

REVIEW

AMPK as a mediator of hormonal signalling

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Abstract

AMP-activated protein kinase (AMPK) is a key molecular player in energy homeostasis at both cellular and whole-body levels. AMPK has been shown to mediate the metabolic effects of hormones such as leptin, ghrelin, adiponectin, glucocorticoids and insulin as well as cannabinoids. Generally, activated AMPK stimulates catabolic pathways (glycolysis, fatty acid oxidation and mitochondrial biogenesis) and inhibits anabolic pathways (gluconeogenesis, glycogen, fatty acid and protein synthesis), and has a direct appetite-regulating effect in the hypothalamus. Drugs that activate AMPK, namely metformin and thiazolidinediones, are often used to treat metabolic disorders. Thus, AMPK is now recognised as a potential target for the treatment of obesity and associated co-morbidities.

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Introduction

AMP-activated protein kinase (AMPK) has emerged as a key molecular player in energy homeostasis at both cellular and whole-body levels (Kahn *et al.* 2005). Initially, AMPK was shown to have lipid-related effects: it inactivates acetyl-CoA carboxylase (ACC; Carlson & Kim 1973) and 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase (Beg *et al.* 1973), the key regulatory enzymes of fatty acid and cholesterol synthesis. Later, the role of AMPK in carbohydrate and protein metabolism, cell cycle regulation and mitochondrial biogenesis was also described. AMPK is an evolutionarily conserved serine/threonine kinase with a catalytic α -subunit and regulatory β - and γ -subunits, forming a heterotrimeric complex. The upstream regulation of AMPK is summarised in Figs 1 and 2.

Role of AMPK in the central control of appetite

AMPK is expressed throughout the brain: all isoforms are expressed in neuronal tissues including areas that are involved in the control of food intake and neuroendocrine function, such as the hypothalamus and the hindbrain (Turnley *et al.* 1999, Kola 2008). In the last 5 years, AMPK has emerged as a nutrient and glucose sensor in the hypothalamus (Momcilovic *et al.* 2006). Hypothalamic AMPK activity is increased during

fasting and decreased during refeeding (Minokoshi *et al.* 2004). Pharmacological activation of AMPK in the rodent hypothalamus with 5-aminoimidazole-4-carboxamide riboside (AICAR) causes an increase in food intake (Xue & Kahn 2006). Alteration in ventromedial hypothalamic AMPK activity with recombinant adenoviruses expressing dominant negative (DN) or constitutively active (CA) AMPK- $\alpha 1/\alpha 2$ subunit (Minokoshi *et al.* 2004) changed body weight and food intake. DN-AMPK adenovirus-treated mice ate less and had lower body weight compared with control mice. DN-AMPK mice also had decreased neuropeptide Y (NPY) and agouti-related peptide (AgRP) mRNA levels in the arcuate nucleus (ARC) under *ad libitum* fed conditions. In contrast, CA-AMPK adenovirus-treated mice ate significantly more and had higher body weight with increased expression of NPY and AgRP mRNA in ARC, as well as increased orexigenic melanin-concentrating hormone expression in lateral hypothalamus. This suggests that high AMPK activity enhances orexigenic signals, whereas low AMPK activity suppresses these signals under *ad libitum* fed conditions. In agreement with this, deletion of AMPK $\alpha 2$ in AgRP neurons led to the development of an age-dependent lean phenotype.

Peripheral hormones from the gastrointestinal tract (peptide YY, ghrelin, cholecystokinin, glucagon-like peptide 1 (GLP-1) and oxyntomodulin) and adipose tissue (leptin, resistin and adiponectin) are important in influencing the activity of the appetite-regulating

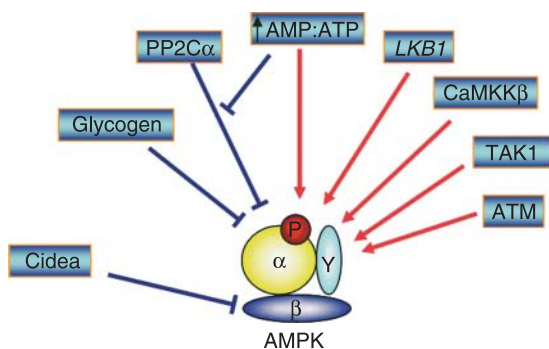


Figure 1 AMPK regulation: AMPK's upstream kinases phosphorylate the Thr172 residue of the α -kinase subunit. *LKB1*, a tumour-suppressor gene (Hawley *et al.* 2003, Woods *et al.* 2003, Shaw *et al.* 2004), calmodulin kinase kinase (CaMKK; Hawley *et al.* 2005, Hurley *et al.* 2005, Woods *et al.* 2005), and the recently described kinases transforming growth factor- β -activated kinase (TAK1) and ataxia telangiectasia mutated (ATM) are the known AMPK kinases. CaMKK2 (also known as CaMKK β) is expressed primarily in the brain, suggesting that this Ca^{2+} -mediated pathway may be operating in neurons (Towler & Hardie 2007). TAK1 is a member of the mitogen-activated protein kinase kinase kinase family and is reported to phosphorylate AMPK in HeLa cells, which lack *LKB1* expression (Momcilovic *et al.* 2006). ATM, which stimulates mitochondrial biogenesis in response to double-stranded DNA breaks, also can phosphorylate AMPK (Fu *et al.* 2008). A rise in AMP:ATP ratio activates AMPK i) allosterically and ii) by inhibition of dephosphorylation by protein phosphatases (Hardie *et al.* 1999, Sanders *et al.* 2007). Recently, it has been shown that AMPK can be regulated directly by glycogen and may act as a glycogen sensor (McBride *et al.* 2009). The α 1–6 linkage-branched forms of glycogen allosterically inhibit AMPK more potently than the linear α 1–4-linked forms (Carling 2009, McBride *et al.* 2009). In addition to the allosteric effect, the branched glycogen also inhibits *LKB1*- or CaMKK2-induced Thr172 phosphorylation, while dephosphorylation is not affected by glycogen. A new model of AMPK regulation, which is independent of AMP, has recently been identified. It is shown that cell death-inducing DFFA-like effector- α (Cidea) forms a complex with the β -subunit of AMPK, eliciting a ubiquitination-mediated degradation of AMPK (Qi *et al.* 2008).

neuronal populations in the hypothalamus. In addition, a number of these hormones have been shown to influence AMPK activity. In the short term, anorectic agents such as glucose, GLP-1 and oxyntomodulin decrease hypothalamic AMPK activity (Andersson *et al.* 2004, Minokoshi *et al.* 2004, Seo *et al.* 2008), leading to reduction in food intake during satiation, while orexigenic agents such as ghrelin lead to AMPK activation and increased food intake (Andersson *et al.* 2004, Kola *et al.* 2005). In the long term, the circulating anorectic insulin and leptin determine the energy and adiposity profile.

The hypothalamus is not the only location in the brain important for appetite regulation. Emerging data suggest that the nucleus tractus solitarius (NTS) in the hindbrain also has an important role. Fasting increases AMPK activity in the NTS and leptin inhibits it (Hayes *et al.* 2009a). Ghrelin is known to activate neurons in

the NTS (Date *et al.* 2006). GLP-1 (7–36) amide, an anorectic hormone, acts both in the hypothalamus and the NTS (Goldstone *et al.* 2000, Seo *et al.* 2008, Hayes *et al.* 2009b).

Role of AMPK in peripheral tissues

AMPK is ubiquitously expressed and plays an important role in the peripheral metabolism of the skeletal muscle, liver, fat, myocardium and other tissues. In general, activated AMPK switches on catabolic processes that produce ATP and switches off ATP-consuming processes, thus restoring the AMP:ATP ratio.

AMPK plays a key role in regulating lipid metabolism. Activated AMPK phosphorylates and inhibits ACC1 and HMG-CoA, decreases fatty acid synthase (FAS) expression and activates malonyl-CoA carboxylase, thereby leading to a decrease in fatty acid and cholesterol synthesis (Woods *et al.* 2000, Kahn *et al.* 2005, Lopez *et al.* 2007). Activated AMPK stimulates fatty acid oxidation by decreasing malonyl-CoA levels through the inhibition of ACC2 (Merrill *et al.* 1997, Kahn *et al.* 2005, Lopez *et al.* 2007). This leads to an increase in carnitine palmitoyltransferase 1 (CPT1) activity and the subsequent activation of fatty acid oxidation (Kahn *et al.* 2005, Lopez *et al.* 2007). The decreased AMPK activity in visceral fat could enhance lipolysis as well as lipogenesis, although the effect on lipogenesis prevails (Divertie *et al.* 1991, Djurhuus *et al.* 2002). AMPK thus plays a key role in regulating lipid metabolism. AMPK has been suggested to inhibit catecholamine-stimulated lipolysis in adipocytes (Corton *et al.* 1995, Daval *et al.* 2005), thus lowering the plasma level of fatty acids. Activated AMPK also stimulates and upregulates the expression of peroxisome proliferator-activated receptor- γ coactivator-1 α , which consequently increases mitochondrial biogenesis (Terada *et al.* 2002, Zong *et al.* 2002).

AMPK also regulates glucose homeostasis. Activation of AMPK by contraction in fast-twitching muscles increases hexokinase II expression (Holmes *et al.* 1999), and enhances glucose uptake through the translocation of glucose transporter 4 (GLUT4) to the cell membrane and the upregulation of *Glut4* gene expression (Holmes *et al.* 1999, Derave *et al.* 2000, Wright *et al.* 2005). Interestingly, these effects were not observed in slow-twitching soleus muscle (Derave *et al.* 2000, Wright *et al.* 2005).

AMPK regulates hepatic gluconeogenesis by inhibiting the transcription of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase; Lochhead *et al.* 2000, Cool *et al.* 2006). AMPK α 2-knockout (KO) and *LKB1*-KO mice were shown to have glucose intolerance and fasting-induced hyperglycaemia, possibly caused by increased

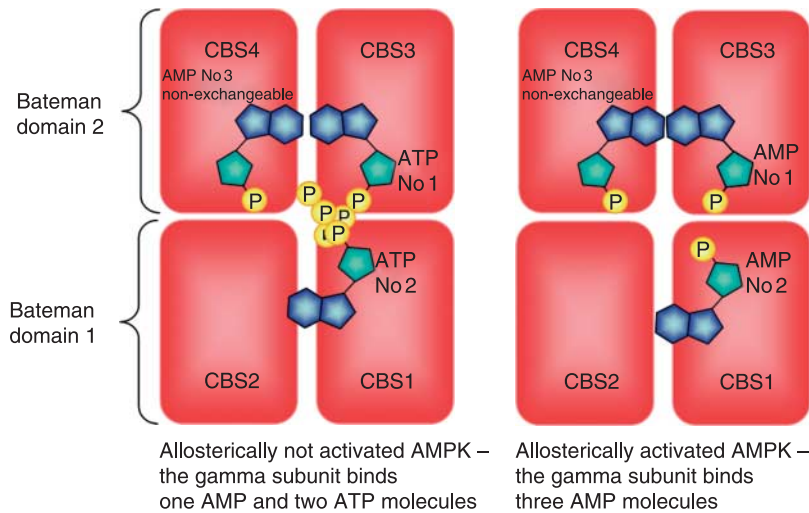


Figure 2 The γ -subunit of AMPK contains three AMP-binding sites. These are formed in the interface of its two pairs of CBS domains, also called Bateman domains. Two of these binding sites can bind either AMP or ATP, whereas a third site contains a tightly bound AMP that does not exchange (Xiao *et al.* 2007). When AMPK is inactive under physiological conditions, it binds two ATP and one AMP molecule, while in low energy states it binds three AMP molecules. It is now proposed that the interaction of the catalytically active kinase domain with the AMP-bound γ -subunit protects the phosphorylated Thr172 residue from dephosphorylation.

gluconeogenesis associated with increased PEPCK and G6Pase activity (Lochhead *et al.* 2000, Cool *et al.* 2006). Activated AMPK in skeletal muscle phosphorylates and inhibits glycogen synthase, thereby leading to a decrease in glycogen synthesis (Wojtaszewski *et al.* 2002).

Hypothalamic AMPK has been linked to the regulation of peripheral metabolism, suggesting that AMPK is a key enzyme in coordinating the interaction between peripheral and central energy regulation. Central AICAR treatment has been shown to increase both insulin-mediated and non-insulin-mediated glycogen synthesis (Perrin *et al.* 2004), thus implicating the role of hypothalamic AMPK in regulating muscle glycogen synthesis. Central insulin infusion also increased muscle glycogen synthesis and this effect was blocked by the co-administration of glucose, possibly mediated by AMPK (Perrin *et al.* 2004). Central adiponectin treatment leads to hypothalamic AMPK activation and decreases energy expenditure, possibly via a reduced expression of uncoupling protein-1 (UCP-1) in brown adipose tissue (Kubota *et al.* 2007). Central α -lipoic acid, which inhibits hypothalamic AMPK activity, increases UCP-1 expression and energy expenditure in brown adipose tissue (Kim *et al.* 2004b). Central ghrelin treatment, independently from the effect on food intake, has been shown to increase glucose utilisation rate of white and brown adipose tissues and counteract the effects of intracerebroventricular leptin treatment on fat weight, plasma glucose and insulin (Kim *et al.* 2004a, Theander-Carrillo *et al.* 2006).

Role of AMPK as mediator of hormonal signals

Interestingly, several hormones have tissue-specific, often opposite, effects on AMPK activity (Fig. 3).

Leptin

Leptin increases AMPK activity in the skeletal muscle directly as well as indirectly through stimulation of the hypothalamo-sympathetic axis (Minokoshi *et al.* 2002). Chronic s.c. administration of leptin also increases the expression of AMPK in skeletal muscle (Steinberg *et al.* 2003). Leptin- or leptin receptor-deficient rodents showed a decreased AMPK activity in the liver (Yu *et al.* 2004). In lean animals, leptin has been shown to attenuate hepatic glucose production and insulin resistance under normal conditions and to slightly increase AMPK activity (Brabant *et al.* 2005). However, these effects are lost in diet-induced obese rats, thereby suggesting an important physiological dysregulation of leptin effects in obese animals. Leptin inhibits triacylglycerol storage and stimulates fatty acid oxidation in the heart, and both AMPK-dependent (Lee *et al.* 2004) and AMPK-independent (Atkinson *et al.* 2002) pathways have been suggested.

Central injection of leptin into ventromedial hypothalamus (VMH) of rats has been shown to increase glucose uptake in the heart, brown adipose tissue and skeletal muscle, but not in white adipose tissue (Kamohara

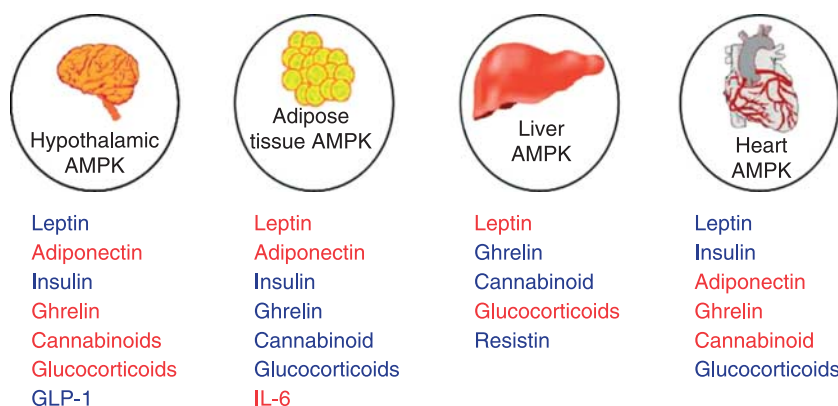


Figure 3 Hormones that activate (red) or inhibit (blue) AMPK in various tissues.

et al. 1997, Haque *et al.* 1999, Minokoshi *et al.* 1999). Central infusion of leptin decreases hepatic glycogen content (Kamohara *et al.* 1997, Haque *et al.* 1999).

In the hypothalamus, leptin has an opposite effect: it decreases AMPK activity in the ARC and paraventricular (PVC) nuclei (Minokoshi *et al.* 2002, 2004, Andersson *et al.* 2004, Mountjoy *et al.* 2007). By reducing the appetite centrally and increasing the peripheral fatty acid consumption, these tissue-specific effects of leptin lead to an overall negative energy balance and reduction in body weight.

Adiponectin

Adiponectin activates and stimulates liver and muscle AMPK activity *in vivo* and *in vitro*, leading to stimulation of glucose uptake, fatty acid oxidation and PEPCK (Yamauchi *et al.* 2002). These lead to an improvement in insulin sensitivity. Globular adiponectin was also shown to activate AMPK in primary rat adipocytes (Tomas *et al.* 2002, Yamauchi *et al.* 2002, Wu *et al.* 2003, Huypens *et al.* 2005).

Adiponectin protects the heart from ischaemic injury via AMPK- and cyclooxygenase-2-dependent mechanisms (Shibata *et al.* 2005). Adiponectin is also suggested to play a beneficial role in cardiac remodelling through multiple mechanisms, one of which is possibly via the activation of AMPK (Liao *et al.* 2005, Shibata *et al.* 2005). Impaired regulation of AMPK and glucose metabolism in adiponectin-deficient mice result in the development of heart failure (Liao *et al.* 2005). In endothelial cells, adiponectin stimulates nitric oxide production via AMPK activation, leading to beneficial vasoprotective effects (Chen *et al.* 2003).

It has been suggested that adiponectin may be involved in the stimulation of food intake (Kadowaki *et al.* 2008). Serum and cerebrospinal fluid (CSF) adiponectin concentrations increase under fasting conditions, as does expression of adiponectin receptor-1 in ARC. Central adiponectin administration leads to increased

phosphorylation of AMPK and ACC in the hypothalamus (Carling 2005, Xue & Kahn 2006, Kadowaki *et al.* 2008). Adiponectin KO mice were shown to have reduced food intake and decreased AMPK activity in ARC (Kadowaki *et al.* 2008). Thus, unlike leptin, adiponectin stimulates both central and peripheral AMPK activity. Adiponectin-transgenic ob/ob mice, which have serum adiponectin levels two- to threefold higher than ob/ob mice, have significantly higher body weight, but an improved metabolic state compared with ob/ob mice (Kim *et al.* 2007). Adiponectin is considered a starvation hormone: under fasting conditions, high adiponectin levels stimulate central and peripheral AMPK leading to increased food intake and decreased energy expenditure, promoting fat storage. After refeeding, adiponectin levels would fall with a consequent decrease in AMPK activity leading to reduced food intake and an increase in energy expenditure.

Resistin

Resistin is an adipokine secreted in rodents and humans, which generally seems to have opposite effects to those of adiponectin. Resistin induces insulin resistance and stimulates hepatic glucose production. These effects are thought to be mediated by a reduction in liver AMPK activity (Banerjee *et al.* 2004, Muse *et al.* 2004). It has been shown to decrease fatty acid uptake and oxidation in skeletal muscle via a reduction in the membrane content of fatty acid translocase/CD36, possibly mediated by inhibition of AMPK (Palanivel & Sweeney 2005). Resistin, despite its anorectic effect, has been shown to phosphorylate hypothalamic AMPK and ACC. The consequent inactivation of ACC, after AMPK activation, might represent a physiological compensatory mechanism that prevents deleteriously high levels of malonyl-CoA occurring in the hypothalamus after resistin-induced FAS inhibition in the VMH (Vazquez *et al.* 2008). Time course-dependent resistin treatments would probably be needed to clarify this issue.

Ghrelin and cannabinoids

Ghrelin has been shown to regulate AMPK activity in hypothalamus and peripheral tissues (Andersson *et al.* 2004, Kola *et al.* 2005). Cannabinoids are known to regulate appetite and peripheral metabolism, and AMPK has been shown to mediate these effects (Kola *et al.* 2005, 2008). Both ghrelin and cannabinoids have similar effects on AMPK activity in various tissues: they stimulate hypothalamic and heart AMPK activity, while inhibit adipose tissue and liver AMPK activity (Kola *et al.* 2005, 2008).

The mechanism of central effect of ghrelin includes the activation of Ca^{2+} signalling in NPY neurons in the ARC (Kohno *et al.* 2003, 2008). The Ca^{2+} rise leads to CAMKK2 activation, which can stimulate hypothalamic AMPK (Anderson *et al.* 2008, Sleeman & Latres 2008). AMPK activation leads to inhibition of malonyl-CoA and stimulation of CPT1, leading to increased mitochondrial oxidation and activation of the UCP-2, which can increase NPY/AgRP neuronal activity and ultimately stimulate appetite (Andrews *et al.* 2008, Lopez *et al.* 2008).

Endocannabinoids are synthesised locally on demand, and the level in the hypothalamus varies in response to feeding and fasting (Kirkham *et al.* 2002). The variation in hypothalamic endocannabinoid levels seems to play an important role in mediating the anorectic effects of leptin (Di Marzo *et al.* 2001) and the orexigenic effects of ghrelin (Kola *et al.* 2008). Recently, we have shown that the effects of ghrelin on hypothalamic AMPK activity and appetite are abolished in the absence of cannabinoid type 1 receptor (CB1) or in the presence of a CB1 antagonist rimonabant (Kola *et al.* 2008). These data suggest that an intact cannabinoid-signalling pathway is required for the effects of ghrelin on AMPK activity and appetite. Interestingly, i.p. injection of cannabinoids in rats results in increased plasma ghrelin levels (Zbucki *et al.* 2008). This suggests that the stimulation of appetite by cannabinoids may be connected to an increase in ghrelin secretion from the gastric X/A-like cells (Zbucki *et al.* 2008). Further studies are needed to elucidate the details of the ghrelin–cannabinoid interaction.

Insulin

Insulin has a range of metabolic effects in addition to its main role of stimulating glucose uptake into the cells. Centrally, insulin is an anorectic hormone, which has been shown to inhibit hypothalamic AMPK activity (Minokoshi *et al.* 2004). Insulin deficiency has been proposed as one of the factors causing hypothalamic AMPK activation and the subsequent increase in food intake seen in streptozotocin-induced diabetic rats (Namkoong *et al.* 2005). In the periphery, insulin inhibits AMPK in fat by activating protein kinase B/Akt

complex, which can phosphorylate alphaAMPK at Ser485/491, thus leading to reduced phosphorylation at Thr172 (Kovacic *et al.* 2003, Horman *et al.* 2006). Insulin inhibits myocardial AMPK activity during ischaemic events alone or when co-administered with glucose, suggesting that the inhibitory effect of insulin on myocardial AMPK activity might be caused by enhanced glucose metabolism (Russell *et al.* 2004).

Insulin resistance is characterised by the inability of insulin to increase glucose uptake and repress glucose production in the liver, and it often leads to hyperglycaemia. The role of AMPK in this condition has to be considered. While activated AMPK stimulates catabolic pathways and inhibits the energy-consuming anabolic processes, insulin promotes glycogen, lipid and protein synthesis. However, both upregulate glucose uptake in muscle via an effect of GLUT1 or GLUT4 translocation and increase in GLUT4 transcription (Zheng *et al.* 2001, Barnes *et al.* 2002). In skeletal muscle, the two pathways also phosphorylate the protein AS160, which has a Rab GTPase-activating protein domain that can increase translocation of GLUT4 to the plasma membrane (Kurth-Kraczek *et al.* 1999, Kramer *et al.* 2006, Treebak *et al.* 2006). These factors will lead to an increase in glucose uptake, which is an important homeostatic feature of plasma glucose regulation. Furthermore, AMPK activation is thought to upregulate insulin receptor substrate-1 (Harrington *et al.* 2004, Shah *et al.* 2004, Um *et al.* 2004) through inhibition of the insulin–mammalian target of rapamycin (mTOR) pathway (Fisher *et al.* 2002, Inoki *et al.* 2003). This will improve the insulin sensitivity profile. Both insulin and activated AMPK repress the expression of the gluconeogenic enzymes PEPCK and G6Pase (Lochhead *et al.* 2000). Phosphorylation of AMPK is thought to result in translocation of the transcriptional coactivator TORC2 to the cytoplasm (Koo *et al.* 2005), thus repressing the expression of the TORC2-target enzymes

Glucagon-like peptide-1

GLP-1 is produced from pre-proglucagon mRNA, which is expressed in NTS cell bodies in the brainstem, with projections to the PVN and other hypothalamic nuclei involved in the control of feeding (Goldstone *et al.* 2000). Central nervous system GLP-1 is an endogenous inhibitor of feeding acting via the GLP-1 receptor. Hypothalamic GLP-1 peptide content is decreased during fasting. Fasting-induced increase in hypothalamic AMPK activity is inhibited by GLP-1 and this could be the mechanism of its anorectic effects (Seo *et al.* 2008). It has been shown that the anorectic effects of leptin are at least partly via GLP-1. GLP-1 acts in both the hypothalamus and the NTS, as GLP-1 receptors located in the NTS are also suggested to regulate food intake (Hayes *et al.* 2009b).

Glucocorticoids

Glucocorticoids increase appetite and lead to increased availability of metabolic fuels such as amino acids and fatty acids. Chronic or excessive exposure to glucocorticoids will result in insulin resistance, truncal obesity, hyperlipidaemia and symptoms similar to the metabolic syndrome. We have suggested that AMPK is involved in the central and peripheral effects of glucocorticoids. Glucocorticoids activate hypothalamic AMPK activity *in vivo* (Christ-Crain *et al.* 2008) either directly or via stimulation of endocannabinoid synthesis (Di *et al.* 2005, Christ-Crain *et al.* 2008), and these effects could lead to appetite stimulation (Tataranni *et al.* 1996). In the periphery, glucocorticoids inhibit AMPK activity in adipose tissue, leading to increased lipogenesis and fat storage (Christ-Crain *et al.* 2008). Glucocorticoids also inhibited AMPK activity in the heart, which might, at least in part, mediate the detrimental effects of glucocorticoid excess on the heart. Surprisingly, and somewhat unexpectedly, glucocorticoids were shown to stimulate AMPK activity in rat liver *in vivo* as well as in a liver cell line (Viana *et al.* 2006, Christ-Crain *et al.* 2008). This could be the result of the balance of local lipolysis, lipid oxidation and the flux of fatty acids into the liver (Foretz *et al.* 2005). It has been shown that an increase in free fatty acids leads to fatty acid esterification, an energy-demanding process, and this can increase the cellular AMP:ATP ratio and therefore AMPK activity (Gauthier *et al.* 2008).

Inflammatory mediators and AMPK

Interleukin-6 (IL-6) treatment was shown to increase AMPK phosphorylation in cultured rodent myocytes and adipocytes, as well as in muscle, liver and adipose tissue *in vivo* (Keller *et al.* 2001, Park *et al.* 2002, Kahn *et al.* 2005). Moreover, IL-6-KO mice have decreased AMPK activity in muscle (Kelly *et al.* 2004). Conversely, adiponectin was shown to increase IL-6 production in human synovial fibroblasts partly via AMPK regulation (Tang *et al.* 2007). Exercise increases AMPK activity in skeletal muscle primarily in response to changes in the AMP:ATP ratio (Ruderman *et al.* 2006). Exercise affects AMPK activity in fat and liver tissue (Takekoshi *et al.* 2006) possibly due to an increase in circulating levels of IL-6 (Keller *et al.* 2001). Furthermore, AMPK activity is often still increased after exercise at times when the energy state of the muscle is presumably no longer altered, and IL-6 is suggested to be involved in AMPK activation during this 'late-phase' stage (Ruderman *et al.* 2006).

Ciliary neurotrophic factor (CNTF) is a cytokine, which was found to induce severe anorectic effect (Miller *et al.* 1996), possibly via hypothalamic neurogenesis that

leads to sustained reduction in caloric intake and prolonged maintenance of weight loss (Kokoeva *et al.* 2005). CNTF was shown to activate AMPK through the CNTFR α -IL-6R-gp130 β receptor complex and ultimately increasing fatty acid oxidation and reducing insulin resistance in skeletal muscle (Steinberg *et al.* 2006b). CNTF can also suppress inflammatory signalling cascades associated with lipid accumulation in the liver and skeletal muscle (Febbraio 2007).

The ischaemic heart releases macrophage inhibitory factor (MIF), an upstream regulator of inflammation. MIF stimulates AMPK through CD74 during ischaemia, and shows impaired ischaemic AMPK signalling in the heart of mice with germline deletion of the *MIF* gene (Miller *et al.* 2008). MIF promotes glucose uptake and protects the heart during ischaemia-reperfusion injury (Miller *et al.* 2008). Human fibroblasts with a low-activity MIF promoter polymorphism also have diminished MIF release and AMPK activation during hypoxia, thus linking inflammation with metabolism in the heart (Miller *et al.* 2008).

Tumour necrosis factor α (TNF α) signalling via TNF-receptor has been shown to suppress skeletal muscle AMPK activity both *in vivo* and *in vitro* (Steinberg *et al.* 2006a). This happens via upregulation of protein phosphatase 2C transcription, which in turn reduces ACC phosphorylation and fatty acid oxidation as well as increasing diacylglycerol accumulation in the muscle. Suppressive effects of TNF α on AMPK activity, seen in obese mice with pathologically elevated levels of TNF α , could be reversed in null mice for both TNF receptor-1 and -2 or following treatment with a TNF α -neutralising antibody (Steinberg *et al.* 2006a). This indicates that AMPK is an important target for TNF α signalling.

Metabolic syndrome and AMPK

The metabolic abnormalities observed in metabolic syndrome are insulin resistance, hypertriglyceridaemia, abdominal obesity, hypertension, reduced levels of the beneficial high-density lipoprotein and disturbances in glucose metabolism (Trevisan *et al.* 1998). Patients with metabolic syndrome have higher risks of developing cardiovascular disease (Isomaa *et al.* 2001) and have higher rates of mortality from all causes (Trevisan *et al.* 1998). Downstream targets of AMPK such as genes regulating carbohydrate metabolism (e.g. glycogen synthetase, ChREBP) or lipid metabolism (e.g. HMG-CoA, FAS, ACC, SREBP1) play an important role in features of the metabolic syndrome. Therefore, AMPK emerged as a target for treatment of the metabolic syndrome. Two major anti-diabetic drugs that exert effects via the AMPK pathway will be considered in this review: metformin and rosiglitazone.

Metformin

Metformin, a biguanide agent, is widely used as an anti-diabetic drug. The biguanide class of anti-diabetic drugs originates from the French lilac (*Galega officinalis*) plant, known for several centuries to reduce the symptoms of diabetes mellitus (Witters 2001). These were first introduced in 1957 and marketed in France in 1979.

Metformin is shown to stimulate AMPK in the liver and in the muscle (Zhou *et al.* 2001, Zang *et al.* 2004, Shaw *et al.* 2005). This consequently stimulates glucose uptake in the muscle, induces hepatic fatty acid oxidation and inhibits hepatic glucose production and expression of lipogenic enzymes. Metformin does not activate AMPK directly, but indirectly via inhibition of complex I of the respiratory chain and the consequent increase in AMP:ATP ratio. LKB1 has also been reported to mediate the activation of AMPK in the liver by metformin (Zhou *et al.* 2001, Zang *et al.* 2004, Shaw *et al.* 2005).

In contrast, metformin inhibits AMPK in the hypothalamus (Chau-Van *et al.* 2007). Metformin inhibits low glucose-induced AMPK phosphorylation and NPY mRNA expression. These may explain the anorectic effects of metformin.

Metformin is shown to reduce mitochondrial ATP synthesis in the pancreatic β -cell, resulting in impaired glucose responsiveness, inhibition of insulin release and possibly apoptosis (Kefas *et al.* 2004, Leclerc *et al.* 2004). These findings of metformin are clearly undesired and further studies are needed to reassess the long-term effects of metformin on β -cells.

Rosiglitazone

Rosiglitazone belongs to a class of anti-diabetic drugs known as thiazolidinediones (TZDs). TZDs are used to reverse insulin resistance and improve glucose tolerance. It is known that TZDs improve insulin sensitivity by activating nuclear PPAR- γ and the consequent regulation of gene transcription. However, it is also believed that TZDs can improve insulin sensitivity via PPAR- γ -independent mechanisms, one of which is AMPK activation (Kahn *et al.* 2005).

Rosiglitazone was shown to increase AMPK activity in muscle cell lines (Fryer *et al.* 2002), and chronic rosiglitazone treatment was reported to restore skeletal muscle AMPK α 2 activity in obese, insulin-resistant Zucker rats (Lessard *et al.* 2006). Rosiglitazone activates AMPK indirectly by inhibiting complex I of the respiratory chain, which consequently leads to an increase in cellular AMP:ATP ratio (El-Mir *et al.* 2000, Owen *et al.* 2000, Brunmair *et al.* 2004).

TZDs also decrease the levels of resistin and stimulate release of adiponectin via action on PPAR- γ in adipocytes (Samaha *et al.* 2006). These effects might also contribute to the stimulatory effect of TZDs on AMPK.

Conclusion

AMPK is one of the key regulators in energy homeostasis and is known to mediate the effects of several metabolic hormones. AMPK is now recognised as a potential target for the treatment of obesity and the metabolic syndrome.

Declaration of interest

The authors have nothing to declare.

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