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REVIEW

Insulin resistance and fuel homeostasis: the role of AMP-activated protein kinase

B. D. Hegarty, N. Turner, G. J. Cooney and E. W. Kraegen

Diabetes and Obesity Research Program, Garvan Institute of Medical Research, Sydney, NSW, Australia

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Correspondence: B. D. Hegarty, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia.
E-mail: b.hegarty@garvan.org.au

Abstract

The worldwide prevalence of type 2 diabetes (T2D) and related disorders of the metabolic syndrome (MS) has reached epidemic proportions. Insulin resistance (IR) is a major perturbation that characterizes these disorders. Extra-adipose accumulation of lipid, particularly within the liver and skeletal muscle, is closely linked with the development of IR. The AMP-activated protein kinase (AMPK) pathway plays an important role in the regulation of both lipid and glucose metabolism. Through its effects to increase fatty acid oxidation and inhibit lipogenesis, AMPK activity in the liver and skeletal muscle could be expected to ameliorate lipid accumulation and associated IR in these tissues. In addition, AMPK promotes glucose uptake into skeletal muscle and suppresses glucose output from the liver via insulin-independent mechanisms. These characteristics make AMPK a highly attractive target for the development of strategies to curb the prevalence and costs of T2D. Recent insights into the regulation of AMPK and mechanisms by which it modulates fuel metabolism in liver and skeletal muscle are discussed here. In addition, we consider the arguments for and against the hypothesis that dysfunctional AMPK contributes to IR. Finally we review studies which assess AMPK as an appropriate target for the prevention and treatment of T2D and MS.

Keywords AMPK, energy homeostasis, insulin resistance, lipid accumulation, liver, muscle.

Type 2 diabetes (T2D) currently affects close to 250 million people worldwide. By the year 2025, its prevalence is expected to reach 380 million and the associated costs are forecast to exceed US$300 billion (International Diabetes Federation, 2006). Clearly, it is imperative that we develop new strategies to curb and treat this epidemic.

Type 2 diabetes is one of several metabolic disorders that are collectively referred to as the metabolic syndrome (MS). T2D and other disorders of MS are characterized by an underlying resistance to the actions of insulin in target tissues, including the liver and skeletal muscle (Eckel *et al.* 2005). The liver plays an integral role in the coordination of fuel homeostasis. Through mechanisms that are regulated by insulin and counter-regulatory hormones, the liver releases glucose into the circulation when fuel is limited, and converts glucose into lipid when fuel is in excess (Postic *et al.* 2004). The skeletal muscle is the largest organ in the body and the most important site of insulin-stimulated glucose disposal (DeFronzo *et al.* 1981). Insulin resistance (IR) in these tissues therefore promotes the development of hyperglycaemia, due to both inadequate suppression of hepatic glucose production (HGP) and reduced glucose uptake into the skeletal muscle. Although hepatic lipogenesis is normally stimulated by insulin, interestingly, this pathway is generally not attenuated in states of IR (Brown & Goldstein 2008). In combination with reduced suppression of lipolysis in white adipose tissue (WAT), IR thus also promotes dyslipidaemia.

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There are a number of theories as to what causes IR in the MS, but it is clear that abnormalities in lipid metabolism plays a key role (McGarry 1992, Eckel *et al.* 2005). In particular, accumulation of lipid in the liver and skeletal muscle has been closely linked with both tissue-specific and whole-body IR (Hegarty *et al.* 2003, Savage *et al.* 2007, Kraegen & Cooney 2008).

AMP-activated protein kinase (AMPK) is a key metabolic enzyme that regulates both glucose and lipid metabolism. AMPK is activated under conditions of energetic stress. In line with a role of energy sensor, AMPK phosphorylates a wide range of downstream targets which, globally speaking, has the effect to stimulate energy-producing pathways [e.g. fatty acid (FA) oxidation and glucose utilization], and to inhibit energy-consuming pathways (e.g. lipogenesis and gluconeogeneis) (Hardie 2008). These effects could be expected to ameliorate dysregulated fuel metabolism in the MS via not only promoting insulin-independent improvements of glucose homeostasis but also by reducing extra-adipose lipid accumulation. This scenario has been largely based on animal and *in vitro* investigation, but should it prove the case in humans, then AMPK activation would be an attractive target for the prevention and treatment of T2D and other disorders of the MS. Conversely, it could also be proposed that dysregulation of the AMPK pathway may contribute to the metabolic perturbations characterizing IR.

The purpose of this review is to give an update on the regulation of AMPK and its metabolic actions, focusing on the liver and skeletal muscle. We discuss findings for and against a contributing role of AMPK dysfunction to the development of IR. Finally, we review the evidence that AMPK is a valid target for the treatment of T2D and other disorders of the MS.

The role of lipids in insulin resistance: location, location, location!

Over the last 15–20 years, numerous studies have revealed a close relationship between the accumulation of lipid in extra-adipose tissues, particularly the liver and skeletal muscle, and the development of IR (reviewed in Hegarty *et al.* 2003, Savage *et al.* 2007, Kraegen & Cooney 2008). Whilst first demonstrated in animal models, this relationship is now well established in clinical states of human IR through the use of direct muscle biopsy and via noninvasive magnetic resonance spectroscopy (Boesch *et al.* 1997, Gan *et al.* 2002, Savage *et al.* 2007).

Accumulation of lipid within the liver and muscle results from an imbalance of energy supply and/or demand within these individual tissues. It can manifest over prolonged periods, for example in association with the development of diet-induced obesity (Greco *et al.* 2002) or acutely (3–5 h), as in the case of experimental models involving glucose or lipid infusions (Itani *et al.* 2002, Ye *et al.* 2002, Kraegen *et al.* 2006). Interestingly, even where overall caloric intake remains 'normal', simply increasing the proportion of energy in the diet that is derived from fats leads to lipid accumulation in the liver and skeletal muscle (Storlien *et al.* 1991, Kim *et al.* 2003). Indeed, our own studies of high-fat fed rats show that the skeletal muscle adapts to the diet to better extract and oxidize circulating lipids, presumably in order to best utilize the most predominant fuel available (Hegarty *et al.* 2002, Turner *et al.* 2007). Lipodystrophy represents an extreme example of lipid oversupply, where the lack of adipose storage tissue drives lipid into the skeletal muscle and liver, and is associated with severe IR (Simha & Garg 2006). Recently, much attention has been focused upon the possibility that reduced mitochondrial oxidative capacity of the skeletal muscle may be a causal factor leading to lipid accumulation and the development of IR. While some forms of IR are clearly associated with reduced number of mitochondria in the skeletal muscle or impaired mitochondrial function, the primacy of this defect in the development of lipid accumulation and IR remains unclear (Bonnard *et al.* 2008, Kim *et al.* 2008a, Turner & Heilbronn 2008).

Hepatic and intramuscular triglyceride content is commonly used as a marker of lipid accumulation in these tissues; however, evidence suggests that it is more likely that lipid intermediates, including diacylglycerols (DAG), ceramides and long-chain acyl-CoAs (LCACoA) are responsible for perturbations of insulin action rather than metabolically inert triglycerides (Kraegen & Cooney 2008, Timmers *et al.* 2008). Indeed, the upregulation of DGAT1 in mouse skeletal muscle, which causes the sequestration of lipid out of DAG and ceramide into intramuscular triglyceride pools, has recently been associated with increased insulin sensitivity (Liu *et al.* 2007). Mechanisms by which these lipid intermediates are believed to interfere with insulin signalling have been recently reviewed by Holland *et al.* (2007).

Overall, in terms of insulin sensitivity the *location* of lipid appears to be more important than the quantity of lipid. i.e. at the cellular level, it is best to sequester lipid into triglyceride rather than accumulating large pools of DAG, ceramide or LCACoAs (Liu *et al.* 2007, Kraegen & Cooney 2008); at the whole body level, it is best to store lipid in WAT rather than in liver or skeletal muscle (Kim *et al.* 2007).

Given the well-described effects of AMPK to stimulate FA oxidation in the liver and skeletal muscle as well as to suppress hepatic lipogenesis, this kinase has become a particularly interesting target in the search for strategies that can reduce extra-adipose lipid accumulation and associated IR (Hardie 2008).

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AMPK structure and activation

AMPK is a serine/threonine kinase with a diverse range of downstream metabolic targets (Carling *et al.* 2008). It has a heterotrimeric structure comprising a catalytic α subunit and regulatory β and γ subunits. For maximal kinase activity, AMPK must be phosphorylated on the Thr172 residue of the α subunit (αThr172). The level of phosphorylation at αThr172 depends upon the balance of activities of upstream kinases [e.g. LKB1 and calmodulin-dependent kinase β (CaMKKβ)] and phosphatases [e.g. protein phosphatase 2C (PP2C)] that act at this site (Carling *et al.* 2008).

AMPK is classically activated by an increase in the intracellular AMP : ATP ratio, which occurs under conditions of metabolic stress, and is thereby believed to function as a cellular energy sensor. The exact mechanism of activation by AMP remains to be fully understood; however, current evidence suggests that AMP binds to the γ-subunit of AMPK inducing a conformational change. This causes allosteric activation of the enzyme and also reduces phosphatase access to the αThr172 site, leading to a net increase in phosphorylation via constitutive LKB1 activity (Suter *et al.* 2006, Sanders *et al.* 2007, Xiao *et al.* 2007).

AMPK can also be phosphorylated at αThr172 by CaMKKβ, which is itself activated by increases in intracellular calcium levels. Whilst this calcium-dependent pathway of AMPK activation has been demonstrated in neurones, endothelial cells and t-lymphocytes (Hawley *et al.* 2005, Stahmann *et al.* 2006, Tamás *et al.* 2006), there appears to be little contribution of CaMKKβ to AMPK phosphorylation in the major metabolic tissues involved in fuel homeostasis. Indeed, all evidence points to LKB1 as the main kinase responsible for AMPK activation in liver and WAT, as well as for phosphorylation of the α2-isoform in the skeletal muscle (Sakamoto *et al.* 2005, Shaw *et al.* 2005, Daval *et al.* 2006).

Metabolic targets of AMPK

The list of AMPK target genes is long and steadily growing (see Hardie 2007 for a comprehensive list of targets). It is expressed in a wide range of tissues, and affects metabolic pathways through both central and peripheral actions (Xue & Kahn 2006). Here we will focus, however, on the direct effects of AMPK within the liver and skeletal muscle that are of particular interest in terms of fuel homeostasis and insulin sensitivity. Specifically, we will discuss the targets of AMPK involved in the stimulation of FA oxidation and inhibition of lipid synthesis, which, according to the arguments above, would be expected to reduce ectopic lipid accumulation and improve insulin action. We will also address the actions of AMPK to promote glucose uptake and oxidation in the skeletal muscle, as well as to suppress HGP, which can improve glucose homeostasis independently of insulin.

Fatty acid oxidation and lipid synthesis

One of the major effects of AMPK on lipid metabolism is centred around its ability to regulate the intracellular content of malonyl-CoA. Malonyl-CoA is both a substrate for lipogenesis and an inhibitor of carnitine palmitoyltransferase 1 (CPT-1), the rate-limiting enzyme that controls the transfer of cytosolic FA into mitochondria for oxidation (Fig. 1) (Ruderman & Saha 2006). AMPK reduces malonyl-CoA levels through inhibitory phosphorylation of acetyl-CoA carboxylase [ACC; one of the first targets of AMPK to be identified (Carling *et al.* 1987)], and secondly, by the stimulatory phosphorylation of malonyl-CoA decarboxylase (MCD), which catalyses malonyl CoA degradation (Saha *et al.* 2000). The decrease in malonyl-CoA resulting from these dual effects, along with the coincident inhibition of sn-glycerol-3-phosphate acyltransferase (GPAT), promotes FA oxidation and inhibits FA and glycerolipid synthesis in skeletal muscle and liver (Merrill *et al.* 1997, Velasco *et al.* 1997, Muoio *et al.* 1999, Park 2002). Although these actions of AMPK are well established in cell-systems and animal models, there are only a few studies that have shown a direct link between AMPK activation and FA metabolism in human tissues. Steinberg *et al.* (2004, 2006a) however demonstrated that the rate of FA oxidation in isolated human muscle strips and cultured human skeletal muscle cells is increased when AMPK is activated using the pharmacological agent AICAR.

Recently, Puljak *et al.* (2008) reported that AMPK rapidly increases lipoprotein exocytosis from a liver cell line, and demonstrated that this effect can significantly reduce intracellular lipid content. Although potentially beneficial for the liver, one might expect that this effect could exacerbate hypertriglyceridaemia and lipid accumulation in skeletal muscle *in vivo*.

AMPK activation in WAT acutely suppresses lipolysis (Daval *et al.* 2006), thereby reducing the exposure of the liver and skeletal muscle to FA from the circulation. A major mechanism contributing to this anti-lipolytic effect is likely to be the inhibitory regulation of hormone-sensitive lipase (HSL), the enzyme that catalyses the breakdown of DAG to glycerol and FA. AMPK has been shown to phosphorylate HSL at Ser565 which inhibits both basal and stimulated lipolysis (Watt & Steinberg 2008).

In addition to these acute actions, AMPK also has longer-term effects on lipid synthesis and utilization via regulation of gene transcription (Ferré *et al.* 2003).

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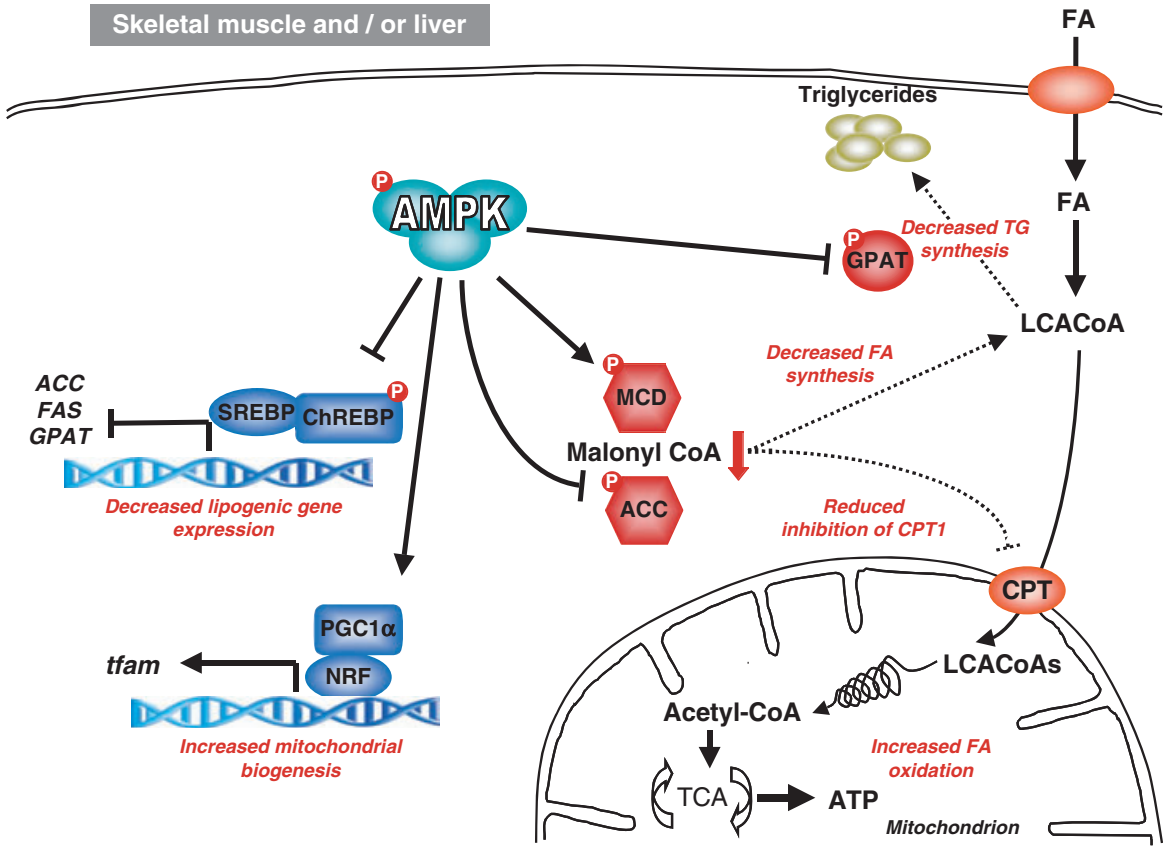

Figure 1 Regulation of lipid metabolism by AMP-activated protein kinase (AMPK). The combined effects of AMPK to inactivate acetyl-CoA carboxylase (ACC) and stimulate malonyl-CoA decarboxylase (MCD) leads to a reduction in intracellular malonyl-CoA levels. As malonyl-CoA is both an allosteric inhibitor of carnitine palmitoyltransferase (CPT) and a substrate for lipogenesis, the reduction in malonyl-CoA levels promotes fatty acid (FA) entry into mitochondria for oxidation, and simultaneously reduces lipogenesis. AMPK also reduces triglyceride synthesis through the inhibitory phosphorylation of GPAT. These acute effects of AMPK on enzyme activities are supported in the long-term by the stimulation of mitochondrial biogenesis and suppression of lipogenic gene expression.

AMPK activation reduces the abundance of hepatic lipogenic genes including ACC, fatty acid synthase (FAS) and GPAT (Foretz *et al.* 1998, Leclerc *et al.* 1998, Woods *et al.* 2000, Zhou *et al.* 2001). AMPK is thought to induce these effects through regulation of transcription factors, including inhibitory phosphorylation of ChREBP (Kawaguchi *et al.* 2002), and reduced expression of both sterol regulatory-element-binding protein-1 (SREBP) and hepatic nuclear factor α (HNFα) (Leclerc *et al.* 2001, Zhou *et al.* 2001) (Fig. 1). Chronic AMPK activation has also been reported to stimulate mitochondrial biogenesis and mitochondrial enzyme content in skeletal muscle (Winder *et al.* 2000, Bergeron *et al.* 2001, Zong *et al.* 2002). This appears to be mediated by an increase in the abundance of peroxisome proliferation-activated receptor (PPAR) γ coactivator 1α (PGC1α) and enhanced binding of the nuclear respiratory factor-1 (NFR-1) transcription factor (Bergeron *et al.* 2001, Zong *et al.* 2002) (Fig. 1). These effects of AMPK activation on mitochondria are consistent with an action to increase the capacity to oxidize FA.

Overall, the short and long-term effects of AMPK on lipid metabolism promote the oxidation of FA and reduce glycerolipid synthesis in the liver and skeletal muscle.

Glucose uptake and utilization in skeletal muscle

In addition to its effects on FA synthesis and utilization, AMPK also regulates aspects of carbohydrate metabolism. The finding that exercise, *ex vivo* contraction and AICAR, all of which activate AMPK, stimulate glucose uptake into skeletal muscle independently of insulin led to the hypothesis that AMPK mediates this effect (Hayashi *et al.* 1998). Studies on mice lacking either the α1 or α2 AMPK catalytic subunits demonstrated that AMPK is required for the effect of AICAR on glucose uptake; however, exercise/contraction stimulated glucose uptake involves additional AMPK-independent

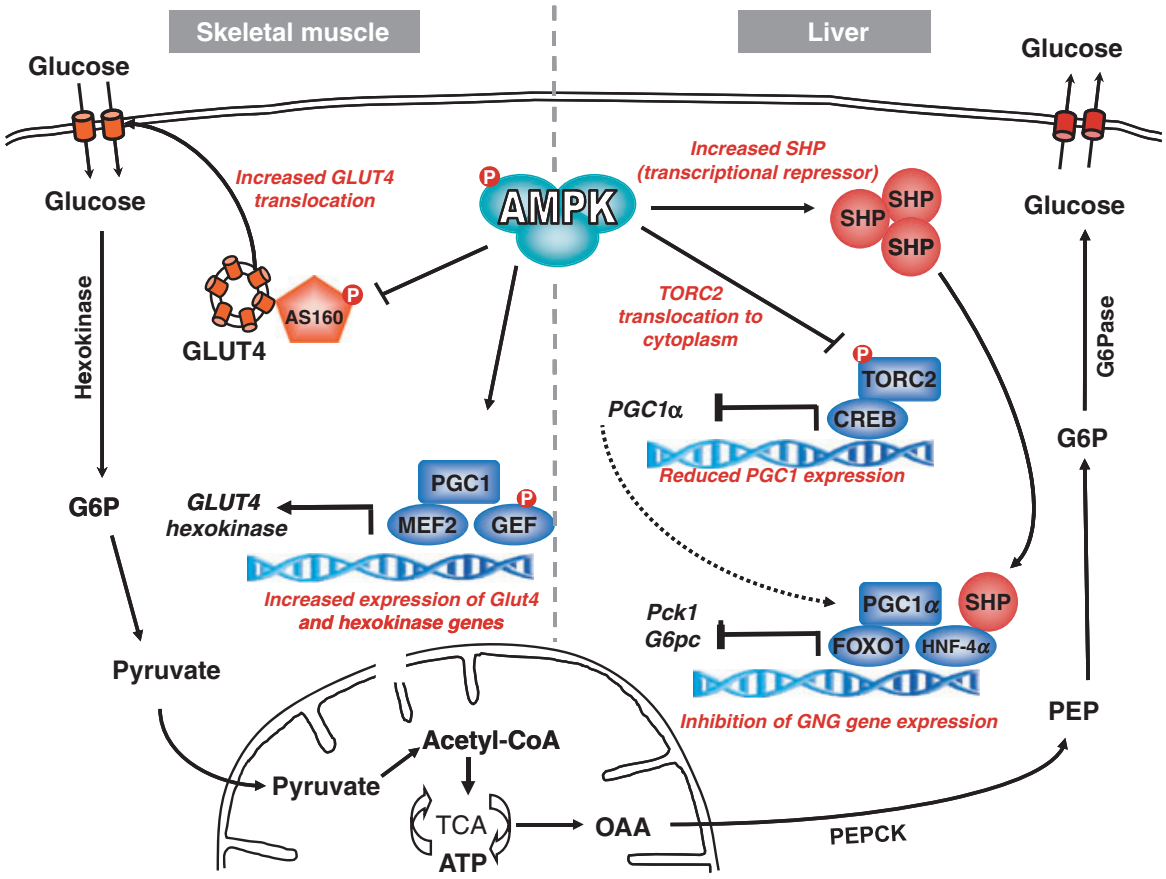

Figure 2 Regulation of glucose metabolism in skeletal muscle and liver by AMP-activated protein kinase (AMPK). In the skeletal muscle, AMPK promotes glucose uptake and utilization by stimulating GLUT4 translocation to the plasma membrane. This is mediated, at least in part, via the inhibitory phosphorylation of the Rab GTPase-activating protein AS160. AMPK also increases the expression of genes encoding GLUT4 and hexokinase. In the liver, AMPK reduces hepatic glucose production by inhibiting the transcription of genes encoding PEPCK and G6Pase. Mechanisms include the sequestration of TORC2 to the cytoplasm and increasing the expression of the small heterodimer partner (SHP) transcriptional repressor. G6P, glucose-6-phosphate; OAA, oxaloacetate; PEP, phosphoenolpyruvate.

pathways (Jørgensen *et al.* 2004b). AICAR has also been shown to stimulate glucose uptake into skeletal muscle in humans *in vivo*; however, in this study no increase in AMPK activity was detected (Cuthbertson *et al.* 2007). In perfused rat hindlimb and isolated human muscle strips, AICAR-induced glucose uptake is associated with increased translocation of the glucose transporter, GLUT4, to the plasma membrane (Kurth-Kraczek *et al.* 1999, Koistinen *et al.* 2003) (Fig. 2). This GLUT4 translocation appears to result, at least in part, from the inhibitory phosphorylation of AS160/TBC1D4 and/or TBC1D1 by AMPK (Sano *et al.* 2003, Treebak *et al.* 2006, Chavez *et al.* 2008, Chen *et al.* 2008). In their active form, these two Rab GTPase-activating proteins (RabGAPs) function to retain GLUT4-laden vesicles within the cytoplasm (Sakamoto & Holman 2008). The phosphorylation and inhibition of AS160/TBC1D4 and TBC1D1 RabGAP activity by insulin and/or AMPK relieves this anchoring function and sets in motion the trafficking, docking and fusion of GLUT4 vesicles with the plasma membrane (Sakamoto & Holman 2008). The intricacies of this regulation are still far from clear; however, variations in tissue expression of the two RabGAPs and differential targeting of their multiple phosphorylation sites appear to contribute to the complementary roles of insulin and AMPK to regulate GLUT4 translocation under different experimental and physiological conditions (Chavez *et al.* 2008, Chen *et al.* 2008). AMPK activation by AICAR and hypoxia has also been shown to mediate an increase in glucose uptake via increasing the glucose transporter activity of GLUT1 (Jing *et al.* 2008). Although it appears that AMPK induced activation of MAPK pathways may be involved in this effect, the exact mechanisms remain to be elucidated (Jing *et al.* 2008).

In line with an acute stimulation of GLUT4 translocation, AMPK also has a more prolonged effect on

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glucose uptake by increasing the expression of both GLUT4 and hexokinase (Holmes *et al.* 1999) (Fig. 2). The transcriptional coactivator PGC1α appears to play an essential role in the induction of GLUT4 expression by AMPK (Jäger *et al.* 2007). In addition, binding of the transcriptions factors myocyte enhancer factor 2 (MEF2) and GLUT4 enhancer factor (GEF) to the GLUT4 promoter is reportedly required for transcription of this gene (Karnieli & Armoni 2008) (Fig. 2). MEF2 activity at the GLUT4 promoter is suppressed through an association with the transcriptional repressor histone deacetylase 5 (HDAC5) in human skeletal muscle (McGee & Hargreaves 2004). Recently McGee *et al.* (2007) reported that AMPK phosphorylates HDAC5 at Ser259 and Ser498 in human primary myocytes. Phosphorylation at these residues causes HDAC5 dissociation from the GLUT4 promoter and sequestration to the cytoplasm, thereby alleviating its inhibition of MEF2 and inducing GLUT4 transcription (McGee *et al.* 2007). Holmes (2005) demonstrated *in vitro* that AICAR also directly phosphorylates GEF and increases its nuclear content. Importantly, by increasing the expression of GLUT4 in the skeletal muscle, AMPK increases the intracellular pool of this transporter available for translocation in response to insulin, and could thereby enhance insulin-stimulated glucose uptake into skeletal muscle.

In line with its role as an intracellular energy rheostat, AMPK also phosphorylates and inactivates glycogen synthase (GS) in skeletal muscle, thereby suppressing the energetically expensive process of glycogen synthesis, and partitioning glucose towards oxidation (Jørgensen *et al.* 2004a, Miyamoto *et al.* 2007). Although this effect can be understood in terms of AMP : ATP homeostasis, it could be seen as detrimental to the action of insulin, a potent activator of glycogen synthesis. Indeed, Miyamoto *et al.* (2007) showed that insulin-stimulated GS activity in isolated rat skeletal muscle was reduced after AICAR treatment. However, as these authors pointed out, AICAR administration to rats is associated with both increased glycogen content and improved insulin action in skeletal muscle (Holmes *et al.* 1999, Iglesias *et al.* 2002), and therefore it would appear that other effects of AMPK activation, including the promotion of glucose uptake via increased GLUT4 and hexokinase expression, as well as increased muscle content of glucose-6-phosphate (G6P; a potent allosteric activator of GS) must prevail over the acute inhibition of GS activity (Miyamoto *et al.* 2007).

Hepatic glucose production

Whilst AMPK promotes glucose uptake into skeletal muscle, within the liver, the kinase is more important in regulating glucose output (Fig. 2). The liver plays an important role in releasing glucose into the bloodstream when circulating levels do not meet metabolic demands, e.g. during fasting, stress and exercise. This altruistic role of the liver is fulfilled acutely through the breakdown of glycogen stores (glycogenolysis), and in the more long term, through the production of glucose from 3-carbon precursors (gluconeogenesis, GNG) (Fig. 2). When glucose is abundant and HGP is no longer required, insulin acts to suppress both of these processes. During IR however, the suppression of HGP, particularly GNG, is impaired, promoting the chronic elevation of circulating glucose.

Various stimuli that activate AMPK have been found to suppress HGP. This has been primarily attributed to AMPK-mediated reduction in the expression of genes encoding the GNG enzymes phosphenolpyruvate carboxykinase (PEPCK, *Pck1*) and glucose-6-phosphatase (G-6-Pase, *G6pc*) (Lochhead *et al.* 2000, Yamauchi *et al.* 2002, Cool *et al.* 2006) (Fig. 2). The transcriptional regulation of these genes is complex and incompletely understood; however, CREB (cAMP-response element binding protein), HNF-4α (hepatocyte nuclear factor-4α) and FOXO1 have been identified as key transcription factors involved in the induction of *Pck1* and *G6pc* expression (Mayr & Montminy 2001, Hirota *et al.* 2008, Nakae *et al.* 2008). CREB has an additional role to increase the transcription of PGC1α, an important co-activator of GNG gene transcription (Herzig *et al.* 2001, Yoon *et al.* 2001). AMPK has been reported to inhibit the activity of this group of transcriptional regulators via several mechanisms. One such mechanism involves phosphorylation of the CREB co-activator, TORC2 (transducer of regulated CREB activity 2) which normally interacts with CREB, markedly enhancing its induction of cAMP-responsive genes. By phosphorylating TORC2 at Ser171, AMPK causes its cytosolic sequestration, precluding its interaction with nuclear CREB and reducing CREB transcriptional activity (Fig. 2) (Koo *et al.* 2005). Horike *et al.* (2009) have recently proposed that AMPK also indirectly inhibits CREB-induced gene transcription through the phosphorylation and inhibition of GS kinase 3β (GSK3β). This action would reduce the ability of GSK3β to activate CREB by phosphorylation at Ser129 (Horike *et al.* 2009). AMPK reportedly increases the expression of orphan nuclear receptor SHP (small heterodimer partner), which can interact with HNF4α and FOXO1, repressing their transcriptional activities (Kim *et al.* 2008b) (Fig. 2). Finally, Barthel *et al.* (2002) propose that AMPK may stimulate the rapid degradation of FOXO1; however, no mechanism for this effect was presented.

Studies investigating the regulation of HGP by AMPK have almost exclusively focused on the regulation of gluconeogenic gene expression. In our own studies

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however, we have recently explored the possibility of more acute mechanisms, and have found that several AMPK activators suppress net glucose output from cultured mouse hepatocytes within 2–3 h, independently of a change in PEPCK and G6Pase protein content. Interestingly, although this acute suppression of glucose output showed a close temporal association with the activation of AMPK by these stimuli, the effect was completely maintained in hepatocytes isolated from mice lacking both AMPK α1 and α2 catalytic subunits in the liver, indicating an AMPK-independent mechanism (Hegarty *et al.* 2008).

Summary of metabolic targets

Taken together, the vast majority of AMPK's actions described above have the potential of ameliorating IR and improving glucose homeostasis. By stimulating FA oxidation and inhibiting lipogenesis, AMPK activity within the liver and skeletal muscle would be expected to reduce lipid accumulation and its associated interference with insulin action in these tissues. In addition, by stimulating skeletal muscle glucose uptake and the suppression of HGP independently of insulin signalling, AMPK activation has the potential to counteract the effects of IR via bypassing defective insulin signalling. Importantly however, as these functions have mainly been derived from *in vitro* and animal-based findings, their relevance to human physiology needs further study.

Physiological regulation of AMPK

Conditions of metabolic stress that activate AMPK include hypoxia, ischaemia and hyperosmolarity (Hardie 2008). More common physiological stresses include fasting (Assifi *et al.* 2005) and, of particular importance, exercise (Park 2002). Quite recently, it has become clear that in addition to metabolic stress, AMPK activity can also be modulated by a number of hormones, particularly 'adipokines' secreted from WAT, introducing a whole new paradigm of metabolic regulation via AMPK (Kola *et al.* 2006, Xue & Kahn 2006).

Regulation of AMPK by exercise

One of the most important physiological regulators of fuel utilization is exercise, which rapidly increases whole body energy metabolism by up to 20-fold, and increases requirements for metabolic substrates in the working muscle (Kiens 2006). An acute bout of exercise increases FA oxidation in contracting muscle as well as insulin-independent glucose uptake. Furthermore, chronic exercise training promotes mitochondrial biogenesis, increases oxidative capacity of the skeletal muscle and improves insulin sensitivity (Long & Zierath 2006). For these reasons exercise is recognized as an important intervention for the treatment of obesity and T2D. Interestingly, these effects show remarkable similarity to those of AMPK activation (see above). Indeed a recent study purports that AMPK activation can act as an exercise mimetic in terms of chronic alterations in gene expression (Narkar *et al.* 2008). In healthy humans, an acute bout of exercise activates AMPK in an isoform and intensity-dependent manner (Fujii *et al.* 2000, Wojtaszewski *et al.* 2000). AMPK complexes containing the α2-catalytic subunit are predominantly activated, with α1-containing complexes being recruited only during intense power output (Jorgensen & Rose 2008). It would appear reasonable therefore to propose that AMPK mediates the exercise-induced changes in glucose and FA metabolism (Hayashi *et al.* 1998, Holmes *et al.* 1999). Providing evidence to support this hypothesis has however been somewhat difficult. Whereas a variety of AMPK transgenic and knockout models show that AMPK is required for hypoxia or AICAR-induced regulation of glucose and FA metabolism in the skeletal muscle, the lack of AMPK is reported to cause only a partial, if any, defect in the regulation of fuel metabolism with exercise/contraction (Mu *et al.* 2001, Jørgensen *et al.* 2004b, Long & Zierath 2006, Jorgensen & Rose 2008). Whether these animal models reflect a true redundancy of AMPK in mediating the acute and chronic metabolic effects of exercise, or whether these models are compromised by residual AMPK activity or compensatory mechanisms, remains to be clarified.

Adipokine regulation of AMPK

In recent years it has become clear that WAT is not only a storage site for triglycerides but is also an active endocrine organ, secreting a suite of molecules that play important roles in the regulation of whole-body energy homeostasis, immunity and inflammation (Antuna-Puente *et al.* 2008). Several of these 'adipokines' mediate at least some of their effects via modulating AMPK activity in target tissues (Kola *et al.* 2006).

Leptin, the protein product of the *ob* gene and one of the first adipokines identified, has been demonstrated to increase FA oxidation and reduce lipid accumulation in skeletal muscle and liver via an AMPK-dependent mechanism (Minokoshi *et al.* 2002, Yu *et al.* 2004, Brabant *et al.* 2005, Tanaka *et al.* 2005). Leptin reportedly induces a biphasic activation of AMPK in skeletal muscle; the initial activity is attributed to leptin interaction with its receptors in the muscle, whereas the second peak is mediated via central mechanisms after stimulation of leptin receptors in the hypothalamus

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(Minokoshi *et al.* 2002). The physiological significance of leptin's direct interaction with receptors in muscle and liver is unclear. At least in the case of the liver, leptin's effects appear to be predominantly mediated via central signalling pathways (Bjørbaek & Kahn 2004). Interestingly, in the feeding centres of the hypothalamus, leptin actually *inhibits* AMPK, and this contributes to leptin's well-described effects to suppress appetite (Minokoshi *et al.* 2004, 2008).

Another adipokine, adiponectin, has been shown to increase FA oxidation in both skeletal muscle and liver via activation of AMPK, although the upregulation of PPARα signalling is also involved (Yamauchi *et al.* 2002, 2007). In addition to its effects on FA metabolism, adiponectin promotes glucose uptake into skeletal muscle and suppresses glucose output from the liver via AMPK-dependent mechanisms (Yamauchi *et al.* 2002, 2007). Adiponectin is thought to exert these effects through interaction with two cell surface receptors, coined AdipoR1 and AdipoR2 (Yamauchi *et al.* 2003). Experiments in mice in which the receptors were either overexpressed or knocked out led to the proposal that the activation of AMPK by adiponectin is mediated via AdipoR1 (Yamauchi *et al.* 2007). A separate study of mice null for either AdipoR1 or AdipoR2 however demonstrates phenotypes that do not support this paradigm (Bjursell *et al.* 2007). There is also virtually nothing known about the intracellular signalling pathways lying downstream of these receptors, thus the mechanism by which adiponectin activates AMPK remains unknown. To add to the complexity of this system, adiponectin circulates in the form of low-, medium- and high-molecular weight multimers, which appear to have distinct physiological effects and target tissues (Yamauchi *et al.* 2003, Schraw *et al.* 2008, Swarbrick & Havel 2008).

Not all adipokines activate AMPK. Evidence suggests that resistin suppresses AMPK activity in both liver and skeletal muscle (Banerjee *et al.* 2004, Muse *et al.* 2004, Palanivel & Sweeney 2005); however, the mechanism by which resistin mediates this effect remains unknown. Tumour necrosis factor α (TNFα) has also been shown to suppress AMPK activity in skeletal muscle (Steinberg *et al.* 2006b). This is reportedly mediated by the upregulation of PP2C expression, the enzyme that dephosphorylates and inactivates AMPK (Steinberg *et al.* 2006b). The inhibition of AMPK activity in skeletal muscle and/or liver by both resistin and TNFα is associated with reduced FA oxidation and increased lipid accumulation in these tissues (Banerjee *et al.* 2004, Muse *et al.* 2004, Palanivel & Sweeney 2005, Steinberg *et al.* 2006b).

The regulation of AMPK and resultant effects on fuel metabolism induced by these adipokines are accompanied by corresponding modulation of insulin sensitivity; thus adiponectin and leptin improve insulin action, whereas resistin and TNFα cause IR. Although other mechanisms may contribute to the metabolic actions of the adipokines, the consistent and coordinated modulation of AMPK activity suggests that this kinase plays a significant role. Further evidence for this comes from studies which show that leptin and adiponectin can, to a certain extent, compensate for each other by virtue of their similar effects on AMPK. For example, a lack of leptin signalling, as seen in the ob/ob mouse, is associated with obesity, dyslipidaemia, systemic inflammation and marked IR. Moderate overexpression of adiponectin on top of the ob/ob background markedly improves the metabolic profile of these animals, despite their remaining obese (Shklyaev *et al.* 2003). Conversely, the surprisingly mild IR observed in adiponectin knockout mice has recently been attributed to an increased sensitivity of these animals to endogenous leptin (Yano *et al.* 2008).

Does AMPK dysregulation contribute to insulin resistance?

It is clear that AMPK has a number of actions which could potentially alter insulin sensitivity. The question therefore arises as to whether the AMPK pathway may be dysregulated in insulin-resistant states and be a significant contributing factor to defective insulin action.

Studies investigating the status of AMPK signalling pathways in insulin resistant human tissue or primary cells are contradictory. Whereas some studies find that skeletal muscle AMPK expression, activity and activation by exercise are attenuated in obese and/or T2D patients (Bruce *et al.* 2005, Bandyopadhyay *et al.* 2006, Sriwijitkamol *et al.* 2007), other studies find that there is no defect (Musi *et al.* 2001, Hojlund 2003, Koistinen *et al.* 2003, Steinberg *et al.* 2004). Even where AMPK dysregulation is demonstrated, these studies remain only correlative and show no evidence that a reduction in AMPK activity causes or contributes to IR.

In order to address this issue, animals have been generated with genetic manipulations to knockout global α1 or α2 AMPK expression (Viollet *et al.* 2003a,b), or to abolish total AMPK activity specifically within the skeletal muscle (Mu *et al.* 2001). Surprisingly, only the global α2 knockout demonstrated any apparent defect in whole-body fuel homeostasis, and none of these models showed a reduction in insulin sensitivity of skeletal muscle or liver (Mu *et al.* 2001, Viollet *et al.* 2003a,b). Recently however, Fujii *et al.* (2008) reported that transgenic mice expressing an inactive form of AMPK α2 subunit specifically in skeletal muscle (α2i TG) develop impaired whole-body

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glucose tolerance and IR in skeletal muscle, particularly when fed a high-fat diet. The exact mechanism mediating the exacerbated high-fat diet induced IR in α2i TG mice is unclear. For example, it does not seem to be a result of accumulation of intramuscular lipid due to the reduced AMPK activity, as muscle triglycerides, DAG and ceramides were all similar between genotypes on the high-fat diet (Fujii *et al.* 2008).

It is worth mentioning that a polymorphism in the gene encoding AMPK α2 subunit has been linked with IR and T2D in the Japanese population (Horikoshi *et al.* 2006). Conversely, a gain-of-function mutation in the gene encoding human AMPK γ3-subunit is reported to confer beneficial effects on muscle fuel metabolism (Costford *et al.* 2007).

Interestingly, studies performed using primary myotubes isolated from lean, obese and T2D patients show that activation of AMPK in these cells by AICAR was similar across groups; however, the ability of both adiponectin and leptin to activate AMPK was compromised in myotubes from the obese and T2D patients (Chen *et al.* 2005, Steinberg *et al.* 2006a). Similarly, reduced stimulation of FA oxidation by leptin and adiponectin, as well as suppressed adiponectin-induced glucose uptake has been demonstrated in muscle isolated from obese humans, providing direct evidence for the presence of leptin and adiponectin resistance in skeletal muscle (Steinberg *et al.* 2002, Bruce *et al.* 2005). This suggests that while there may be no intrinsic defect in AMPK signalling in obesity or T2D (and hence responses to external activators such as AICAR appear normal), it is possible that there is dysregulation of endogenous hormonal regulation (e.g. via adiponectin and leptin).

AMPK as a therapeutic target for the treatment of insulin resistance

Approximately a decade ago AMPK was suggested as a putative target for the treatment of IR and T2D (Winder & Hardie 1999). Since that time a substantial body of literature has accumulated in support of this concept. *In vitro* studies in skeletal muscle have demonstrated enhanced insulin-mediated glucose transport after administration of the AMPK agonist AICAR (Fisher *et al.* 2002, Ju *et al.* 2007). Administration of AICAR *in vivo* has been shown to improve insulin action in muscle and liver of insulin-resistant high-fat fed rats (Iglesias *et al.* 2002). AICAR also diminished ectopic lipid deposition in liver and muscle of Zucker Diabetic Fatty (ZDF) rats and slowed the progression to T2D in these animals (Yu *et al.* 2004). Furthermore, treatment of obese Zucker rats with AICAR for seven weeks markedly improved a number of metabolic parameters to near Lean control values, including plasma lipids, glucose and insulin, glucose tolerance and systolic blood pressure (Buhl *et al.* 2002).

The finding that the commonly used antidiabetic drug metformin also activates AMPK (Zhou *et al.* 2001, Musi *et al.* 2002) added further support to the idea that targeting the AMPK pathway could have beneficial effects on insulin sensitivity (Cleasby *et al.* 2004). Metformin is one of the most commonly used drugs for the treatment of T2D, reducing blood glucose concentrations by both suppressing HGP (Hundal *et al.* 2000) and increasing glucose disposal into skeletal muscle (Musi *et al.* 2002). These beneficial effects of metformin on glycaemic control are thought to be largely dependent on activation of the AMPK cascade (Zang *et al.* 2004, Shaw *et al.* 2005). Interestingly, the mechanism by which metformin has been proposed to activate AMPK is through inhibition of complex I of the mitochondrial respiratory chain (Owen *et al.* 2000), which causes a subsequent change in the energy status of the cell (Musi *et al.* 2002, Zhang *et al.* 2007).

Thiazolidinediones (TZDs), another class of insulin-sensitizing drugs, have also been shown to exert their beneficial metabolic effects, in part by increasing AMPK activity (Fryer *et al.* 2002, Saha *et al.* 2004, Bandyopadhyay *et al.* 2006). Recently it was shown in T2D patients that activation of AMPK in muscle by TZDs ultimately results in a repartitioning of FAs away from storage and towards oxidation (Bandyopadhyay *et al.* 2006). Hence, at least part of the effect of TZDs to reduce lipid accumulation in peripheral tissues is likely due to increased FA oxidation, secondary to AMPK activation. The mechanism of activation of AMPK by TZDs has been proposed to be principally related to enhanced production of adiponectin from adipose tissue (Nawrocki *et al.* 2006) although more direct mechanisms may be involved (LeBrasseur *et al.* 2006), including perturbation of mitochondrial function (Brunmair *et al.* 2004, Turner *et al.* 2008).

The list of compounds which can activate AMPK and improve insulin sensitivity continues to expand. Berberine, a natural plant product which has been used as a traditional medicine in Asia, was recently shown to enhance insulin sensitivity in rodent models of IR and diabetes (Lee *et al.* 2006) and also activate AMPK (Cheng *et al.* 2006, Yin *et al.* 2008). Recent work has shown that berberine likely acts in a similar fashion to metformin (and TZDs) to inhibit complex I of the mitochondrial electron transport chain (Turner *et al.* 2008, Yin *et al.* 2008), thus indirectly activating AMPK via alteration in the energy nucleotides. Although it may seem somewhat counterintuitive that inhibiting electron transport in the mitochondria may actually promote FA oxidation, a key factor is the site at which these agents act in the electron transport chain. Partial inhibition of complex I could lead to an increased reliance on

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FADH-linked electron transfer that is largely coupled to FA beta-oxidation, bypassing complex I of the electron transport chain. Therefore it is likely that with partial inhibition of complex I, increased FA oxidation (facilitated by AMPK activation) and fat loss may result as a consequence of the increased reliance on less efficient FADH-linked electron transport to support oxidative phosphorylation.

Recently another class of compounds, the triterpenoids, isolated from bitter melon, were found to be highly potent AMPK activators with antidiabetic properties, although the mechanism of action of the triterpenoids is yet to be resolved (Tan *et al.* 2008). Polyphenolic compounds, such as resveratrol, have received great interest recently due to their ability to mimic the effects of calorie restriction (Howitz *et al.* 2003, Baur *et al.* 2006). Polyphenols have been shown to improve insulin sensitivity in high-fat fed rodents as well as activate AMPK (Baur *et al.* 2006, Lagouge *et al.* 2006, Zang *et al.* 2006). While the metabolic properties of these compounds have been largely linked with their ability to activate the SIRT1 deacetylase enzyme (Lagouge *et al.* 2006), recent work has shown that many of the favourable effects of polyphenols, particularly in relation to hepatic lipid metabolism, occur in an AMPK-dependent manner (Hou *et al.* 2008), indicating that AMPK signalling is likely involved in the insulin-sensitizing effects of these compounds. An additional agent that can activate AMPK is beta-guanidinopropionic acid (beta-GPA) (Zong *et al.* 2002), which appears to simulate a condition of energy stress by depleting creatine phosphate storage. Consistent with their role to activate AMPK, beta-GPA and its derivatives have also been shown to improve insulin sensitivity and to promote weight loss in diabetic animals (Vaillancourt *et al.* 2001).

The recent development and identification of the thienopyridones (e.g. A-769662) as small molecule direct activators of AMPK, has been an exciting development in the field (Cool *et al.* 2006, Zhao *et al.* 2007). In cell culture studies A-769662 has been shown to reduce FA synthesis in primary rat hepatocytes (Cool *et al.* 2006) and increase glucose uptake in both L6 myotubes and primary human myotubes (Guigas *et al.* 2009). Cool *et al.* (2006) also demonstrated that acute intraperitoneal injection of A-769662 in Sprague–Dawley rats rapidly decreased hepatic malonyl CoA levels and stimulated FA oxidation. Furthermore, treatment of ob/ob mice with A-769662 for 5 days decreased plasma glucose and triglyceride concentrations, lowered hepatic triglyceride content and reduced expression of GNG genes in the liver (Cool *et al.* 2006). Availability of direct selective AMPK activators such as A-769662, and the recently described PT1 compound (Pang *et al.* 2008) should help to clarify the specific effects of AMPK activation on insulin sensitivity, avoiding any confounding effects that may be observed with 'indirect' activators such as AICAR or metformin.

Conclusion

The AMPK signalling pathway integrates hormonal and chemical signals of energy status to regulate converging aspects of fuel metabolism. Of interest with regard to T2D and disorders of the MS are the actions of AMPK on lipid and glucose metabolism in the liver and skeletal muscle, which have the potential to ameliorate IR and improve whole-body fuel homeostasis. IR is closely associated with the accumulation of lipids within the liver and skeletal muscle, which are major targets of insulin and important regulators of whole-body energy homeostasis. An increased content of lipid intermediates within the liver and skeletal muscle is believed to interfere with insulin signalling in these organs. By stimulating FA oxidation and inhibiting lipogenesis, AMPK activation within the liver and skeletal muscle has the potential to reduce intracellular lipid accumulation and thereby improve insulin action in these tissues. Furthermore, defective insulin signalling in insulin-resistant states could be bypassed by actions of AMPK to promote skeletal muscle glucose uptake and suppress HGP via insulin-independent mechanisms. These properties of the AMPK signalling pathway make it an attractive target for development of new therapies in the fight against T2D and the MS. It should be noted that other, as yet unidentified, actions of AMPK may also contribute to its promotion of insulin sensitivity (Fujii *et al.* 2008). Although it remains unclear as to whether defects in the intrinsic activity of AMPK play a significant role in the development of IR, a growing body of evidence suggests that AMPK activation does indeed ameliorate metabolic dysregulation and IR in animal models as well as humans (e.g. metformin and TZDs). An important consideration for the development of pharmacological activators however is the diverse list of AMPK targets. Here we have focused on AMPK regulation of fuel metabolism; however, AMPK does have a wide variety of effects across different physiological systems, thus off-target effects are not only likely, they are seemingly unavoidable. A greater understanding of specific AMPK signalling pathways and their regulation will be crucial for the development of high-performance therapies for T2D.

Conflict of interest

There is no conflict of interest.

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