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REVIEW ARTICLE

AMPK inhibition in health and disease

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Abstract

All living organisms depend on dynamic mechanisms that repeatedly reassess the status of amassed energy, in order to adapt energy supply to demand. The AMP-activated protein kinase (AMPK) αβγ heterotrimer has emerged as an important integrator of signals managing energy balance. Control of AMPK activity involves allosteric AMP and ATP regulation, auto-inhibitory features and phosphorylation of its catalytic (α) and regulatory (β and γ) subunits. AMPK has a prominent role not only as a peripheral sensor but also in the central nervous system as a multifunctional metabolic regulator. AMPK represents an ideal second messenger for reporting cellular energy state. For this reason, activated AMPK acts as a protective response to energy stress in numerous systems. However, AMPK inhibition also actively participates in the control of whole body energy homeostasis. In this review, we discuss recent findings that support the role and function of AMPK inhibition under physiological and pathological states.

Keywords: *Energy balance; metabolism; inhibition; AMPK; metabolic diseases*

Introduction

The survival of all organisms depends on the dynamic control of energy metabolism during acute or prolonged shortage of nutrient supply. Over the past years, the AMP-activated protein kinase 5'-adenosine monophosphate-activated protein kinase (AMPK) has emerged as an important regulator of cellular energy homeostasis that coordinates metabolic pathways in order to balance nutrient supply with energy demand in mammalian cells. AMPK is a homolog of Snf1 kinase, a *Saccharomyces cerevisiae* metabolic stress sensing kinase that is critical for yeast survival under conditions of glucose starvation (Woods *et al.*, 1994). AMPK is an energy-sensing protein complex, activated in response to an increase in the adenosine 5'-monophosphate (AMP): adenosine 5'-triphosphate (ATP) ratio during hypoxia, starvation, glucose deprivation or muscle contraction (Kahn *et al.*, 2005). AMPK integrates nutritional and hormonal signals to maintain cellular energy balance and execute appropriate metabolic functions (e.g. regulation of fatty acids partitioning between oxidative and biosynthetic pathways) in response to nutritional and environmental variations (Viollet *et al.*, 2009). One mechanism by which AMPK regulates lipid metabolism is phosphorylation and inactivation of acetyl CoA carboxylase (ACC), an important rate-controlling enzyme for the synthesis of malonyl-CoA. ACC is both a critical precursor for biosynthesis of fatty acids and a potent inhibitor of long-chain fatty acyl-CoA transport to mitochondria for β-oxidation. Knockdown/knockout of ACC1 and ACC2 (predominantly expressed in liver and skeletal muscle, respectively), has been reported to cause continuous fatty acid oxidation, increased energy expenditure and reduced fat mass (Abu-Elheiga *et al.*, 2001; Choi *et al.*, 2007; Savage *et al.*, 2006). But recent studies have reported limited effects of ACC2 deletion on fatty acid oxidation in skeletal muscle and overall energy expenditure or adiposity (Hoehn *et al.*, 2010; Olson *et al.*, 2010). These recent reports indicate that increased fatty

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acid oxidation in skeletal muscle does not cause leanness and raises questions regarding the use of ACC2 inhibitors in the treatment of obesity. However, the use of a small molecule direct AMPK activator A-769662 was shown to have beneficial effects on both hepatic steatosis and insulin resistance, thus emphasizing the potential therapeutic implications for AMPK activation in type 2 diabetes (Cool *et al.*, 2006). Regardless, AMPK activation results in inhibition of energy-consuming biosynthetic pathways (such as fatty acid synthesis in adipocytes, cholesterol synthesis in the liver and insulin secretion from β-cells) and activation of ATP-producing catabolic pathways (such as fatty acid uptake and oxidation in multiple tissues, glycolysis in heart and mitochondrial biogenesis in muscle). AMPK also modulates transcription of specific genes involved in energy metabolism, thereby exerting long-term metabolic control (Viollet *et al.*, 2006). It is also implicated in the central regulation of food intake and energy expenditure in response to hormonal cues including leptin, ghrelin and adiponectin. Thus, AMPK not only governs cellular energy, but regulates overall organismal bio-energetics by coordinating the response in and inbetween tissues according to nutritional input. Its position at crossroads of energy metabolism makes AMPK an attractive therapeutic target in metabolic diseases, with its pharmaceutical potential in situations of insulin resistance already confirmed. It has therefore emerged as a promising new drug target for the treatment of metabolic disorders including obesity, Type 2 diabetes and cardiovascular disease.

Due to its role in maintaining energy balance, a dysfunction in AMPK signaling pathway may result in perturbations at the systemic level that contribute to development of metabolic disorders. In support, there is a strong correlation between low AMPK activation state, mainly due to over-nutrition and lack of exercise, and metabolic disorders associated with insulin resistance, obesity and sedentary lifestyle (Ruderman and Prentki, 2004). Furthermore, decreased AMPK activation is implicated in human metabolic disorders associated with increased cancer risk. Numerous studies show links between AMPK and cancer, both at the organism and molecular levels. Although the contribution of AMPK to the etiology of these disorders is unclear, pharmacologic AMPK activators are effective in their treatment. Interestingly, recent evidence indicates that AMPK participates in the regulation of non-metabolic processes such as cell growth, cell cycle progression and organization of the cytoskeleton (Williams and Brenman, 2008). Since all these are highly energy-consuming processes, AMPK's involvement is certain, due to its ability to directly sense and regulate cellular energy homeostasis. AMPK signaling pathway serves as a metabolic checkpoint in the cell, arresting cell growth when there is low energy status, such as in low nutrient conditions (Jones *et al.*, 2005). Moreover, recent studies in lower eukaryotes demonstrated that AMPK is involved in the regulation of epithelial cell polarity and mitotic cell division (Lee, JH *et al.*, 2007).

Although AMPK activation is an adaptive response to energy stress in numerous systems, AMPK plays a role in both physiological and pathophysiological states. Here, we review our understanding of AMPK inhibition in response to cellular and organismal energy challenges and describe the beneficial as well as adverse consequences of global AMPK modulation.

A. Structure and regulation of AMPK

1. Structure and subunit composition

AMPK exists in the cell as a heterotrimeric complex with one catalytic (α, 63 kDa) and two regulatory subunits (β, 30 kDa, and γ, 38–63 kDa) in a 1α:1β:1γ ratio (Figure 1). The α subunit contains a conventional serine/threonine kinase domain at the N-terminus followed by an auto-inhibitory domain, and a C-terminus containing the domains required for binding of β and γ subunits (Crute *et al.*, 1998). The β subunit contains two characterized elements, a central domain ensuring the binding of AMPK complexes to glycogen (Hudson *et al.*, 2003; Polekhina *et al.*, 2003) and a C-terminal region acting as a tethering domain for α and γ subunits (Iseli *et al.*, 2005; Townley and Shapiro, 2007). The γ subunit contains a variable N-terminal region followed by four highly conserved cystathionine-β-synthase (CBS) sequence repeats (small motifs found in tandem pairs termed Bateman domains (Bateman, 1997)), capable of binding adenine nucleotides, such as AMP or ATP (Scott *et al.*, 2004; Townley and Shapiro, 2007; Xiao *et al.*, 2007). Further isoforms have been identified for each of the three AMPK subunits (α1, α2, β1, β2, γ1, γ2, γ3 with splice variants for the γ2 and γ3 isoforms adding to the diversity), encoded by distinct genes and theoretically leading to formation of at least 12 different complexes. These combinations confer different properties to the AMPK complexes through differences in subcellular localization and signaling functions (Cheung *et al.*, 2000; Salt *et al.*, 1998a). Thus, the tissue-specific subunit composition may be important to determine a specialized cellular and systemic response to different metabolic stresses. Recent investigation of isoform composition of AMPK complexes in human skeletal muscle found that only three of the 12 potential AMPK complexes were present (α2β2γ1 >> α2β2γ3 = α1β2γ1) and were activated differently, depending on exercise intensity and duration (Birk and Wojtaszewski, 2006). AMPKα1 catalytic subunit expression is relatively distributed across adipose tissue, pancreas, lung, spleen and kidney. Skeletal

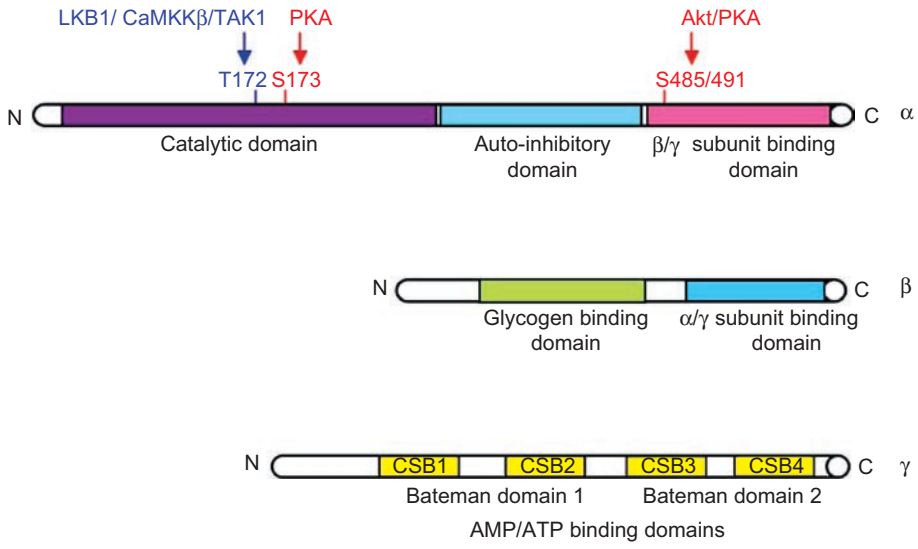

Figure 1. Domain organization of the catalytic and regulatory and subunits of AMPK. Residues phosphorylated by AMPKK (LKB1, CaMKK, TAK1), PKA and Akt are shown within the subunit.

and cardiac muscles predominantly express AMPKα2 catalytic subunit. While the β1 subunit is ubiquitously expressed, AMPK β2 subunit is abundantly expressed in skeletal muscle and heart. Interestingly, expression of the γ3 subunit appears highly specific to glycolytic skeletal muscle whereas γ1 and γ2 show broad tissue distribution (Cheung *et al.*, 2000).

2. Allosteric regulation by AMP and ATP

AMPK is allosterically activated by AMP, which binds to the regulatory γ subunit, resulting in a 2–5-fold increase in activity compared to basal activity (Hardie *et al.*, 1999). The degree of activation by AMP is markedly affected by nature of the catalytic α and regulatory γ iso-forms constituting the AMPK complex, illustrating the complexity of AMPK signaling regulation. The greatest scale of activation is observed in AMPK complexes containing the α2 and γ2 subunits, while complexes containing the γ3 isoform are only weakly activated by AMP (Cheung *et al.*, 2000). Binding of AMP to the γ subunit causes direct allosteric activation of the kinase and also induces a conformational change in the kinase domain that protects AMPK from dephosphorylation of Thr-172 (Suter *et al.*, 2006; Sanders *et al.*, 2007; Riek *et al.*, 2008), favoring accumulation of the phosphorylated active form of AMPK (see below). Interestingly, it has been demonstrated that high concentrations of ATP oppose activation of the AMPK complex by AMP, suggesting that the allosteric sites bind AMP and ATP in a mutually exclusive manner (Corton *et al.*, 1995). Thus, AMPK can be considered more as a sensor of the intracellular AMP/ATP ratio, than a direct sensor of AMP levels. AMP and ATP vary reciprocally in cells, due to the action of adenylate kinase (AMP + ATP ↔ 2ADP [adenosine 5'-diphosphate]), thus, AMP:ATP ratio may be a more sensitive indicator of cellular energy status than ADP:ATP ratio. The finding that AMPK activation is altered in contracting muscles from adenylate kinase-deficient mice also supports a role for adenylate kinase in generation of an AMPK activating signal (Hancock *et al.*, 2006).

The crystal structures of mammalian γ and yeast homolog revealed the structural and conformational elements required for binding the regulatory nucleotides AMP and ATP (Townley and Shapiro, 2007; Xiao *et al.*, 2007). Out of all the CBS domains present, two CBS domains appear to bind AMP or ATP reversibly and may correspond to the two regulatory sites identified from previous binding studies (Scott *et al.*, 2004). A third CBS domain binds AMP very strongly and does not readily exchange with ATP, but its physiological role is unclear. The fourth CBS domain remains unoccupied even in the presence of high concentrations of AMP or ATP (Xiao *et al.*, 2007). Several naturally occurring point mutations in the CBS domains of human γ2 isoform have been reported to cause an inherited syndrome of hypertrophic cardiomyopathy of varying severity associated with excessive glycogen storage in cardio myocytes, accompanied with Wolff–Parkinson–White syndrome, a pre-excitation disorder (Arad *et al.*, 2007). Biochemical studies demonstrate that some of these mutations interfere with the binding of AMP and allosteric activation (Scott *et al.*, 2004) of AMPK. This supports the evidence that Bateman domains (CBS domains) constitute the regulatory binding sites for AMP. Interestingly, although mutations in the γ2 subunit reduce binding of the activating nucleotide AMP, they also appear to increase the basal activity associated with elevated Thr-172 phosphorylation (Arad

et al., 2002; Burwinkel *et al.*, 2005). This is presumably due to a concomitant reduction in binding of the inhibitory nucleotide ATP and consequent reduction in phosphatase activity, thus hindering kinase inactivation (see below). Therefore, γ2 mutants, unoccupied by ATP, behave in a partially active conformation and this "gain-of-function" effect could explain the dominant nature of γ subunit mutations (Hamilton *et al.*, 2001; Burwinkel *et al.*, 2005). Hence, AMPK dysregulation could be connected with impaired binding or interaction of both AMP and ATP on the γ subunit.

AMPK is also allosterically inhibited by physiological concentrations of phosphocreatine (Ponticos *et al.*, 1998), consistent with the proposed physiological role of the kinase as a sensor of cellular energy status. As it decreases during muscle contraction, phosphocreatine, rather than AMP, may be the key regulator of the AMPK system during short-term exercise.

3. Autoregulation of AMPK complexes

AMPK, like other protein kinases, autoregulates its own activity through structural elements that directly block its catalytic site. Within the catalytic α subunit, a region that is C-terminal to the kinase domain appears to act as an auto-inhibitory domain (AID) by interfering with kinase substrate binding and catalytic function (Crute *et al.*, 1998). Detailed mutagenesis studies provide evidence that a conserved short segment of the α subunit [α1-(313–335)], forming an α helix, binds to the kinase domain in an inactive conformation and is responsible for auto-inhibition (Pang *et al.*, 2007). Furthermore, three-dimensional structural studies revealed that hydrophobic contacts between the kinase domain and the AID have a predominant role in the allosteric control by AMP (Chen, L *et al.*, 2009). Upon binding of AMP, conformational change between low and high activity forms of AMPK alters the interaction between AID and kinase domains and eventually removes the effect of AID on kinase activation and also Thr172 dephosphorylation (Chen, L *et al.*, 2009). This mechanism of AMPK inhibition highlights the potential to develop small compounds that activate AMPK by antagonizing the auto-inhibitory role of AID (Pang *et al.*, 2008). In addition to the AID, it has been suggested that AMPK is also inhibited by an internal auto-inhibitory sequence similar to the consensus recognition motif for AMPK substrates but lacking a phosphoryl-able amino acid. Scott and coworkers proposed that, in the absence of AMP, a pseudo-substrate sequence within the γ2 CBS2 sub-domain binds to the catalytic groove of AMPKα, preventing phosphorylation by the upstream kinase, and therefore access to downstream targets (Scott *et al.*, 2007). When AMP binds to the γ subunit, a conformational change prevents the interaction of the pseudo-substrate sequence with the kinase domain, and thus causes activation of AMPK.

4. Regulation by phosphorylation/dephosphorylation

In addition to allosteric activation, AMPK is regulated by reversible phosphorylation (Figure 2). The key step in AMPK activation is its phosphorylation on threonine residue 172 (Thr-172), within the catalytic domain, by upstream kinases. The combination of the allosteric and phosphorylation effects causes > 1000-fold increase in kinase activity (compared to ≤ 5-fold for allosteric activation alone), allowing high sensitivity in responses to small changes in cellular energy status (Suter *et al.*, 2006). Three AMPK upstream kinases (AMPKKs) have been identified to date. The primary AMPKK is a complex between the tumor suppressor, LKB1, and two accessory subunits, STRAD and MO25 (Hawley *et al.*, 2003; Woods *et al.*, 2003a). LKB1 also functions upstream of 12 other kinases (AMPK-related kinases) situated on the same family as AMPK by phylogenetic analysis of kinase domain sequences (Lizcano *et al.*, 2004). The LKB1 protein kinase activity appears to be constitutively active and is not regulated by AMP (Lizcano *et al.*, 2004; Sakamoto *et al.*, 2004). This view was recently challenged with studies showing that the subcellular localization of LKB1 and consequently its activity may be modifiable. It has been suggested that SIRT1, one of the seven mammalian NAD(+)-dependent deacetylase silent mating type information regulator 2 ortholog (sirtuin) genes (Howitz *et al.*, 2003), promotes LKB1-dependent AMPK stimulation through direct deacetylation and increased cytoplasmic/nuclear ratio of LKB1 (Lan *et al.*, 2008). Also, recently it was shown that Fyn kinase phosphorylation of LKB1 on Tyr265 and Tyr365 residues results in cytoplasmic distribution of LKB1 and increased AMPK phosphorylation (Yamada *et al.*, 2010). Binding of AMP to AMPK promotes LKB1-dependent phosphorylation of Thr-172 through inhibition of dephosphorylation (by making AMPK complex a less efficient substrate for protein phosphatases) and produces a large effect on kinase activity by allosterically activating the phosphorylated form of AMPK. In addition, Ca2+/calmodulin-dependent protein kinase kinase β (CaMKKβ) has also been identified as a separate AMPK kinase (Hawley *et al.*, 2005, Hurley *et al.*, 2005, Woods *et al.*, 2005), that phosphorylates and activates AMPK in response to elevated intracellular Ca2+ concentrations, independent of any change in cellular AMP/ATP ratio. TGF-β-activated kinase 1 (TAK1) has also been recently implicated in the regulation of AMPK activity, although the physiological conditions during which TAK1 regulates AMPK are unclear (Xie *et al.*, 2006, Momcilovic *et al.*, 2006).

While α-Thr-172 is the major AMPK phosphorylation and activation site, α and β subunits are phosphorylated at multiple sites (Mitchelhill *et al.*, 1997; Woods *et al.*, 2003b; Villen *et al.*, 2007); however, the physiological relevance of these sites remains unclear. Recent studies provide evidence that direct phosphorylation of AMPKα1/α2 at Ser485/491 antagonizes its activation and correlates

with inhibition of AMPK activity during insulin signaling in the heart (Horman *et al.*, 2006) and cAMP-mediated signaling in an insulin-secreting cell line (Hurley *et al.*, 2006). A hierarchical control by insulin was proposed for the reduction of AMPK activation in ischemic heart via PKB-induced phosphorylation of Ser485/491 (see below). The inhibitory effect of cAMP was linked to reduction in phosphorylation of AMPK at Thr172 and appears to be due, in part, to cAMP-dependent inhibition of the upstream AMPK kinase CaMKK, but not LKB1 (Hurley *et al.*, 2006). On the other hand, cAMP-dependent attenuation of AMPK activity has also been correlated with increased phosphorylation of AMPKα1 Ser485/491 by PKA (Hurley *et al.*, 2006). This is in contrast to studies in adipocytes, showing that agents stimulating PKA-mediated cAMP signaling (isoproterenol, isobutylmethylxanthine, forskolin, β-adrenergic agonist and adrenaline) result in increased AMPK activity (Moule and Denton, 1998; Yin *et al.*, 2003; Daval *et al.*, 2005; Koh *et al.*, 2007; Omar *et al.*, 2009). Since cAMP-stimulated lipolysis in adipocytes was accompanied by an increase in oxidative stress (i.e. an increased AMP:ATP ratio), AMPK activation could be a consequence of lipolysis and the associated relative change in cellular energy balance rather than a direct effect of PKA (Gauthier *et al.*, 2008). Similarly, it has been shown that IL-6 activates AMPK in skeletal muscle by increasing the concentration of cAMP and, secondarily, the AMP:ATP ratio (Kelly *et al.*, 2009). However, potential crosstalk between PKA and AMPK signaling pathways underlying negative action of PKA on AMPK signaling has been recently reported in context of adipocytes. Central to this mechanism is the phosphorylation of AMPKα1 by PKA at Ser173 (Djouder *et al.*, 2010). This site is highly conserved, located directly adjacent to the critical activation loop Thr172, and its phosphorylation may create constraints by steric hindrance or charge incompatible with subsequent phosphorylation at the Thr172 residue. This mechanism is critically important for the control of the lipolytic response. Stimulation of adipocyte lipolysis, via PKA activation, triggers a negative feedback mechanism involving AMPK to restrain the energy depletion and oxidative stress caused by lipolysis (Gauthier *et al.*, 2008). By opposing the activity of AMPK-mediated negative feedback loop, PKA allows fine-tuning of lipolysis (Djouder *et al.*, 2010).

Protein phosphatases have an important role in regulating AMPK phosphorylation at Thr-172 and consequently AMPK activity, although the exact mechanisms that modulate their action remains poorly understood. Their ability to dephosphorylate Thr172 on AMPK is inhibited by AMP binding to the γ subunit (Davies *et al.*, 1995; Sanders *et al.*, 2007). Both protein phosphatases 2A (PP2A) and 2C were shown to dephosphorylate AMPK *in vitro* (Davies *et al.*, 1995; Kudo *et al.*, 1996). Recent findings revealed the important role of protein phosphatase activation in the suppression of AMPK activity by dephosphorylation in

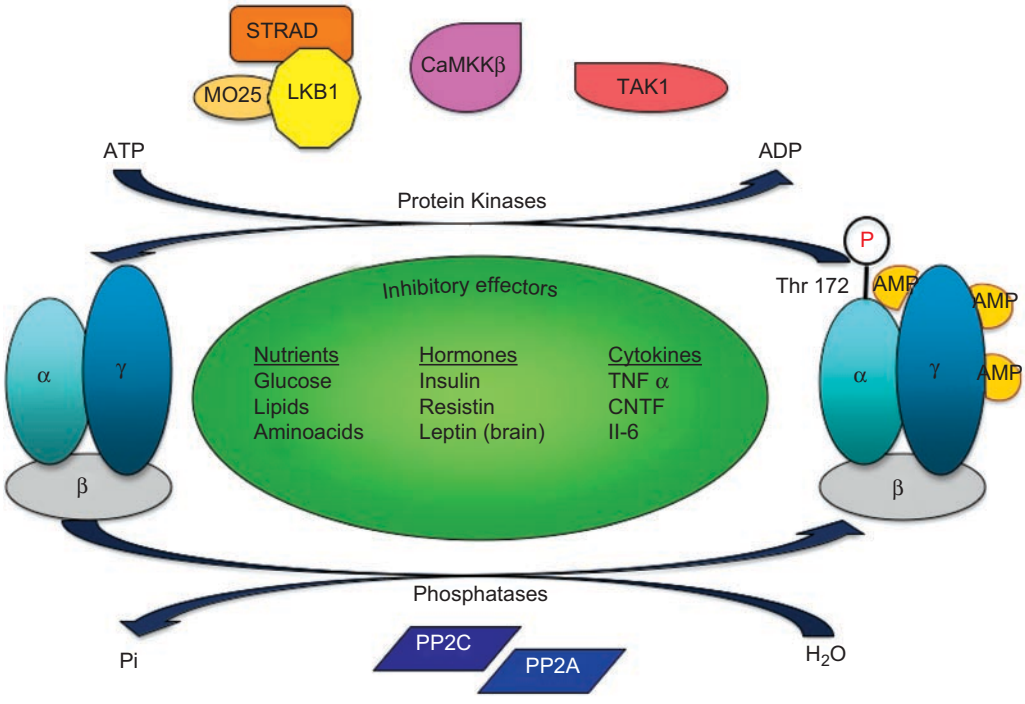

Figure 2. Regulation of AMPK activation by phosphorylation/dephosphorylation. Phosphorylation of AMPK at Thr-172 is regulated by the upstream protein kinases LKB1, CaMKK and possibly TAK1. Dephosphorylation of AMPK at Thr172 is modulated by protein phosphatases PP2A and PP2C. Multiple effectors (nutrients, hormones and cytokines) regulating AMPK phosphorylation and activity are listed.

different species, organs, and nutrition types (Wang and Unger, 2005; Ravnskjaer *et al.*, 2006; Wu *et al.*, 2007). In support with these results, it has been reported that PP2A is involved in regulating the interaction between AMPK α2 and γ1 (Gimeno-Alcaniz and Sanz, 2003).

5. Regulation by subcellular localization

Intracellular distribution of AMPK complexes appears to shuttle between the nucleus and the cytoplasm in response to specific stimuli. In Hela cells, AMPK translocated to the nucleus upon stimulation by agents inducing cellular stress (Kodiha *et al.*, 2007). In human skeletal muscle, the AMPKα2 subunit translocated to the nucleus following intense exercise (McGee *et al.*, 2003). Interestingly, the two AMPKα subunits, α1 and α2, have been shown to have different localization patterns in mammalian cells, with the α1 subunit being localized to the non-nuclear fraction and the α2 subunit localized to both the nucleus and the non-nuclear fractions. AMPKα1 is hence likely to phosphorylate cytosolic and plasma membrane substrates, whereas AMPKα2 may be primarily involved in the conversion of metabolic signals into transcriptional regulation (Salt *et al.*, 1998a). Another mechanism to localize signaling events is the association with scaffold proteins. The β subunits act as targeting scaffolds, influencing subcellular localization through an N-terminal myristoylation site (Mitchelhill *et al.*, 1997) that can target AMPK to membrane (Warden *et al.*, 2001; Gregor *et al.*, 2006). AMPKα2 bound to AMPKβ1 is anchored in the cytoplasm at the outer mitochondrial membrane through the myristoylation site of β1 subunit. In contrast, AMPKα2 bound to AMPKβ2 translocates to the nucleus in a manner driven by a nuclear localization signal present in AMPKα2 but not in AMPKα1 subunit (Suzuki *et al.*, 2007). The γ1 subunit also exhibits preferential nuclear localization over the other γ subunits (Turnley *et al.*, 1999). These data suggest that activation of AMPK complexes may elicit distinct metabolic as well as signaling effects in tissues and cells depending on the expression of different catalytic and regulatory subunits.

6. Regulation of protein stability

Recent data revealed a new mechanism that regulates AMPK activity independently of AMP and of phosphorylation or dephosphorylation processes. Modulation of AMPK complex stability via ubiquitination-mediating degradation has emerged through a complex containing cell-death-inducing like-effector A Cell death-inducing DNA fragmentation factor α-like effector A (CIDEA) and AMPK (Qi *et al.*, 2008). Cidea and AMPK have been shown to co-localize in the endoplasmic reticulum and form a complex *in vivo* through specific interaction with the AMPKβ subunit to promote ubiquitin-mediated AMPK degradation and down-regulation of its activity. Truncated AMPKβ proteins lacking the region required for its interaction with Cidea no longer undergo Cidea-mediated protein degradation (Qi *et al.*, 2008).

B. AMPK inhibition in physiology

1. Regulation by nutrient

1.a. Inhibition by lipid overload

An increasing body of evidence indicates that dysregulation of AMPK activity and its consequential signaling network may have sustained and deleterious effects at the systemic level that underlie the pathogenesis of metabolic syndrome (Ruderman and Prentki, 2004). A strong correlation between low activation state of AMPK and metabolic disorders associated with insulin resistance, obesity and sedentary activities has been established in a variety of rodent models with aspects of the metabolic syndrome (Kelly *et al.*, 2004; Yu *et al.*, 2004). In addition, feeding mice with a high fat diet causes dysregulation of AMPK, associated with impaired AMPK phosphorylation and protein expression in skeletal muscle, heart, liver, aortic endothelium and hypothalamus (Muse *et al.*, 2004; Lee, WJ *et al.*, 2005; Wang and Unger, 2005; Wilkes *et al.*, 2005; Lessard *et al.*, 2006; Liu *et al.*, 2006; Martin *et al.*, 2006). Furthermore, inhibition of AMPK was found to occur in mice fed with a high fat diet rich in palmitate (Wu *et al.*, 2007), raising the possibility that chronic exposure to fatty acids inhibits AMPK activation in a feed-forward effect of lipid overload. It was reported that palmitate inhibited AMPK in endothelial cells via ceramide-dependent PP2A activation (Wu *et al.*, 2007). Interestingly, AMPK inhibition by PP2C upregulation accounted for decreased AMPK activity in the heart of obese rodents with cardiac lipotoxicity (Wang and Unger, 2005). These data provide new insights into the mechanisms of lipo-regulatory dysfunction, leading to lipid metabolism disorders in obesity.

If decreased AMPK activity contributes to the pathogenesis of obesity, as suggested by dysregulation of AMPK signaling in obese rodent models, one would expect that mice lacking AMPK will be more sensitive to deleterious effects of over-nutrition. Consistent with this hypothesis, whole-body ablation of AMPKα2 activity exacerbates high fat diet-induced obesity, while the glucose disposal rates are similar to those of wild-type mice (Villena *et al.*, 2004). The fact that these mice have similar triglyceride content in liver and muscle, either on high-fat or normal diets, rules out the lipid accumulation in these tissues as a major determinant of their glucose homeostasis (Villena *et al.*, 2004). More recently, Jorgensen and coworkers investigated whether reduced levels of muscle AMPK promoted lipid accumulation and insulin resistance during high-fat

diet (Jorgensen *et al.*, 2009). High-fat feeding increased body mass and adiposity, and impaired insulin and glucose tolerance, however, there was no difference between wild-type and transgenic litter-mates overexpressing an AMPKα2 kinase-dead (KD) in muscle. High-fat feeding decreased insulin-stimulated muscle glucose uptake and Akt-phosphorylation, while increasing muscle triacylglycerol, diacylglycerol and ceramide. These effects, as well as obesity-induced lipid accumulation and insulin resistance were not exacerbated in AMPK KD mice, suggesting that reduced levels of muscle AMPKα2 did not promote insulin resistance in the early phase of obesity-related diabetes. Another study by Fujii and coworkers demonstrated that mice overexpressing a muscle-specific KD AMPKα2 Asp157Ala mutation developed more severe muscle insulin resistance after 30 weeks on high-fat diet (Fujii *et al.*, 2008). However, the observation that the genotype effect occurred 26 weeks later than the first evidence of glucose intolerance suggested that AMPK did not play a primary role in the development of insulin resistance. Thus, while AMPK function is impaired with severe obesity, it does not appear to influence the development of insulin resistance in diet-induced obesity.

1.b. Inhibition by high glucose concentration

AMPK can be negatively regulated by chronic exposure to high glucose. Acute hyperglycemia reduces AMPK activation in muscle, liver (Kraegen *et al.*, 2006) and kidney (Lee, MJ *et al.*, 2007). Decreased AMPK activity observed after glucose infusion does not depend on changes in plasma insulin and FFA levels, as alterations in AMPK activity are also observed following incubation with high glucose concentrations in isolated muscles (Itani *et al.*, 2003) as well as in cultured HepG2 hepatocytes (Zang *et al.*, 2004), human umbilical vein endothelial cells (Ido *et al.*, 2002), β-cells (da Silva Xavier *et al.*, 2003; Gleason *et al.*, 2007; Salt *et al.*, 1998b) and islets (Leclerc *et al.*, 2004). Upon elevation of glucose concentration over the physiological range, AMPK activity is rapidly down-regulated, concomitant with decrease of phosphorylation at Thr172. According to the classic view, glucose-dependent regulation of AMPK activity and phosphorylation is presumably induced by the activation of ATP synthesis and consequent changes in AMP/ATP ratio (da Silva Xavier *et al.*, 2000; 2003; Salt *et al.*, 1998b). However, no change in creatine phosphate or adenine nucleotides was reported in muscle incubated with a high concentration of glucose (Itani *et al.*, 2003), indicating that novel regulation mechanisms of AMPK may be operative in response to glucose oversupply. Under circumstances where no significant change in high-energy phosphate molecules was observed, diminished AMPK activity and phosphorylation were attributed to alterations in phosphorylation and inhibition of AMPK by Akt (Hahn-Windgassen *et al.*, 2005; Lee, MJ *et al.*, 2007), action of specific phosphatases on phosphorylated AMPK (Ravnskjaer *et al.*, 2006), changes in redox state (Rafaeloff-Phail *et al.*, 2004), modification in intracellular free Ca^{2+} concentration (Leclerc and Rutter, 2004) and alterations in glycogen content (Jorgensen *et al.*, 2004). Regulation of AMPK by glucose might be important to limit glucose uptake into tissues and to protect cells against the adverse effects of sustained hyperglycemia, such as oxidative stress.

Recent work in animal models demonstrated that glucose and fasting/refeeding change AMPK activity in several hypothalamic nuclei (Kim, MS *et al.*, 2004; Minokoshi *et al.*, 2004). These studies described reduced AMPK activity and phosphorylation state in the basomedial hypothalamus in response to intracerebroventricular (icv) injection of glucose, and showed reciprocal effects of AMPK activation or inhibition on feeding behavior (Kim, MS *et al.*, 2004; Minokoshi *et al.*, 2004). Hypothalamic neurons appear to mediate the effects of glucose via changes in AMPK activity (Mountjoy *et al.*, 2007). It was established that AMPK responds to changes in blood glucose and functions in transmitting the malonyl-CoA signal (Wolfgang *et al.*, 2007). AMPK activation allows the dephoshorylation/activation of acetyl-CoA carboxylase (ACC) which increases the level of hypothalamic malonyl-CoA resulting in food intake suppression and increased energy expenditure. Interestingly, AMPK has been shown to play an important role in the glucose-sensing mechanism used by the ventromedial hypothalamus, a key brain region involved in the detection of hypoglycemia (Fan *et al.*, 2009). These findings indicate that minute changes in neuron glucose concentration modulate AMP/ATP ratio which can be sensed by AMPK signaling pathway in discrete hypothalamic regions to generate hunger or satiety signals (see below).

1.c. Inhibition by glycogen accumulation

In skeletal muscle, some studies found that high glycogen content repressed AMPK activation (Derave *et al.*, 2000; Wojtaszewski *et al.*, 2002), suggesting that the AMPK system may monitor availability of this energy store by virtue of the glycogen-binding domain on its β subunit (McBride *et al.*, 2009). The degree of AMPK activation was immense during the glycogen-depleted state in both rat and human muscles (Wojtaszewski *et al.*, 2002; Viollet *et al.*, 2003). However, this inverse correlation was not evident under all circumstances. In a human training study, AMPK activity was not found to be directly correlated with muscle glycogen content (McConell *et al.*, 2008). Furthermore, in patients with McArdle's disease (glycogen storage disease V), the activation of AMPK in response to moderate exercise was exaggerated despite high skeletal muscle glycogen levels (Nielsen *et al.*, 2002). Other paradoxes exist as AMPK can inactivate glycogen synthase (GS) by phosphorylation on Ser7 (site 2)

(Jorgensen *et al.*, 2004). Although AMPK is activated by exercise, glycogen synthase was found dephosphorylated as well as activated after exercise. McBride and coworkers proposed a single hypothesis to explain the physiological role of glycogen binding to AMPK complex based on its structure (McBride *et al.*, 2009). Glycogen preparations with high branching content were found to cause allosteric inhibition of AMPK, due to its binding to the glycogen-binding domain (McBride *et al.*, 2009). It was demonstrated that oligosaccharides with single α1-6 branch points, but not α1-4, are potent allosteric inhibitors of AMPK that also inhibit phosphorylation and activation by upstream kinases. AMPK bound to fully synthesized glycogen particle is probably in an active state due to inaccessibility of internal branch points. This will lead to phosphorylation and inhibition of GS, providing feedback inhibition of further extension of glycogen particles. However, when glycogen is depleted, AMPK becomes inhibited after binding to exposed α1-6 branch points. This allows dephosphorylation of GS on site 2, promoting rapid resynthesis of glycogen. This model implies that different pools of AMPK (glycogen-bound versus glycogen-free) can phosphorylate some targets but not others (Jorgensen *et al.*, 2004).

1.d. Inhibition by amino acids

Several reports have suggested a possible interplay between the mammalian target of rapamycin (mTOR) and AMPK signaling pathways coordinating amino acid- and energy-sensing. The mTOR pathway has recently emerged as a crucial point of convergence for signaling by amino acids, growth factors and cellular energy (Wullschleger *et al.*, 2006). Whereas mTOR was presumed to be a direct cellular sensor for ATP levels, mounting evidence implicated AMPK in the regulation of mTOR activity. AMPK inhibits mTOR through direct phosphorylation of TSC2 tumor suppressor (Inoki *et al.*, 2003) as well as critical mTOR-binding subunit raptor (Gwinn *et al.*, 2008). Thus, mTOR activation and AMPK activity are inversely related (Aguilar *et al.*, 2007). Recent studies demonstrated that AMPK activity is suppressed by amino acids (Leclerc and Rutter, 2004; Gleason *et al.*, 2007). Treatment of C2C12 myoblast cells with leucine enhanced the phosphorylation of mTOR and concomitantly reduced the phosphorylation of AMPK and inhibited its activity (Du *et al.*, 2007). The ability of leucine to dramatically reduce AMPK activity is linked to a consequent drop in the level of AMP and a subsequent decrease in AMP/ATP ratio. In the liver, the increase of protein intake induces metabolic adaptation characterized by concomitant increase of mTOR phosphorylation and decrease of AMPK phosphorylation (Chotechuang *et al.*, 2009). Similarly, high protein diet decreases AMPK and increases mTOR activity in the hypothalamus, leading to reduction in food intake (Ropelle *et al.*, 2008). Consistent with a cross-regulation between AMPK and mTOR to control food intake, hypothalamic ATP levels are increased and AMP/ATP ratio reduced after high-protein feeding.

2. Regulation by hormones and cytokines

2.a. Inhibition by insulin in the heart

The energy necessary to maintain the myocardial contraction/relaxation cycle is derived from the mitochondrial oxidation of carbohydrates and long chain fatty acids. Under physiological conditions, fatty acid oxidation provides 60–70% of the heart energy requirements (Bertrand *et al.*, 2008). This substrate preference can be attributed to inhibition of glucose uptake and catabolism via the Randle cycle (Randle *et al.*, 1963). Following myocardial infarction, fatty acid oxidation accounts for almost all the heart ATP production (Neely and Morgan, 1974; Opie, 1975). This over-reliance on fatty acid oxidation is detrimental to functional reperfusion recovery of ischemic hearts (Lopaschuk *et al.*, 1990; 1993). Under such conditions, the beneficial effects of insulin are important for maintaining proper cardiac function. Insulin can increase glucose use by the heart both by activating key steps of glycolysis, namely the recruitment of GLUT-4 to the plasma membrane and the activation of 6-phosphofructo-2-kinase (Rider and Hue, 1984; Russell *et al.*, 1999; Bertrand *et al.*, 2008) and by decreasing the extracellular fatty acid concentration. Also, insulin can directly alter fatty acid oxidation in the normoxic heart. The mechanism behind this involves inactivation of AMPK (Kudo *et al.*, 1995; Gamble and Lopaschuk, 1997) which contributes to accelerated fatty acid oxidation via direct phosphorylation and inactivation of ACC (Carling *et al.*, 1989; Hardie, 1992) resulting in decreased malonyl-CoA, a potent inhibitor of fatty acid transport into the mitochondrial matrix (McGarry *et al.*, 1989). Witters and Kemp have previously observed this inhibitory effect of insulin on AMPK activity in hepatoma cells (Witters and Kemp, 1992).

As insulin is a very potent PKB/Akt activator in the heart (Lefebvre *et al.*, 1996), Kovacic and coworkers investigated whether increased PKB/Akt activity could lead to inactivation of AMPK. They demonstrated that hearts from transgenic mice expressing constitutively active PKB/Akt show a dramatic reduction in AMPK phosphorylation, when compared to control hearts that do not express the transgene, indicating that insulin-induced down-regulation of AMPK is mediated by Akt-dependent pathways (Kovacic *et al.*, 2003). PKB/Akt and AMPK have been shown to be inversely correlated in other occurrences too. For example, ischemia in heart causes activation of AMPK and inhibits insulin signaling (Beauloye *et al.*, 2001a), whereas priming of the hearts by insulin pre-treatment in the aerobic period blunts the AMPK response to a subsequent period of ischemia (Beauloye *et al.*, 2001b; Bertrand *et al.*, 2006). The molecular

mechanism of the effect of insulin on AMPK signaling pathways has been elucidated as direct phosphorylation of AMPK by PKB/Akt on Ser 485/491 (Horman *et al.*, 2006). This phosphorylation can prevent subsequent activation of AMPK at Th172 by LKB1. It is possible, as suggested by Zou and coworkers, that phosphorylation at Ser 485/491 hinders the physical association of AMPK with LKB1 (Zou *et al.*, 2004). Although insulin inhibits AMPK under ischemia the glycolysis should remain elevated because both insulin and ischemia stimulate glycolysis by activating the same key steps. Physiological relevance of this inhibition in ischemic hearts could also modulate other targets of AMPK, as yet unknown. The ability of PKB/Akt to negatively regulate AMPK activity becomes especially relevant in the physiology of myocardial ischemia-reperfusion. It is possible that PKB/Akt regulates fatty acid oxidation rates secondarily to inhibition of AMPK activity. In addition, PKB/Akt is supposed to be protective by promoting the post-ischemic synthesis of contractile proteins and by inhibiting myocyte apoptosis (Fujio *et al.*, 2000; Miao *et al.*, 2000; Ruan *et al.*, 2009), two processes conversely regulated by AMPK (Horman *et al.*, 2003; Meisse *et al.*, 2002). However, the role of PKB/Akt remains controversial and needs to be further investigated, as others argue against the beneficial effects of PKB/Akt negatively regulating AMPK (Nagoshi *et al.*, 2005). Although the metabolic effects of AMPK and PKB/Akt have been largely studied, the ability of PKB/Akt to inhibit AMPK has implications beyond cardiac metabolism. Insulin and IGF-1 have been shown to induce protein synthesis and cardiac hypertrophy via PKB/Akt activation (Proud and Denton, 1997). AMPK antagonizes the stimulating effect of insulin by inhibiting the TSC2/mTOR/p70S6K (Inoki *et al.*, 2003) and eEF2 pathway (Horman *et al.*, 2002). It is therefore possible that the reduction of AMPK activity may be a contributing factor to PKB/Akt-induced cardiac hypertrophy. Studies are ongoing to investigate this relationship.

2.b. Inhibition by inflammatory signals

Recent studies have suggested that AMPK plays a crucial role in the inflammatory signaling pathways. AMPK activity has been shown to be down-regulated upon pro-inflammatory stimulus (LPS) and up-regulated upon anti-inflammatory cytokine stimulation (IL-10 and TGF-β) (Sag *et al.*, 2008). Also, inhibition of AMPK activity or expression increases the production of TNFα, IL-6 and IL-1 upon pro-inflammatory stimulus, whereas overexpression of AMPK results in the dampening of inflammatory response and increases the production of IL-10 (Sag *et al.*, 2008; Jeong *et al.*, 2009). The effects of AMPK deficiency on the regulation of inflammatory status indicates that the presence of AMPK and its activation is important to counteract inflammation. Furthermore, increasing AMPK activity with AICAR, or by transfection of a constitutively active AMPK catalytic subunit, blunts the ability of free fatty acids (palmitate) or TNFα to activate NFκB (Cacicedo *et al.*, 2004). Accordingly, *in vivo* AMPK activation decreases severity of LPS-induced lung injury (Zhao *et al.*, 2008) and the expression of pro-inflammatory genes in adipose tissue of obese *db/db* mice (Bai *et al.*, 2010). A defect in AMPK function has been found in various cells in animals with metabolic diseases. In diabetes and obesity, it is likely that AMPK activation is compromised in inflammation-related cells and leads to the development of inflammatory diseases. Thus, AMPK may be a promising pharmacologic target for the treatment of various chronic inflammatory diseases.

Obesity is a morbid condition characterized by an excess in fat mass and myriad co-morbidities. Among them, it is recognized that insulin resistance promotes the development of type 2 diabetes. Interestingly, insulin resistance varies greatly among obese people, some patients being severely insulin resistant while other remains insulin sensitive despite accumulation of body fat (Brochu *et al.*, 2001; Guilherme *et al.*, 2008). Different hypotheses have been discussed to explain this variability. One of them postulated that obesity-related insulin resistance can be recognized as a state of chronic low-grade inflammation (Xu *et al.*, 2003; Rasouli *et al.*, 2005; Permana *et al.*, 2006; Lumeng *et al.*, 2007). Macrophages in obese patients are in an inflammatory state and display increased NFκB and TNFα expression. TNFα induces insulin resistance through the serine phosphorylation of IRS protein by JNK and Iκ/NFκB and increases the expression of STAT3-suppressor of cytokine signaling 3 (SOCS3) (Kern *et al.*, 2001; Shi *et al.*, 2004). Obesity favors increased rates of fatty acid uptake and esterification leading to storage of bioactive lipids such as ceramides, diacylglycerol (DAG) and fatty acyl-CoA in tissues. These lipids contribute to the activation of inflammatory serine threonine kinases such as conventional PKCs, IKK-β and JNK (Schenk *et al.*, 2008). Rates of fatty acid oxidation in skeletal muscle are also reduced in obese humans and rodents and this defect has been correlated with reduced AMPK activity. Nevertheless, the mechanism connecting excess of lipids and decreased AMPK activity in skeletal muscle has not been completely elucidated. To address this question, it has been shown in cultured L6 muscle cells that TNFα reduced AMPK activity without change in LKB1 activity. TNFα suppresses AMPK activity which leads to defective fatty-acid metabolism, an important contributing factor to the development of insulin resistance in obesity (Steinberg *et al.*, 2006a). TNFα mediates its action through TNF receptor (TNFR) 1 to attenuate AMPK activity via transcriptional upregulation of PP2C, which results in reduction of ACC phosphorylation, suppressing fatty-acid oxidation, increasing intramuscular diacylglycerol accumulation and causing insulin resistance in skeletal muscle. Using *in vitro* and *in vivo*

approaches, Steinberg and coworkers provided for the first time conclusive evidence of AMPK as a link between inflammation and metabolic disease. According to these results, *ob/ob* mice also have reduced muscular AMPK activity, inhibited fatty acid oxidation, increased PP2C expression in their skeletal muscle and reduced muscular insulin sensitivity *in vivo*. In contrast, AMPK activity is not altered in *ob/ob* TNFR–/– mice, indicating that disruption of TNF signaling prevents AMPK inhibition in this genetically obese mice model.

Circulating free fatty acids (FFA) are often increased in obesity and they activate TLR4 signaling-NFκB-inflammation cascade (TNFα production) in adipocytes and macrophages, which contributes to insulin resistance in skeletal muscle (Shi *et al.*, 2006). Interestingly, ablation of TLR4 signaling using TLR4 knockout mice protects against high fat diet-induced insulin resistance, due to reduced inflammation, linking innate immune system and metabolism. Activation of TLR4 by endotoxin also leads to loss of AMPK phosphorylation under similar conditions where NFκB pathway is activated in macrophage (Nath *et al.*, 2009). These studies delineate a novel FFA/endotoxin-TLR4-NFκB-TNFα-loss of AMPK-insulin resistance pathway which could be implicated in metabolic disorders (Figure 3). In addition, it has been demonstrated that resistin, an adipocytokine elevated in obesity, inhibited skeletal muscle AMPK activity. Consequent accumulation of lipids and their mediators probably explains resistin-mediated insulin resistance during obesity. When insulin resistance occurs, reduced adiponectin levels can also contribute to continuous suppression of AMPK activity. Because AMPK is a critical factor for mitochondrial biogenesis, long-term reduction of its activity can lead to reduction of mitochondrial density/function in skeletal muscle, as observed in insulin resistance associated with obesity. Supporting this hypothesis, treatment of *ob/ob* mice by rosiglitazone or by adiponectin reduced TNFα synthesis and increased muscle mitochondrial biogenesis in parallel to metabolic improvement. In summary, it has been shown that excess of lipids can inhibit muscular AMPK activity through increased proinflammatory cytokines pathway. Similar conclusions have been obtained in hearts of mice during excess lipids availability. Indeed, acute lipid excess (5 hours of lipid infusion) or diet-induced obesity was both associated with blunted myocardial glucose metabolism concomitantly with reduction of AMPK phosphorylation in heart (Ko *et al.*, 2009). These deleterious effects of long-term or acute exposure to lipids *in vivo* are based on elevation of inflammatory cytokines (TNFα and IL-6) and increase in their myocardial signaling (Senn *et al.*, 2002). Myocardial levels of STAT3, CD68 and SOCS3, reduction of AMPK activity and down-regulation of myocardial glucose metabolism are attenuated in IL-6 KO mice following high fat diet. This suggests that IL-6 is a key component of the diet-induced myocardial inflammation and subsequent metabolic changes in heart. Chronic exposure of IL-6 (as observed in obesity) promotes insulin resistance both *in vitro* and *in vivo* (Nieto-Vazquez *et al.*, 2008). In contrast, during prolonged exercise, IL-6 is released acutely from the skeletal muscle (Kelly *et al.*, 2004; Febbraio and Pedersen, 2005) and AMPK is activated (Kelly *et al.*, 2009), leading to improved peripheral glucose uptake and insulin sensitivity at the whole body level (Glund *et al.*, 2007; Ruderman *et al.*, 2006). This dual effect of IL-6 on insulin sensitivity probably explains some conflicting results recently discussed in more detail elsewhere (Nieto-Vazquez *et al.*, 2008).

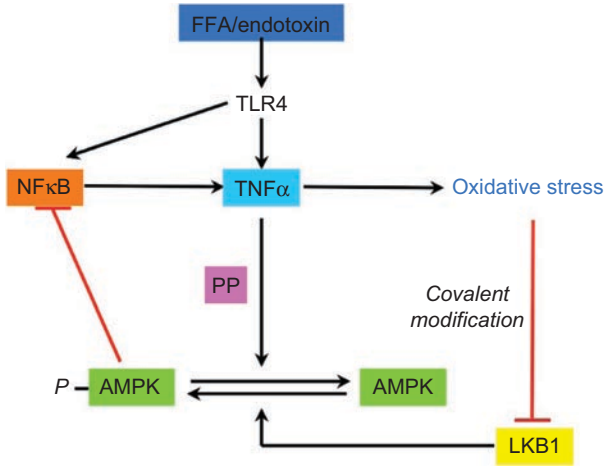

Figure 3. Regulation of AMPK activity by inflammatory signals. Activation of TLR4 by endotoxin and free fatty acid (FFA) regulates AMPK phosphorylation status through the action of protein phosphatase (PP).

In general, AMPK functions solely to restore energy balance after depletion of energy stores. However, in T cells, Tamas and coworkers (Tamas *et al.*, 2006) proposed that its unique ability to anticipate energy-consuming processes could be useful for immune cells that need a rapid response to an increased demand for ATP. Activation of AMPK by TCR engagement was shown to be abrogated by CaMKK inhibitor (STO-609) but not when it was activated by AMP/ATP ratio, suggesting two independent pathways for the regulation of AMPK in T cells. Recently, it was reported that the AMPKα1 protein is lost in spleen macrophages, total T cells and their subsets (CD4, CD8 and regulatory T cells) isolated from experimental autoimmune encephalomyelitis (EAE) afflicted animals, compared to the control, without affecting its mRNA levels (Nath *et al.*, 2009), suggesting a posttranscriptional modification. Genetic ablation of AMPKα1 in mice exhibited severe disease with profound infiltration of mononuclear cells in central nervous system (CNS) compare to wild-type mice. Interestingly, the AMPKα2 isoform does not participate in enhancing the severity of the disease.

2.c. Inhibition of AMPK in the regulation of food intake

Recently, AMPK has emerged as a regulator of appetite. Indeed, hypothalamic AMPK is now recognized not only as a nutrient and glucose sensor in the central nervous system (CNS) but also as a key regulator of appetite. Because the brain has an extremely high metabolic rate and is a high lipid-containing tissue, the distribution of the AMPK isoforms throughout its various areas was considered as an exciting area of research. Turnley and coworkers first reported the cellular distribution of AMPK isoforms in mouse CNS (Turnley *et al.*, 1999). They demonstrated that these are widely expressed in neurons and in activated astrocytes. In addition, several groups showed that AMPK isoforms are expressed in hypothalamus and hindbrain, both areas controlling food intake (Kola, 2008). Studies pertaining to pharmacological or genetic activation as well as inhibition of hypothalamic AMPK lead to a better knowledge of hypothalamic AMPK function as a regulator of food intake. It was first recognized that hypothalamic AMPK activation by AICAR infusion into the third ventricle significantly increased food intake (Andersson *et al.*, 2004). Confirming this first study, expression of dominant negative AMPK in the hypothalamus was reported to be sufficient to reduce food intake and body weight, whereas hypothalamic expression of constitutively active AMPK isoform increased both (Minokoshi *et al.*, 2004). In contrast with these previous studies, some conflicting data came from rodent models, especially α2 catalytic subunit specific knock out in hypothalamic Agouti-related peptide (AgRP) neurons or in hypothalamic pro-opiomelanocortin (POMC) neurons. Indeed, in contrast to what could be expected from the data previously published, AMPK-α2 specific deletion in AgRP neurons did not change food intake or energy expenditure, whereas mice were lean. Furthermore, AMPK-α2 specific deletion in POMC neurons unexpectedly increased body weight and adiposity (Claret *et al.*, 2007). To explain some of these surprising data, it was argued that AICAR or Compound C (as used previously in many studies) was not specific of AMPK pathway and that genetically modified mice models may provide new insights into hypothalamic AMPK functions. In this regard, study from Claret and coworkers clearly suggests that loss of AMPK in orexigenic (AgRP) neurons leads to reduced body weight whereas loss of this enzyme in anorexigenic (POMC) neurons leads to increased body weight. Importantly, electrophysiological studies showed that leptin or insulin action are both preserved in AMPKα2-deficient POMC or AgRP neurons. In consequence, this paper challenged the concept of hypothalamic AMPK as a general sensor and integrator of energy homeostasis in the mediobasal hypothalamus.

Hypothalamic AMPK is regulated by various metabolic signals coming from the periphery (Ahima and Antwi, 2008). It is now well accepted that fasting results in activation of AMPK whereas re-feeding inhibits AMPK activity in multiple hypothalamic regions in mice (Kola, 2008). Specific effects of nutrients and hormones on hypothalamic AMPK activity have been investigated by different groups. Peripheral or central hyperglycemia is known to inhibit AMPK in all brain areas controlling appetite (such as the arcuate nucleus, the ventro- and dorso-mediobasal hypothalamus, the paraventricular nucleus and the lateral hypothalamus (Kim, MS *et al.*, 2004; Minokoshi *et al.*, 2004). In contrast, hypothalamic AMPK activity was increased (with greater food intake as a consequence) during insulin-induced hypoglycemia or by inhibition of intracellular glucose utilization (administration of 2-deoxyglucose (2-DG)) (Han *et al.*, 2005; Kim, MS *et al.*, 2004). These data indicate that intraneuronal glucose concentration is a key modulator of hypothalamic AMPK activity. In order to dissociate the respective effects of glucose and insulin on hypothalamic AMPK activity, icv insulin infusion can be used to study the effects of insulin without any changes in glucose concentration. It has been demonstrated in this way that insulin inhibits hypothalamic AMPK activity (Minokoshi *et al.*, 2004). In consequence, hyperinsulinemia and/or hyperglycemia are now recognized as potent inhibitors of hypothalamic AMPK while hypoglycemia is an activator of this enzyme.

Leptin is a key hormone in the communication between energy stores and the brain. In contrast to what is observed in skeletal muscle, leptin decreases hypothalamic AMPK activity (Minokoshi *et al.*, 2002). Similarly, chronic calorie excess, as observed in diet-induced obese mice, reduced hypothalamic AMPK activity (Martin *et al.*, 2006) probably by the inhibitory effects of combined hyperinsulinemia, hyperglycemia and increased secreted leptin. Presumably, leptin promotes loss of body weight by enhancing fat oxidation in peripheral tissues and by decreasing food intake, suggesting that leptin has tissue-specific effects. It is not known if muscular AMPK activation by leptin and concomitant reduction of hypothalamic AMPK activity by leptin are supported by different AMPK isoforms. However, as discussed above, the hypothesis that hypothalamic AMPK could be a key mediator for the control of appetite by leptin has been recently challenged when a normal response to leptin has been described in selective AMPKα2-deficient POMC or AgRP neurons (Claret *et al.*, 2007). Interestingly, it has been shown that like leptin, ciliary neurotrophic factor (CNTF) also suppresses hypothalamic AMPK signaling and reduces food intake (Steinberg *et al.*, 2006b). Importantly, despite the similarities in signaling between leptin and CNTF, CNTF-mediated suppression of hypothalamic AMPK is maintained in diet-induced obesity, whereas the effects of leptin on AMPK signaling are blunted. Thus, the capacity of CNTF to bypass leptin resistance highlights its potential role in the therapeutic treatment of obesity.

AMPK activity is regulated by cellular energetic status, which can be summarized by the intracellular AMP/ATP ratio. Any modification of glucose and/or lipids availability has consequences on AMPK activity. C75 is a fatty acid synthase (FAS) inhibitor which causes weight loss and anorexia. This effect is linked to increased neuronal ATP content by C75 and reduced level of the phosphorylated AMPKα subunit in the hypothalamus (Kim, EK *et al.*, 2004). Anorectic effect induced by C75 is based on decreased phosphorylation of cAMP response element-binding protein (CREB) in the arcuate nucleus and subsequent reduction in NPY expression (Kim, EK *et al.*, 2004). Similarly, α-lipoic acid, a cofactor of mitochondrial enzymes that possesses antioxidative, antidiabetic and anorectic properties, inhibits AMPK activity in the hypothalamus (Kim, MS *et al.*, 2004).

Taking together the effects of nutrients, hormones and compounds described above, it can be postulated that hypothalamic AMPK is a key sensor of whole-body energy status and regulates fuel availability and appetite. Nevertheless, many questions have to be resolved. The molecular mechanisms involved in the regulation of food intake by hypothalamic AMPK are not clearly understood. It can be noticed that changes in hypothalamic activity AMPK may contribute to modifications of arcuate neuropeptide expression. Thus, reduction of hypothalamic AMPK activity (by glucose, leptin, insulin, C75, α-lipoic acid and melanocortin 4 receptor agonists) suppresses expression of orexigenic neuropeptides, NPY and AgRP in arcuate nucleus. In contrast, increase in hypothalamic AMPK activity (by hypoglycemia, ghrelin, cannabinoids and adiponectin) enhances the expression of orexigenic NPY and AgRP in arcuate nucleus and melanin-concentrating hormone in the lateral hypothalamus (Minokoshi *et al.*, 2004). In additional studies, it was shown that hypothalamic AMPK and melanocortin pathways are interrelated. Indeed, melanocortin 4 receptor agonists decrease hypothalamic AMPK activity whereas melanocortin receptor antagonists (as AgRP) increase hypothalamic AMPK (Kola, 2008). In these cases, it is difficult to understand if AMPK activity is regulated by AgRP or melanocortin signaling independently of neuronal AMP/ATP ratio changes. Lastly, beyond unspecific effects of AICAR or Compound C, rodent models overexpressing or deleted for hypothalamic AMPK provide evidence of changes of AMPK activity and food intake. However, the extent of physiological relevance of these models could be discussed.

2.d. Inhibition by resistin: implication for the regulation of glucose homeostasis

Resistin is a 12.5-kDa cysteine-rich protein secreted by adipose tissue of rodents and macrophages of humans (Steppan *et al.*, 2001). The hypothesis that resistin could be a possible link between obesity and insulin resistance is controversial in humans in the light of recent studies (Nagaev and Smith, 2001; Lee, JH *et al.*, 2003). In contrast, consistent findings in rodents suggest that resistin plays a causative role in the development of diet-induced insulin resistance. Additionally, some studies support a link between deleterious metabolic effects of resistin and reduction of AMPK activity. Indeed, a significant correlation has been shown between plasma resistin levels with high fat feeding (or acute infusion of recombinant resistin), hepatic insulin resistance and diminished AMPK phosphorylation in liver (Muse *et al.*, 2004). Conversely, treatment with resistin-specific antisense oligodeoxynucleotide reversed these effects. In addition, mice lacking resistin exhibit low blood glucose levels after fasting, due to reduced hepatic glucose production (Banerjee *et al.*, 2004). This is partly mediated by activation of AMPK and decreased expression of gluconeogenic enzymes in the liver. Taken together, these data indicated that resistin is a key promoter of hepatic insulin resistance and that this effect could be partly mediated through reduction of hepatic AMPK activity.

Additional studies suggested that resistin acting on hypothalamus modulates hepatic glucose production. Thus, infusion of resistin in the third cerebral ventricle (icv) or in the mediobasal hypothalamus was sufficient to enhance endogenous glucose production through an increase of TNFα, IL-6, and SOCS-3 expression and a decrease of AMPK phosphorylation in the liver (Muse *et al.*, 2007). This suggested that hypothalamus is an important site of resistin action.

It has been also shown that resistin reduces not only insulin-mediated glucose transport *in vivo* (Satoh *et al.*, 2004) and in isolated muscle cells (Palanivel and Sweeney, 2005; Palanivel *et al.*, 2006; Niederwanger *et al.*, 2007; Junkin *et al.*, 2009`); but also AICAR-stimulated glucose uptake in muscle (Jorgensen *et al.*, 2009). Basically, these studies showed that resistin regulates the function of IRS-1 and Akt1 and decreases GLUT4 translocation and glucose uptake in response to insulin. Short-term resistin incubation impairs glycogen synthesis by reducing the rate of glucose-6-phosphate formation by reduction of hexokinase type I activity and reduction of glucose uptake (Niederwanger *et al.*, 2007). Lastly, resistin decreases phosphorylation of muscular AMPK and ACC (Palanivel and Sweeney, 2005). Nevertheless, it can be noted that some studies used supra-physiological concentrations of resistin. This could explain that in a recent study on mouse extensor digitorum longus (EDL), soleus muscles and L6 myotubes, physiological concentrations of resistin impair insulin-stimulated glucose uptake by mechanisms involving reduced plasma membrane GLUT4 translocation but independently of the proximal insulin-signaling cascade, AMPK, and SOCS-3 (Jorgensen *et al.*, 2009).

C. AMPK inhibition in therapeutics

1. Neuroprotection in stroke: slowing down AMPK activation

Lack of blood and oxygen after ischemic stroke causes disruption of cell ion homeostasis and leads to neuronal cell death. To repair the damage and return neurons to homeostasis, a number of energy-consuming processes are activated. Overactivation of these pathways during ischemia can lead to complete energy failure and cell death. Activation of AMPK was initially considered to be an adaptive response due to altered AMP/ATP ratio in response to ischemia, hypoxia, or glucose deprivation (Culmsee *et al.*, 2001; Gadalla *et al.*, 2004; McCullough *et al.*, 2005) but there has been some discordance about the outcome on cell survival and neuroprotection. Some groups proposed that AMPK represents an endogenous neuroprotective pathway conserving cellular energy levels under conditions of intense metabolic stress (Culmsee *et al.*, 2001) or ischemic injury in addition to limiting neuronal injury via excitotoxicity (Kuramoto *et al.*, 2007). Conversely, McCullough and coworkers suggested that AMPK overactivation is detrimental in models of ischemia reperfusion (McCullough *et al.*, 2005). Pharmacological and genetic approaches were used to clarify the role of AMPK in stroke outcome. AMPK inhibition with Compound C or with the fatty acid synthase inhibitor C75 (which reduces AMPK activation indirectly) provided sustained neuroprotection after stroke (Li *et al.*, 2007). Similarly, AMPKα2 knockout mice were protected from stroke damage (Li *et al.*, 2007). Furthermore, the beneficial effect of Compound C was lost in AMPKα2 knockout mice implying that targeting neuronal energy balance during cerebral ischemia may be therapeutic (Li *et al.*, 2007). However, the physiological consequences of AMPK activation after hypoxic stress on cerebral vasculature has been poorly investigated and it is not known if AMPK activation exacerbates or ameliorates cerebral blood flow. In contrast, regarding peripheral vascularture, many studies confirmed the beneficial effects of pharmacological AMPK activation (Evans *et al.*, 2005; Rubin *et al.*, 2005; Davis *et al.*, 2006; Wang *et al.*, 2009; Bradley *et al.*, 2010), thereby favoring blood flow. Some of the protective actions of AMPK have been related to the activation of endothelial NO synthase (eNOS) and formation of NO, which is a central signaling molecule in the vasculature (Zou and Wu, 2008). AMPK has been shown to enhance eNOS activity by direct phosphorylation of Ser1177 (Chen, ZP *et al.*, 1999; 2000), Ser633 (Chen, Z *et al.*, 2009) and by promoting its association with heat shock protein 90 (Davis *et al.*, 2006) leading to endothelial NO production. In addition, AMPK also produces its regulatory effects in the peripheral vasculature through vascular endothelial growth factor (VEGF)-mediated endothelial angiogenesis (Nagata *et al.*, 2003; Ouchi *et al.*, 2005; Stahmann *et al.*, 2010). Interestingly, a recent study has shown increased phosphorylation of AMPK and eNOS in endothelial cells of cerebral arteries following severe subarachnoid hemorrhage (Osuka *et al.*, 2009). Thus, it is likely that AMPK causes beneficial effects in the brain vasculature through eNOS-mediated acute vasodilatation (Osuka *et al.*, 2009) or VEGF-induced angiogenesis (Lopez-Lopez *et al.*, 2007).

2. AMPK inhibition in cancer: a two-edged sword?

Several recent reports support the idea that the stimulation of AMPK with pharmacological compounds exerts antitumoral effect in various experimental settings (reviewed in (Billaud and Viollet, 2008; Fogarty and Hardie, 2009). Furthermore, epidemiological analyses indicate that treatment with the anti-diabetic drug metformin may reduce the cancer burden in diabetic type 2 patients (reviewed in Billaud and Viollet, 2008; Fogarty and Hardie, 2009). These findings have led to the conception that pharmacological activators of AMPK may find clinical applications in cancer chemoprevention and therapy. Since the LKB1-AMPK pathway inhibits mTOR, a kinase overactivated in a broad range of tumors, AMPK activators may prove beneficial in a large spectrum of cancers. This idea is reinforced by the observation that metformin is selectively toxic for malignant cells harboring p53-inactivating mutations (Buzzai *et al.*, 2007). However, during specific stages of the tumorigenic process, activation of AMPK might provide a survival advantage to tumor cells. It is clearly documented that nascent cancer cells and their metastatic counterparts are exposed to harsh microenvironmental conditions since they have to cope with extrinsic cellular stresses such as hypoxia, acidosis, shortage of glucose and nutrients. In this context of energetic stress and hypoxia, AMPK is activated and protects cells from apoptosis as demonstrated for pancreas cancer cells (Kato *et al.*, 2002). Also, a recent report has provided evidence that the AMPK catalytic activity is triggered under low-oxygen conditions and is critical to promote the growth of xenografted tumors prepared from Ras-transformed mouse embryonic fibroblasts (Laderoute *et al.*, 2006). It is thus possible that activation of AMPK is a key event at defined steps of the sequential tumorigenic process. For instance, breast cancer cells overcome anoikis, a cell death mechanism that leads to the self-destruction of epithelial cells detaching from the basement membrane, through an increase of glucose uptake that restores the intracellular level of ATP and reduces reactive oxygen species (Schafer *et al.*, 2009). AMPK stimulates the transport of glucose through the GLUT1 transporter and may be involved in the capacity of tumor cells to override cell death induced by loss of extracellular matrix attachment. In a larger prospect, the multiple metabolic pathways regulated by AMPK possibly place this kinase as one of the main actors contributing to the metabolic reprogramming known as Warburg effect,

which is a hallmark of malignant cells (Vander Heiden *et al.*, 2009). Thus, it is conceivable that at certain stages of cancer progression, and for some types of malignancies, AMPK inhibition rather than activation may represent a potential way of therapeutic intervention. In any case, the experimental arguments supporting a favoring role for AMPK during oncogenesis call for a cautious evaluation of possible protumoral effects of treatments that aims at activating AMPK in cancer prevention and chemotherapy.

3. Pharmacological AMPK inhibitor: the hidden side of Compound C

Compound C is a cell-permeable pyrrazolopyrimidine compound that can act as a reversible and ATP-competitive inhibitor of AMPK (Zhou *et al.*, 2001). This compound is being used increasingly to inhibit AMPK in cell-based assays. However, several studies have reported inhibition of various biological events by Compound C independently of AMPK inhibition, such as inhibition of the hypoxic activation of HIF-1 by suppressing mitochondrial generated reactive oxygen species (ROS) (Emerling *et al.*, 2007) and proliferation of preadipocytes by increasing p21 levels (Nam *et al.*, 2008). Furthermore, Compound C does not inhibit AMPK activation in response to all stimuli. Thus, this pharmacological inhibitor blunted the AICAR-induced but not the dinitrophenol-induced (Fryer *et al.*, 2002) or the LPS-induced (Labuzek *et al.*, 2010) activation of AMPK. Further investigation showed that Compound C inhibits the adenosine transporter (Fryer *et al.*, 2002), the primary transporter for the uptake of AICAR into cells, suggesting that this pharmacological inhibitor should not be used to demonstrate AMPK-dependent effects of AICAR. Lastly, Compound C appears to inhibit a number of other protein kinases with lower IC_{50} values than AMPK, indicating that this compound could certainly have "off-target" effects (Bain *et al.*, 2007). However, despite the uncertain specificity of this pharmacological inhibitor, various reports suggest that in specific circumstances Compound C inhibits AMPK with expected results. For example, the genetic approach combined with the pharmacological approach further confirmed the AMPK-specific action of Compound C during stroke as the effect of this pharmacological inhibitor was lost in AMPKα2 knockout mice (Li *et al.*, 2007).

D. Concluding remarks

Since the initial description of the role of AMPK in modulating energy metabolism (as illustrated by regulation of lipid metabolism), there has been an expanded interest in the role of AMPK in numerous physiological systems. AMPK integrates the activity of several essential processes to maintain energy balance both at the single and the whole body levels. In recent years, additional mechanisms in AMPK regulation have been discovered and it is clear that multiple pathways for activation or inhibition of AMPK are now possible. These various stimuli include nutrients, hormones, cytokines, physiological state as well as pathological events. A large body of experimental evidence has clearly shown the therapeutic potential of pharmacological activation of AMPK in order to prevent or reverse metabolic disorders associated with the metabolic syndrome. However, understanding the consequence of AMPK inhibition may suggest novel therapeutic targets in a number of disease conditions. Thus, studies using genetic models with AMPK deficiency will be a great help to define the role of AMPK in regulating physiological responses *in vivo*.

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Declaration of interest

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