saha, E. Tomas, N. B. Ruderman, G. J. Cooney and E. W. Kraegen: AICAR administration causes an apparent enhancement of muscle and liver insulin action in insulin-resistant high-fat-fed rats. *Diabetes*, 51(10), 2886-94 (2002)

195. A. Y. Chan, C. L. Soltys, M. E. Young, C. G. Proud and J. R. Dyck: Activation of AMP-activated protein kinase inhibits protein synthesis associated with hypertrophy in the cardiac myocyte. *J Biol Chem*, 279(31), 32771-9 (2004)

196. R. Shibata, N. Ouchi, S. Kihara, K. Sato, T. Funahashi and K. Walsh: Adiponectin stimulates angiogenesis in response to tissue ischemia through stimulation of amp-activated protein kinase signaling. *J Biol Chem*, 279(27), 28670-4 (2004)

197. I. Sasaki, M. Tomizawa, B. Kudo and M. Ogasawara: [Study on the typing and epidemiology of a mass occurrence of *Shigella sonnei*]. *Nihon Densenbyo Gakkai Zasshi*, 41(3), 115-21 (1967)

198. R. R. Russell, 3rd, R. Bergeron, G. I. Shulman and L. H. Young: Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR. *Am J Physiol*, 277(2 Pt 2), H643-9 (1999)

199. Y. Xing, N. Musi, N. Fujii, L. Zou, I. Luptak, M. F. Hirshman, L. J. Goodyear and R. Tian: Glucose metabolism and energy homeostasis in mouse hearts

overexpressing dominant negative alpha2 subunit of AMP-activated protein kinase. *J Biol Chem*, 278(31), 28372-7 (2003)

200. R. R. Russell, 3rd, J. Li, D. L. Coven, M. Pypaert, C. Zechner, M. Palmeri, F. J. Giordano, J. Mu, M. J. Birnbaum and L. H. Young: AMP-activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury. *J Clin Invest*, 114(4), 495-503 (2004)

201. J. M. Cacicedo, N. Yagihashi, J. F. Keaney, Jr., N. B. Ruderman and Y. Ido: AMPK inhibits fatty acid-induced increases in NF-kappaB transactivation in cultured human umbilical vein endothelial cells. *Biochem Biophys Res Commun*, 324(4), 1204-9 (2004)

202. M. Dixit, E. Bess, B. Fisslthaler, F. V. Hartel, T. Noll, R. Busse and I. Fleming: Shear stress-induced activation of the AMP-activated protein kinase regulates FoxO1a and angiopoietin-2 in endothelial cells. *Cardiovasc Res*, 77(1), 160-8 (2008)

203. Y. Hattori, Y. Nakano, S. Hattori, A. Tomizawa, K. Inukai and K. Kasai: High molecular weight adiponectin activates AMPK and suppresses cytokine-induced NF-kappaB activation in vascular endothelial cells. *FEBS Lett*, 582(12), 1719-24 (2008)

204. E. Schulz, J. Dopheide, S. Schuhmacher, S. R. Thomas, K. Chen, A. Daiber, P. Wenzel, T. Munzel and J. F. Keaney, Jr.: Suppression of the JNK pathway by induction of a metabolic stress response prevents vascular injury and dysfunction. *Circulation*, 118(13), 1347-57 (2008)

205. S. L. Colombo and S. Moncada: AMPKalpha1 regulates the antioxidant status of vascular endothelial cells. *Biochem J*, 421(2), 163-9 (2009)

206. G. Alba, R. El Bekay, M. Alvarez-Maqueda, P. Chacon, A. Vega, J. Monteseirin, C. Santa Maria, E. Pintado, F. J. Bedoya, R. Bartrons and F. Sobrino: Stimulators of AMP-activated protein kinase inhibit the respiratory burst in human neutrophils. *FEBS Lett*, 573(1-3), 219-25 (2004)

207. G. Ceolotto, A. Gallo, I. Papparella, L. Franco, E. Murphy, E. Iori, E. Pagnin, G. P. Fadini, M. Albiero, A. Semplicini and A. Avogaro: Rosiglitazone reduces glucose-induced oxidative stress mediated by NAD(P)H oxidase via AMPK-dependent mechanism. *Arterioscler Thromb Vasc Biol*, 27(12), 2627-33 (2007)

208. D. Nagata, R. Takeda, M. Sata, H. Satonaka, E. Suzuki, T. Nagano and Y. Hirata: AMP-activated protein kinase inhibits angiotensin II-stimulated vascular smooth muscle cell proliferation. *Circulation*, 110(4), 444-51 (2004)

209. B. J. Stuck, M. Lenski, M. Bohm and U. Laufs: Metabolic switch and hypertrophy of cardiomyocytes

following treatment with angiotensin II are prevented by AMP-activated protein kinase. *J Biol Chem*, 283(47), 32562-9 (2008)

210. Z. Chen, I. C. Peng, W. Sun, M. I. Su, P. H. Hsu, Y. Fu, Y. Zhu, K. DeFea, S. Pan, M. D. Tsai and J. Y. Shyy: AMP-activated protein kinase functionally phosphorylates endothelial nitric oxide synthase Ser633. *Circ Res*, 104(4), 496-505 (2009)

211. B. J. Michell, Z. Chen, T. Tiganis, D. Stapleton, F. Katsis, D. A. Power, A. T. Sim and B. E. Kemp: Coordinated control of endothelial nitric-oxide synthase phosphorylation by protein kinase C and the cAMP-dependent protein kinase. *J Biol Chem*, 276(21), 17625-8 (2001)

212. D. Nagata, M. Mogi and K. Walsh: AMP-activated protein kinase (AMPK) signaling in endothelial cells is essential for angiogenesis in response to hypoxic stress. *J Biol Chem*, 278(33), 31000-6 (2003)

213. B. Thors, H. Halldorsson and G. Thorgeirsson: Thrombin and histamine stimulate endothelial nitric-oxide synthase phosphorylation at Ser1177 via an AMPK mediated pathway independent of PI3K-Akt. *FEBS Lett*, 573(1-3), 175-80 (2004)

214. I. Fleming, B. Fisslthaler, M. Dixit and R. Busse: Role of PECAM-1 in the shear-stress-induced activation of Akt and the endothelial nitric oxide synthase (eNOS) in endothelial cells. *J Cell Sci*, 118(Pt 18), 4103-11 (2005)

215. B. Fisslthaler, I. Fleming, B. Keseru, K. Walsh and R. Busse: Fluid shear stress and NO decrease the activity of the hydroxy-methylglutaryl coenzyme A reductase in endothelial cells via the AMP-activated protein kinase and FoxO1. *Circ Res*, 100(2), e12-21 (2007)

216. V. A. Morrow, F. Fougelle, J. M. Connell, J. R. Petrie, G. W. Gould and I. P. Salt: Direct activation of AMP-activated protein kinase stimulates nitric-oxide synthesis in human aortic endothelial cells. *J Biol Chem*, 278(34), 31629-39 (2003)

217. K. K. Cheng, K. S. Lam, Y. Wang, Y. Huang, D. Carling, D. Wu, C. Wong and A. Xu: Adiponectin-induced endothelial nitric oxide synthase activation and nitric oxide production are mediated by APPL1 in endothelial cells. *Diabetes*, 56(5), 1387-94 (2007)

218. J. A. Reihill, M. A. Ewart, D. G. Hardie and I. P. Salt: AMP-activated protein kinase mediates VEGF-stimulated endothelial NO production. *Biochem Biophys Res Commun*, 354(4), 1084-8 (2007)

219. J. G. Boyle, P. J. Logan, M. A. Ewart, J. A. Reihill, S. A. Ritchie, J. M. Connell, S. J. Cleland and I. P. Salt: Rosiglitazone stimulates nitric oxide synthesis in human aortic endothelial cells via AMP-activated protein kinase. *J Biol Chem*, 283(17), 11210-7 (2008)

220. M. H. Zou, S. S. Kirkpatrick, B. J. Davis, J. S. Nelson, W. G. t. Wiles, U. Schlattner, D. Neumann, M. Brownlee, M. B. Freeman and M. H. Goldman: Activation of the

AMP-activated protein kinase by the anti-diabetic drug metformin *in vivo*. Role of mitochondrial reactive nitrogen species. *J Biol Chem*, 279(42), 43940-51 (2004)

221. B. J. Davis, Z. Xie, B. Viollet and M. H. Zou: Activation of the AMP-activated kinase by antidiabetic drug metformin stimulates nitric oxide synthesis *in vivo* by promoting the association of heat shock protein 90 and endothelial nitric oxide synthase. *Diabetes*, 55(2), 496-505 (2006)

222. J. Zhang, Z. Xie, Y. Dong, S. Wang, C. Liu and M. H. Zou: Identification of nitric oxide as an endogenous activator of the AMP-activated protein kinase in vascular endothelial cells. *J Biol Chem*, 283(41), 27452-61 (2008)

223. A. C. Webler, U. R. Michaelis, R. Popp, E. Barbosa-Sicard, A. Murugan, J. R. Falck, B. Fisslthaler and I. Fleming: Epoxyeicosatrienoic acids are part of the VEGF-activated signaling cascade leading to angiogenesis. *Am J Physiol Cell Physiol*, 295(5), C1292-301 (2008)

224. N. Ouchi, R. Shibata and K. Walsh: AMP-activated protein kinase signaling stimulates VEGF expression and angiogenesis in skeletal muscle. *Circ Res*, 96(8), 838-46 (2005)

225. L. Leick, Y. Hellsten, J. Fentz, S. S. Lyngby, J. F. Wojtaszewski, J. Hidalgo and H. Pilegaard: PGC-1 α mediates exercise-induced skeletal muscle VEGF expression in mice. *Am J Physiol Endocrinol Metab*, 297(1), E92-103 (2009)

226. P. V. Katakam, M. R. Ujhelyi, M. Hoenig and A. W. Miller: Metformin improves vascular function in insulin-resistant rats. *Hypertension*, 35(1 Pt 1), 108-12 (2000)

227. J. L. Sartoretto, G. A. Melo, M. H. Carvalho, D. Nigro, R. T. Passaglia, C. Scavone, R. K. Cuman and Z. B. Fortes: Metformin treatment restores the altered microvascular reactivity in neonatal streptozotocin-induced diabetic rats increasing NOS activity, but not NOS expression. *Life Sci*, 77(21), 2676-89 (2005)

228. S. M. Blattler, F. Rencurel, M. R. Kaufmann and U. A. Meyer: In the regulation of cytochrome P450 genes, phenobarbital targets LKB1 for necessary activation of AMP-activated protein kinase. *Proc Natl Acad Sci U S A*, 104(3), 1045-50 (2007)

229. F. Rencurel, M. Foretz, M. R. Kaufmann, D. Stroka, R. Looser, I. Leclerc, G. da Silva Xavier, G. A. Rutter, B. Viollet and U. A. Meyer: Stimulation of AMP-activated protein kinase is essential for the induction of drug metabolizing enzymes by phenobarbital in human and mouse liver. *Mol Pharmacol*, 70(6), 1925-34 (2006)

230. F. Goirand, M. Solar, Y. Atea, B. Viollet, P. Mateo, D. Fortin, J. Leclerc, J. Hoerter, R. Ventura-Clapier and A. Garnier: Activation of AMP kinase α 1 subunit induces

aortic vasorelaxation in mice. *J Physiol*, 581(Pt 3), 1163-71 (2007)

231. L. J. Rubin, L. Magliola, X. Feng, A. W. Jones and C. C. Hale: Metabolic activation of AMP kinase in vascular smooth muscle. *J Appl Physiol*, 98(1), 296-306 (2005)

232. M. F. McCarty: AMPK activation as a strategy for reversing the endothelial lipotoxicity underlying the increased vascular risk associated with insulin resistance syndrome. *Med Hypotheses*, 64(6), 1211-5 (2005)

233. P. M. Brito, R. Devillard, A. Negre-Salvayre, L. M. Almeida, T. C. Dinis, R. Salvayre and N. Auge: Resveratrol inhibits the mTOR mitogenic signaling evoked by oxidized LDL in smooth muscle cells. *Atherosclerosis*, 205(1), 126-34 (2009)

234. M. H. Zou, X. Y. Hou, C. M. Shi, S. Kirkpatrick, F. Liu, M. H. Goldman and R. A. Cohen: Activation of 5'-AMP-activated kinase is mediated through c-Src and phosphoinositide 3-kinase activity during hypoxia-reoxygenation of bovine aortic endothelial cells. Role of peroxynitrite. *J Biol Chem*, 278(36), 34003-10 (2003)

235. M. Zhang, Y. Dong, J. Xu, Z. Xie, Y. Wu, P. Song, M. Guzman, J. Wu and M. H. Zou: Thromboxane receptor activates the AMP-activated protein kinase in vascular smooth muscle cells via hydrogen peroxide. *Circ Res*, 102(3), 328-37 (2008)

236. Z. Xie, Y. Dong, R. Scholz, D. Neumann and M. H. Zou: Phosphorylation of LKB1 at serine 428 by protein kinase C-zeta is required for metformin-enhanced activation of the AMP-activated protein kinase in endothelial cells. *Circulation*, 117(7), 952-62 (2008)

237. H. C. Choi, P. Song, Z. Xie, Y. Wu, J. Xu, M. Zhang, Y. Dong, S. Wang, K. Lau and M. H. Zou: Reactive nitrogen species is required for the activation of the AMP-activated protein kinase by statin *in vivo*. *J Biol Chem*, 283(29), 20186-97 (2008)

238. B. Viollet, R. Mounier, J. Leclerc, A. Yazigi, M. Foretz and F. Andreelli: Targeting AMP-activated protein kinase as a novel therapeutic approach for the treatment of metabolic disorders. *Diabetes Metab*, 33(6), 395-402 (2007)

239. M. Zang, A. Zuccollo, X. Hou, D. Nagata, K. Walsh, H. Herscovitz, P. Brecher, N. B. Ruderman and R. A. Cohen: AMP-activated protein kinase is required for the lipid-lowering effect of metformin in insulin-resistant human HepG2 cells. *J Biol Chem*, 279(46), 47898-905 (2004)

240. M. Zang, S. Xu, K. A. Maitland-Toolan, A. Zuccollo, X. Hou, B. Jiang, M. Wierzbicki, T. J. Verbeuren and R. A. Cohen: Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. *Diabetes*, 55(8), 2180-91 (2006)

241. C. J. Bailey: Metformin: a multitasking medication. *Diab Vasc Dis Res*, 5(3), 156 (2008)

242. T. Matsumoto, E. Noguchi, K. Ishida, T. Kobayashi, N. Yamada and K. Kamata: Metformin normalizes endothelial function by suppressing vasoconstrictor prostanoids in mesenteric arteries from OLETF rats, a model of type 2 diabetes. *Am J Physiol Heart Circ Physiol*, 295(3), H1165-H1176 (2008)

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Send correspondence to: Ming-Hui Zou, Department of Medicine, University of Oklahoma Health Science Center, 941 Stanton L. Young Blvd., Oklahoma City, OK 73104, USA, Tel: 405-271-3974, Fax: 405-271-3973, E-mail: ming-hui-zou@ouhsc.edu