■ R E V I E W

AMP-activated protein kinase pathway: a potential therapeutic target in cardiometabolic disease

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A B S T R A C T

AMPK (AMP-activated protein kinase) is a heterotrimeric enzyme that is expressed in many tissues, including the heart and vasculature, and plays a central role in the regulation of energy homoeostasis. It is activated in response to stresses that lead to an increase in the cellular AMP/ATP ratio caused either by inhibition of ATP production (i.e. anoxia or ischaemia) or by accelerating ATP consumption (i.e. muscle contraction or fasting). In the heart, AMPK activity increases during ischaemia and functions to sustain ATP, cardiac function and myocardial viability. There is increasing evidence that AMPK is implicated in the pathophysiology of cardiovascular and metabolic diseases. A principle mode of AMPK activation is phosphorylation by upstream kinases [e.g. LKB1 and CaMK (Ca^{2+}/calmodulin-dependent protein kinase], which leads to direct effects on tissues and phosphorylation of various downstream kinases [e.g. eEF2 (eukaryotic elongation factor 2) kinase and p70 S6 kinase]. These upstream and downstream kinases of AMPK have fundamental roles in glucose metabolism, fatty acid oxidation, protein synthesis and tumour suppression; consequently, they have been implicated in cardiac ischaemia, arrhythmias and hypertrophy. Recent mechanistic studies have shown that AMPK has an important role in the mechanism of action of MF (metformin), TDZs (thiazolinediones) and statins. Increased understanding of the beneficial effects of AMPK activation provides the rationale for targeting AMPK in the development of new therapeutic strategies for cardiometabolic disease.

INTRODUCTION

The prevalence of cardiometabolic diseases is reaching epidemic proportions in industrialized nations and in developing countries [1–3]. Despite aggressive treatment of the individual cardiometabolic risk factors, death from cardiometabolic conditions remains unacceptably high. Therefore there is an urgent need to identify new strategies for treating and preventing cardiometabolic diseases. In this respect, the AMPK (AMP-activated

protein kinase) pathway has become the focus of a great deal of attention as a novel therapeutic target in cardiometabolic disease because it has been demonstrated to mediate, at least in part, the effects of a number of physiological and pharmacological factors that exert beneficial effects on the vasculature and the heart. AMPK has several important metabolic effects, including increasing muscle glucose uptake [4,5] and ameliorating insulin resistance [6]. It regulates cardiac muscle glucose and lipid metabolism both directly and indirectly in order to provide ATP in response to energy depletion (specifically a rise in the AMP/ATP ratio). AMPK activity can also be modulated by hormones and adipocytokines which may have protective effects against cardiovascular disease. AMPK has also been shown to regulate transcription of genes involved in lipid and glucose metabolism [7,8]. Dysregulation of this process (for example in obesity) can lead to the development of insulin resistance and dyslipidaemia, both of which are major risks factors for CVD (cardiovascular disease). Thus the identification of a compound that specifically and safely activates the AMPK pathway might contribute significantly to the treatment, management and even prevention of CVD. The aim of the present review is to discuss the direct and indirect role of AMPK in normal cardiac physiology and in cardiometabolic disease, and therapeutic strategies in modulating APMK activity.

STRUCTURE AND REGULATION OF AMPK

Understanding of the role of AMPK in key physiological pathways has increased several fold in recent years. Its discovery can be traced back to two independent findings reported in 1973 that observed that crude preparations of ACC (acetyl-CoA carboxylase) [9] and HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase [9a] became inactivated when incubated with ATP. Both groups predicted that the effects were due to phosphorylation of the enzymes by an endogenous protein kinase that contaminated their preparations. It was subsequently shown that this protein kinase was itself activated by phosphorylation by an upstream kinase [10]. In 1987, Carling et al. [11] made the discovery that the inactivation of ACC and HMG-CoA reductase were both catalysed by a single protein kinase. As it became clear that it was a true multi-substrate kinase, they renamed it AMP-activated protein kinase after its allosteric activator $5'$-AMP [12]. Hardie [13] has described AMPK as a 'fuel gauge' and 'guardian of energy status', implying the fundamental role of AMPK in energy metabolism and maintaining body energy balance. AMPK is a heterotrimeric enzyme complex which consists of α, β and γ subunits, each of which has two or more isoforms that are encoded by distinct genes and are differentially expressed in various tissues. The α subunit contains the catalytic domain,

including the important regulatory Thr[172] residue, which is phosphorylated by upstream kinases. The β subunit has glycogen-binding C-terminal domains that are sufficient on their own to form a complex with the α and γ subunits. High cellular glycogen content exerts an inhibitory effect on AMPK through an interaction with the β subunit in skeletal muscle, although the exact mechanism is unknown [14]. The γ subunit of AMPK was first recognized by Bateman [15] and contains four repeats forming two domains. Each of these domains binds one molecule of AMP or ATP ion in a mutually exclusive manner [16], consistent with the findings that high concentrations of ATP antagonize activation of AMPK by AMP.

For many years, the upstream kinase(s) that phosphorylates Thr[172] on the α subunit of AMPK remained unidentified. In recent years, it has been established that the major upstream kinase in mammalian cells is a complex of the protein kinase LKB1 and two accessory subunits STRAD (Ste20-related adaptor) and MO25 (mouse protein 25) [17–19]. LKB1 also acts as an upstream kinase of at least 12 other AMPK-related kinases [20,21]. It has also been found to be a tumour suppressor and was identified in humans as a gene carrying an autosomal-dominant mutation in Peutz–Jeghers syndrome [19,22]. The STRAD subunit is essential for the ability of the LKB1 complex to phosphorylate Thr[172] on AMPK [18]. Besides LKB1, STRAD and MO25, AMPK can also be activated by an LKB1-independent mechanism involving CaMKs (Ca^{2+}/calmodulin-dependent protein kinases).

AMPK exerts its metabolic effects through its interactions with various metabolic pathways. Activation of these metabolic pathways via AMPK activation leads to remodelling of various components of the metabolic syndrome [23] (Figure 1). AMPK plays a major role in providing ATP in the midst of energy depletion via its interactions with various metabolic pathways (Figure 2). Furthermore, AMPK also has direct and indirect effects on the cardiovascular system, and the understanding of such effects provides the rationale of targeting AMPK as a new therapeutic modality for the treatment and prevention of CVD.

AMPK: DIRECT EFFECTS ON THE CARDIOVASCULAR SYSTEM

Congestive cardiac failure, LV (left ventricular) hypertrophy, myocardial ischaemia and diabetic cardiomyopathy are all associated with disturbances of cardiac energy homoeostasis. In these pathological states, AMPK activity is up-regulated in response to an increased AMP/ATP ratio (energy-depleted state). AMPK switches on energy-generating pathways to increase cardiac myocyte fatty acid uptake [24] and increase glucose uptake by increasing the translocation of GLUT-4 (glucose transporter-4) in a PI3K (phosphoinositide
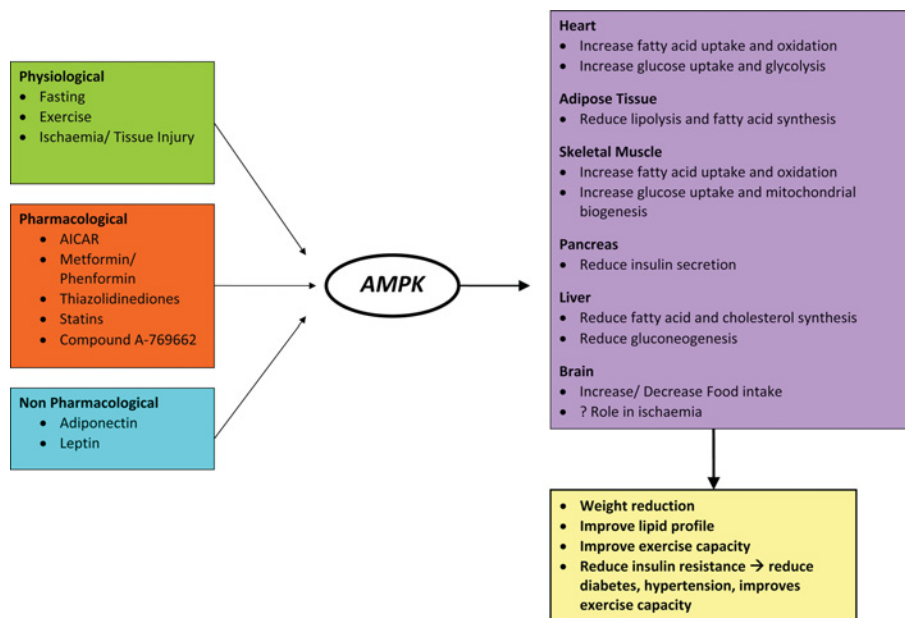
Figure 1   Physiological and pharmacological activation of AMPK results in remodelling of various components of the metabolic syndrome

3-kinase)-independent manner [5], while also enhancing glycolysis via PFK-2 (phosphofructokinase-2) activation [25]. At the same time, AMPK turns off protein synthesis pathways by activating eEF2 (eukaryotic elongation factor 2) kinase, resulting in the phosphorylation and inactivation of eEF2 and by decreasing Thr^{389} phosphorylation of $p70^{RSK}$ (p70 ribosomal protein S6 kinase), another important kinase which is involved in protein synthesis [26] via mTOR (mammalian target of rapamycin) inhibition [26,27] (see Figure 2).

AMPK and cardiac ischaemia

During cardiac ischaemia, the AMP/ATP ratio is increased as a result of decreased oxidative metabolism of both NEFAs [non-esterified fatty acids ('free fatty acids')] and glucose due to diminished oxygen supply in the face of increased glycolytic ATP production and glucose transport [28]. Russell et al. [5] have shown that AMPK activation using AICAR (5-amino-4-imidazole-carboxamide riboside-1-β-D-ribofuranoside) in an *in vitro* rat model increased translocation of glucose transporters (i.e. GLUT-4) into the sarcolemma and, hence, increased glucose uptake. Furthermore, AMPK also phosphorylates and activates PFK-2, leading to the production of fructose 2,6-bisphosphate, a potent stimulator of glycolysis. AMPK may be necessary for adiponectin to exert its cardioprotective effect against ischaemia/perfusion injury [29]. Both the α_1 and α_2 subunits of AMPK are activated during myocardial ischaemia, with the α_2 subunit being activated to a greater extent [30,31].

Previous studies in transgenic mice have shown that decreased α_2 activity resulted in reduced cardiac glucose uptake following ischaemia [32] and impaired recovery of LV systolic function [31]. Additionally, in transgenic mice expressing a kinase-dead form of the enzyme, phosphocreatinine was also lower after reperfusion [31]. These observations suggest that activation of AMPK following ischaemia has a cardioprotective effect and results in lesser cardiac injury and faster recovery. Calvert et al. [33] have also shown that activation of AMPK with MF (metformin) resulted in decreased myocardial injury in both diabetic and non-diabetic mice. This may be a result of deriving ATP from more energy-efficient glucose metabolism from increased AMPK-mediated glucose uptake and glycolytic flux in the face of oxygen deprivation [34,35].

However, ischaemic-induced activation of AMPK may be detrimental to the ischaemic heart, as suggested by Dyck and Lopaschuk [36]. During ischaemia, circulating fatty acid levels are elevated [37], which may be detrimental to the ischaemic heart [38,39]. Activation of AMPK leads to increased fatty acid uptake and inhibition of malonyl-CoA, a potent endogenous inhibitor of mitochondrial fatty acid uptake. This results in accelerated mitochondrial fatty acid uptake and, hence, increased mitochondrial acetyl-CoA production from β-oxidation. High levels of acetyl-CoA have an inhibitory effect on PDH (pyruvate dehydrogenase), reducing the amount of pyruvate being converted into acetyl-CoA and, hence, reduced glucose oxidation (the
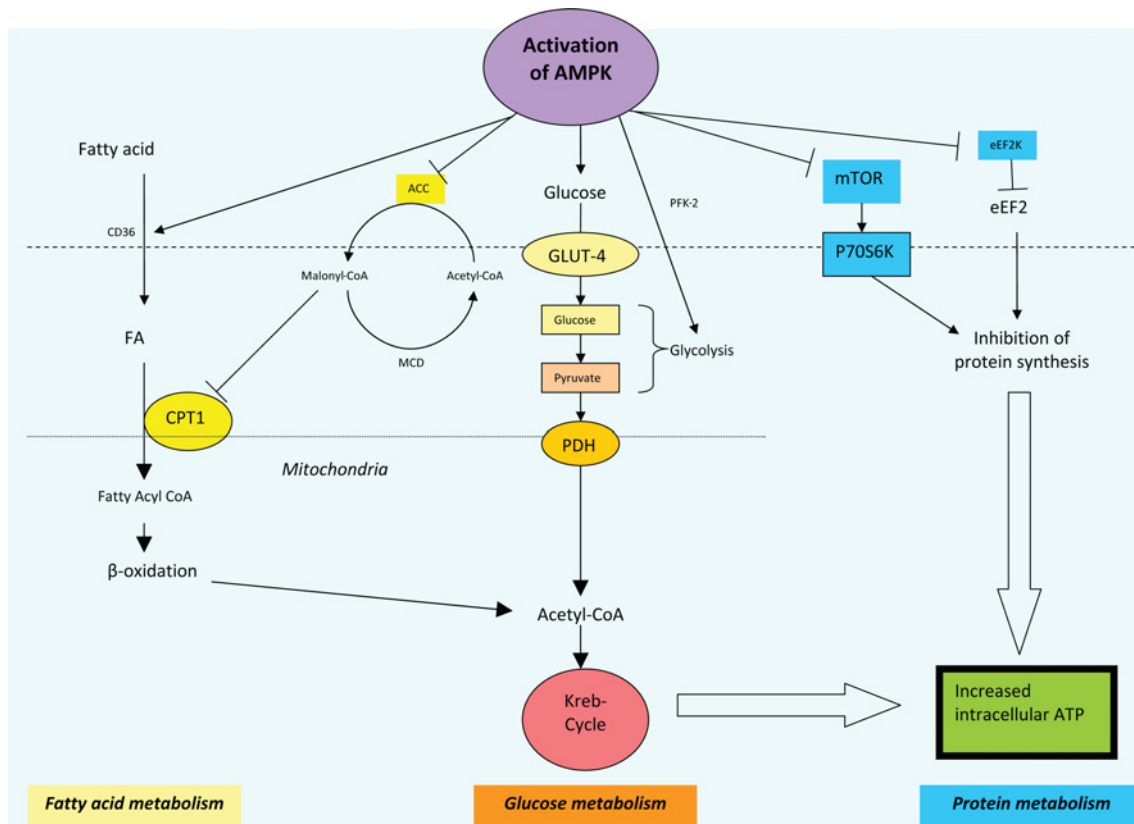
Figure 2   AMPK activation leads to activation of different metabolic pathways

AMPK plays an important role in whole-body energy homoeostasis as it regulates and interacts with different key metabolic pathways. Activation of AMPK, secondary to a change in the AMP/ATP ratio or activation by upstream kinases, such as CAMKK (CaMK kinase) and LKB1 leads to a switching on of energy-production pathways, such as glucose and lipid metabolism, and a turning off of energy-metabolic processes, such as protein synthesis, which is not required for immediate cell survival. (i) Fatty acid metabolism. AMPK activation leads to increased translocation of CD36, a fatty-acid-transport protein, which increases fatty acid (FA) flux into cells and subsequent uptake into mitochondria for β-oxidation. CPT-1 inhibits fatty acid influx and acts as a gatekeeper for the mitochondrial uptake of fatty acids. Activation of AMPK leads to the inhibition of ACC2, which normally converts acetyl-CoA into malonyl-CoA. The inhibitory effect of malonyl-CoA on CPT-1 is hence removed, leading to unopposed intake of fatty acids into mitochondria. Furthermore, phosphorylating and inactivation of ACC1 by AMPK activation reduces fatty acid synthesis and turns off the expression of lipogenic genes, such as fatty acid synthase. MCD, malonyl-CoA decarboxylase. (ii) Glucose metabolism. Activation of AMPK increases translocation and retention of GLUT-4 in the plasma membrane, as well as increased transcription of the GLUT-4 gene, leading to increased glucose uptake. It also enhances glycolysis via activation and phosphorylation of PFK-2. (iii) Protein metabolism. p70RSK (p70S6K) is a one of the key kinases involved in protein synthesis. mTOR activates p70RSK and leads to increased protein synthesis. When AMPK is activated, the activation of p70RSK is blocked as a result of the inhibition of mTOR. Activation of AMPK also results in phosphorylation and inactivation of eEF2, and subsequent inhibition of protein synthesis. eEF2K, eEF2 kinase.

exact mechanisms remain undefined). The proposed mechanisms for these detrimental effects of high circulating fatty acids include: (i) accumulation of toxic intermediates of fatty acid oxidation, such as long-chain acyl-CoA thioesters and long-chain acylcarnitines [38], (ii) inhibition of glucose oxidation via inhibition of the PDH complex by fatty-acid-derived acetyl-CoA, and (iii) accumulation of glycolytic by-products such as protons and lactate. These valuable observations have affirmed the role of AMPK in cardiac ischaemia and suggested a potential role for therapeutic targeting in the treatment of myocardial ischaemia and MI (myocardial infarction).

AMPK and cardiac arrhythmias

Mutations of the γ_2 subunit of AMPK have also been shown to contribute to glycogen storage disease and Wolff–Parkinson–White syndrome [40]. Gollob et al. [40] identified in 2001 a mutation (Arg531Gly) in the AMPK γ_2 subunit (*PRKAG2* gene) to be responsible for Wolff–Parkinson–White Syndrome and early onset of atrial fibrillation and conduction disease. Using a transgenic model targeting the murine gene, Davies et al. [41] demonstrated striking cardiac manifestations, such as hypertrophy, impaired contractile function, electrical conduction abnormalities and marked glycogen accumulation. Furthermore, Sidhu et al. [42] have identified a

distinct atrial ventricular accessory pathway and a pro-longed QRS in this transgenic mice model. However, the effects of the mutations described in this gene on the over-all activity of AMPK varies in the different experimental models [43,44]. It is still uncertain whether these cardiac manifestations are the result of disease-causing mutations or secondary to glycogen deposition. Murphy et al. [45] postulated that the manifestations of AMPK disease may be due to defects in energy utilization or in specific cellular substrates, rather than mere passive deposition of glycogen. Nonetheless, these findings illustrate an important role for AMPK in cardiac hypertrophy and arrhythmias.

AMPK and cardiac hypertrophy, cell growth and gene transcription

AMPK may play a further role in the regulation of normal cardiac cell growth [31,32] and energy regulation in the hypertrophied heart [46], via its effects on protein synthesis [47,48]. Mutations in γ_2 subunits not only cause glycogen overload in the heart and Wolff–Parkinson–White syndrome, but also hypertrophy and HF (heart failure) [40,49–51]. The severity of the defect also correlates with the severity of the disease. eEF2 is the main mediator of the translocation step in protein synthesis and is inhibited through phosphorylation of eEF2 kinase. p70^{RSK} regulates protein synthesis through the same pathway or via phosphorylation of ribosomal protein S6. Chan et al. [48] have shown that AMPK not only regulates eEF2 kinase, but also exerts effects on protein synthesis via the mTOR pathway, ultimately leading to inhibition of p70^{RSK}. Furthermore, Chan et al. [48] have shown that activation of AMPK using MF and AICAR results in inhibition of protein synthesis, and is associated with prevention and regression of cardiac hypertrophy. However, studies in transgenic mice have shown that elevated AMPK activity is associated with the accumulation of large amounts of glycogen, leading to dramatic LV hypertrophy and arrhythmias [46,52]. It remains uncertain, therefore, whether AMPK activation in the hypertrophied heart is beneficial [48,53] or deleterious, and further studies are required.

AMPK and vascular and endothelial function

AMPK also plays an important role in the regulation of vascular function and structure. It activates eNOS (endothelial NO synthase) in endothelial cells and cardiac myocytes by phosphorylation at Ser^{1177} (human sequence) [54,55]. eNOS activation leads to augmentation of vascular tone, platelet aggregation, leucocyte adherence and vascular smooth muscle proliferation [56].

Using a diabetic rat model, Suzuki et al. [57] have shown that activation of AMPK using a cAMP phosphodiesterase inhibitor, cilostazol, restores endothelial function independently of cAMP. Administration of cilostazol leads to phosphorylation of AMPK and subsequent phosphorylation of eNOS and increased NO production. Other AMPK activators, AICAR [58], MF [59] and rosiglitazone [60], have all been shown to increase NO production in human aortic endothelial cells via the AMPK pathway. Additionally, AMPK also appears to have a role in angiogenesis, promoting the action of the HIF-1α (hypoxia-inducible factor-1α)/VEGF (vascular endothelial growth factor) pathway [61,62], and inhibiting AngII (angiotensin II)-induced smooth muscle cell proliferation [63]. Furthermore, activation of AMPK using AICAR has been shown to inhibit palmitate-induced endothelial cell apoptosis through suppression of ROS (reactive oxygen species) [64]. It is clear that AMPK plays a central role in vascular biology.

AMPK: INDIRECT EFFECTS ON THE CARDIOVASCULAR SYSTEM

Recent findings have shown that levels of adipocytokines such as adiponectin and leptin correlate with the development of different components of the metabolic syndrome [65]. AMPK has been suggested to play a role in mediating the metabolic and vascular effects of the key adipocytokines [66,67].

AMPK and leptin

Leptin is an adipocyte-secreted hormone that plays a pivotal role in the regulation of food intake, energy expenditure, body weight and neuroendocrine function [68]. Leptin stimulates fatty acid oxidation [69] and glucose uptake [70], and prevents lipid accumulation outwith adipose tissue, preventing lipotoxicity [71]. Deposition of ectopic fat in pancreatic β-cells, myocardium and skeletal muscle contributes to the pathogenesis of Type 2 diabetes mellitus, cardiomyopathy and insulin resistance respectively. Leptin is known to exert its effects via the AMPK pathway, stimulating phosphorylation and activation of the α_2 catalytic subunit of AMPK selectively in skeletal muscle [69]. Leptin also suppresses ACC2 activity, thereby stimulating fatty acid oxidation in muscle. AMPK also inhibits lipogenesis and ectopic fat deposition in the liver [72]. AMPK is also a key regulator of leptin action in the hypothalamus and a 'master regulator' of food intake. Minokoshi et al. [73] have shown that inhibition of AMPK activity by leptin specifically in the arcuate and paraventricular nuclei is essential for its anorexigenic and weight-loss effects.

AMPK and adiponectin

Adiponectin, an adipose-specific protein present in high concentrations in the circulation, was first identified in 1996. It possesses anti-atherogenic, insulin-sensitizing and anti-inflammatory properties. Yamauchi et al. [66]

Table 1   Different AMPK 'activators' and their limitations in clinical use

For further details of AICAR and MF studies, see Tables 2 and 3 respectively. PKC, protein kinase C.

AMPK activator	Possible mechanism(s) of AMPK activation	Activation of other pathways	Limitation(s)
AICAR	(i) Direct activation followed by allosteric modification	(i) Stimulates adiponectin release; (ii) inhibits cytokines such as TNF-α and IL-6	(i) Short half-life; (ii) variable effectiveness; (iii) only intravenous forms available; (iv) may cause bradycardia and significant hypoglycaemia
MF	(i) Indirect activation; (ii) via alteration of the AMP/ATP ratio as a result of inhibition of Complex I in the respiratory chain; (iii) other unknown mechanisms	(i) Anticancer effects via its effects on p53; (ii) up-regulates eNOS and increases NO bioactivity; (iii) enhances fatty acid oxidation, which leads to alleviation of endothelial lipotoxicity	(i) Indirect AMPK activation; (ii) doses and duration of MF required for AMPK activation are not determined; (iii) higher doses of MF result in intolerable gastrointestinal side effects
TZDs	(i) Indirect activation; (ii) via alteration of the AMP/ATP ratio, possibly similar to MF; (iii) via adiponectin	(i) Anti-atherosclerotic and anti-inflammatory effects via adiponectin; (ii) effects on mitochondrial biogenesis; (iii) exerts antioxidative effects by inhibiting PKC via AMPK activation	(i) Indirect inhibition; (ii) risk of developing fluid retention; (iii) risk of developing cardiovascular events is yet to be determined
Statins	(i) Indirect activation; (ii) does not alter the AMP/ATP ratio; (iii) other unknown mechanisms	(i) HMG-CoA reductase inhibition; (ii) activation of AMPK/eNOS/ACC	(i) Doses required for AMPK activation in humans are still to be determined
Compound A-769662	(i) Direct activation	(i) Increased fatty acid oxidation; (ii) decreased plasma and liver triacylglycerol levels; (iii) inhibits fatty acid synthesis	(i) Poor oral bioavailability; (ii) data on long-term AMPK activation are awaited

have shown that adiponectin stimulates glucose utilization and fatty acid oxidation via the AMPK pathway. Furthermore, adiponectin has been shown to reduce infarct size, improve LV function and remodelling, and increase coronary flow during reperfusion in animal models. The underlying mechanisms are thought to be phosphorylation of eNOS, AMPK Thr[172] and Akt Ser[473] [74]. Adiponectin-deficient mice have been shown to have progressive cardiac remodelling in a pressure-overloaded condition due to reduced AMPK signalling and worsening insulin resistance [75]. Therefore the AMPK pathway is not only critical for the metabolic and insulin-sensitizing actions of adiponectin, but also its cardioprotective effects in myocardial ischaemia and reperfusion.

AMPK ACTIVATORS: PHARMACOLOGICAL TOOLS AND THERAPEUTIC POTENTIAL

As we have seen above, AMPK is a pivotal enzyme that regulates diverse signals in metabolic pathways and has direct and indirect effects on the heart and vasculature. AMPK activation has not only been shown to alleviate various components of the metabolic syndrome, but may also improve LV hypertrophy and reduce cardiac injury in ischaemia. AMPK is also a key mediator of the anti-atherosclerotic and insulin-sensitizing effects of adiponectin. Therefore it is clearly an attractive therapeutic target in cardiometabolic disease. A number

of AMPK activators are available as pharmacological tools and some are in clinical use (Table 1).

AICAR

AICAR is an adenosine analogue which activates AMPK through direct binding, followed by allosteric modification. It is initially taken up by adenosine transporters and subsequently phosphorylated to ZMP (5-aminoimidazole-4-carboxamide-1-β-D-furanosyl 5′-monophosphate) within the cell, which mimics AMP in AMPK signalling [76]. AICAR was first developed to block adenosine reuptake in the ischaemic heart, promoting stimulation of adenosine membrane receptors. In 1997, treatment with acadesine (AICAR) before and during surgery was shown to reduce early cardiac death, MI and combined adverse cardiovascular outcomes [77], although the mode of action via AMPK was not fully appreciated at that time.

AICAR is now widely used in the laboratory setting, particularly in experiments relating to glucose metabolism, insulin signalling pathways and lipid metabolism. In recent years, AICAR has been shown to reverse various aspects of the metabolic syndrome in animal models [23,78–80] and healthy human subjects [81] (Table 2). AICAR has also been shown to stimulate adiponectin and inhibit cytokines, such as TNF-α (tumour necrosis factor-α) and IL-6 (interleukin-6), which have been implicated in the development of obesity-induced insulin resistance [82–85]. Unfortunately, AICAR is far from an ideal activator of the

Table 2 Various studies of AMPK activation using AICAR and their major findings
HDL, high-density lipoprotein; IR, insulin-resistant; OGTT, oral glucose tolerance test; SBP, systolic blood pressure.

Study	Type of subjects	Dosage	Duration	Major finding(s)
Iglesias et al. [80]	IR high-fat-fed rats	Subcutaneous injection of 250 mg/kg of body weight	24 h	(i) Enhanced whole-body, muscle and liver insulin action; (ii) reduced hepatic glucose output
Buhl et al. [23]	Obese Zucker rats exhibiting IR, hyperlipidaemia and hypertension	Subcutaneous injection of 0.5 mg/g of body weight	7 weeks	(i) Decreased plasma triacylglycerol and NEFAs, and increased HDL; (ii) lower SBP; (iii) normalized OGTT and decreased fasting glucose and insulin; (iv) tendency towards decreased intra-abdominal fat content
Bergeron et al. [79]	Obese Zucker rats	Bolus at 100 mg/kg of body weight and constant infusion at 10 mg · kg^{-1} of body weight · min^{-1}	60 min	(i) Increased glucose transport in red gastrocnemius muscle, whereas insulin had no effects; (ii) suppression of endogenous glucose production and lipolysis
Song et al. [78]	*ob/ob* mice	Subcutaneous at 1 mg/g of body weight	7 days	(i) Corrected hyperglycaemia, improved glucose tolerance, and increased GLUT-4 and hexokinase II protein expression in skeletal muscle
Cuthbertson et al. [81]	Healthy men	Intravenous infusion at 10 mg · kg^{-1} of body weight · h^{-1}	9 h	(i) Increased human skeletal muscle 2-deoxyglucose uptake and whole-body glucose disposal

AMPK pathway in the clinical settings because of its short half-life, requirement for intravenous infusion and variable effectiveness. It also causes bradycardia and can lead to hypoglycaemia when administered intravenously. Therefore there is great interest in developing a more potent, safer and more specific activator.

Metformin

MF has been used to treat diabetes for more than 50 years and is associated in observational studies with reduced mortality and improved outcomes in patients with CHF (chronic HF) [86,87]. It is the preferred antidiabetic medication for obese patients with Type 2 diabetes mellitus because of its property to stabilize weight and reduce cardiovascular events when used as monotherapy [88]. Recent clinical studies have shown that the effects of MF may go beyond improving HbA$_{1c}$ (glycated haemoglobin) and may include reductions in cardiovascular end points in Type 2 diabetes mellitus and HF. This wide spectrum of cardiovascular-protective effects may be attributable to its activation of AMPK and its downstream pathways.

MF has been shown to activate AMPK in myocytes [89–91], hepatocytes [92] and skeletal muscle cells [92]. MF decreases hepatic glucose production and increases skeletal muscle glucose disposal. Therapeutic doses of MF have been shown to increase AMPK α_2 subunit activity in human skeletal muscle with an associated increase in phosphorylation of AMPK on Thr^{172} and decreased ACC2 activity [93]. MF can also up-regulate eNOS and increases NO bioactivity via AMPK activation [94]. Furthermore, AMPK activation by MF enhances fatty acid oxidation, which leads to alleviation of endothelial lipotoxicity and improved endothelial function [95]. Moreover, MF has also been shown to have anticancer

effects in a recent study via its indirect AMPK activation [96]. However, the precise mechanisms of how MF activates AMPK are still poorly understood.

Even though MF is regarded as an AMPK activator, it has not been shown to bind directly to AMPK; neither does it regulates its own phosphorylation and dephosphorylation in cell-free assays [97]. One hypothesis is that it activates AMPK by inhibiting Complex I of the respiratory chain, which subsequently causes an increase in the AMP/ATP ratio [98,99]. In fact, inhibition of the respiratory chain in the intestinal mucosa may account for the gastrointestinal side effects of the drug, and this property may account for the propensity of its predecessor biguanides phenformin to cause lactic acidosis. MF is transported into intestinal cells mainly by OCT-1 (organic cation transporter-1), but phenformin penetrates cell membranes without active transport. Identification of polymorphisms in genes encoding cation transporter proteins may ultimately explain differences in tolerance and response to MF [100]. Interestingly, there are also studies suggesting that AMPK can be activated by MF without changes in the AMP/ATP ratio [97,101], and MF can also exert its beneficial metabolic effects on cardiac myocytes in an AMPK-independent manner [102].

However, we should be mindful that extra caution is required if we are to use these results to extrapolate to the effects of MF on AMPK. First, variable doses of MF have been used in these studies. The plasma MF concentration in clinical use is usually approx. 10 μmol/l [103], whereas the doses used in *in vivo* and *in vitro* experiments are consistently higher, in the range of 1–10 mmol/l (Table 3). Saeedi et al. [102] have shown that lower doses of MF (i.e. 2 mmol/l) failed to activate AMPK and caused no

Table 3   Studies of AMPK activation using MF and their major findings

IRI, ischaemia/reperfusion injury; MAPK, mitogen-activated protein kinase; PKC, protein kinase C.

Study	Aim(s)	Subjects	Dosage	Key finding(s)	Clinical application
Calvert et al. [33]	To examine the cardioprotective effects of MF	Murine models	125 μg/kg of body weight compared with saline (286-fold lower than maximum antihyperglycaemic dose)	(i) Reduction in myocardial injury in both diabetic and non-diabetic mice; (ii) increased AMPK activity and eNOS phosphorylation	Cardioprotective effects of MF might be secondary to eNOS activation via AMPK pathway
Solskov et al. [134]	To determine the effects of a single dose of MF on cardiac protection against IRI	Wistar rats	Single dose of MF (250 mg/kg of body weight) compared with saline	(i) Reduction in MI size; (ii) 2-fold increase in AMPK α_1 subunit activity	MF might reduce MI size in pre-treated subjects via AMPK activation
Saeedi et al. [102]	To determine whether MF has effects on the metabolism of heart muscle, independent of the AMPK pathway	Sprague–Dawley rats	2 mmol/l (this dose has greatest cellular metabolic effects without an impact on cellular energy status)	(i) Increased rate of glycolysis, glucose uptake and fatty acid oxidation; (ii) AMPK was not activated by 2 mmol/l MF	MF has AMPK-independent metabolic effects, possibly via PKC and p38 MAPK pathways
Kovacic et al. [91]	To determine whether Akt activation induced by insulin negatively regulates AMPK activities	Akt transgenic mice and adenovirus-infected neonatal rat cardiac myocytes with mutant forms of Akt1 and Akt2	5 mmol/l MF	(i) Insulin increased Akt phosphorylation and reduced AMPK phosphorylation; (ii) administration of MF overcame Akt-dependent AMPK suppression; (iii) suggests a cross-talk between Akt and AMPK pathway	AMPK can be activated by MF via insulin-independent pathways, but higher doses of MF are required
Zhang et al. [89]	To examine whether MF activates AMPK in the heart via increasing cytosolic AMP	Sprague–Dawley rats	10 mmol/l MF	(i) MF increases AMPK activity preceded by and correlated with increased cytosolic AMP, but the overall AMP/ATP ratio remained unchanged	MF activates AMPK without altering the total AMP/ATP ratio; a high dosage of MF is required for AMPK activation.

changes in energetic state. On the contrary, Hawley et al. [97] have shown that lower doses of MF can actually produce AMPK activation without a significant change in the cellular AMP/ATP ratio. Other research groups have reported that AMPK activation required higher doses of MF (i.e. 5–10 mmol/l) [89,91] (Table 3) and suggested that higher doses of MF are required to cause changes in the energetic state and, hence, subsequent AMPK activation. However, these divergent results may be the result of different exposure time of MF. For instance, Yang and Holman [90] have shown that a lower dose of MF (1 mmol/l) activated AMPK and increased cardiac myocyte glucose uptake after a prolonged exposure of 18 h. On the other hand, Bertrand et al. [104] have shown that short exposure (4 h) of MF can result in AMPK activation if much higher doses of MF were used (5–10 mmol/l). Therefore AMPK can be activated by MF in a time- and concentration-dependent manner. Clearly further studies are required to determine the time and concentration of MF which will result in the maximal beneficial effects of AMPK activation without intolerable side effects.

TZDs (thiazolinediones)

TZDs (i.e. rosiglitazone and pioglitazone) are ligands for the nuclear hormone receptor family member PPAR-γ (peroxisome-proliferator-activated receptor-γ) [105]. Both rosiglitazone and pioglitazone have been shown to activate AMPK in intact cells [106,107]. TZDs can also activate AMPK by stimulating the release and expression of circulating adiponectin from adipose tissue [66,67] or indirectly by increasing the cellular AMP/ATP ratio, possibly by a similar mechanism to biguanides [108]. Both rosiglitazone and pioglitazone have been suggested to have additional and protective beneficial anti-atherosclerotic and anti-inflammatory effects [109]. Furthermore, TZDs have also been shown to have diverse beneficial effects on endothelial function, TNF-α, NO

and endothelial cell proliferation via AMPK-dependent and PPAR-γ-independent mechanisms [110–114]. These effects may translate into an improvement in clinical outcomes in patients with cardiometabolic disease. Previous studies have raised the intriguing possibilities that these effects may be mediated via AMPK activation [107,115,116]. However, as with MF, we are not certain whether AMPK activation is the key to these beneficial clinical effects on cardiovascular system. Furthermore, we also need to be very cautious when we try to translate these observations in animal studies to the clinical setting. The doses and type of TZDs that have been shown to activate AMPK vary among different research groups and the doses used in these animal studies may not be applicable to human subjects. Furthermore, the majority of these *in vivo* studies are short-term studies examining the effects of acute AMPK activation and its metabolic effects; however, the effects of long-term AMP activation by TZDs have yet to be determined. Nonetheless, the cardiovascular-protective effects of TZDs are evidenced in the recently published post-hoc analysis from the PROactive (PROspective pioglitAzone Clinical Trial In macroVascular Events) study [116]. This showed that patients who have chronic kidney disease and received pioglitazone are less likely to reach composite end points of all-cause death, MI and stroke, independent of the severity of renal impairment.

However, it should be noted that TZD use is associated with the risk of fluid retention which may exacerbate HF [117]. In a recent meta-analysis, Lago et al. [118] reported that TZDs increased the risk of developing CHF, probably as a result of fluid retention, across a wide background of cardiac risk [relative risk, 1.72 (95 % confidence interval, 1.21–2.42); $P = 0.002$]. There is also a concern that these agents may be associated with additional cardiovascular (MI and stroke) risk in patients with Type 2 diabetes mellitus with rosiglitazone. However, it should be noted that these meta-analyses included many small trials [119], whereas large clinical trial data have shown no sign of these cardiovascular events [RECORD (Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of glycemia in Diabetes) and PROactive] [117,120]. The EMEA (European Medicines Evaluation Agency) for Medicinal Products for Human Use has adopted a scientific opinion in January 2008 recommending the inclusion of a new warning stating that the use of rosiglitazone in patients with ischaemic heart disease and/or peripheral arterial disease is not recommended. A recent FDA (Federal Drug Administration) review suggested that more large randomized studies with active comparators should be conducted by the manufacturers.

Statins

Statins are widely prescribed in patients with the metabolic syndrome owing to the high incidence of hypercholesterolaemia in this group of patients. There is mounting evidence to suggest that the clinical benefits of statins are beyond its lipid-lowering effects. The clinical efficacy of statin treatment in reducing cardiovascular mortality and morbidity in patients without and with diabetes are well-proven in clinical trials, such as the HPS (Heart Protection Study) and the CARDS (Collaborative Atorvastatin Diabetes Study) [121–124]. Besides its cholesterol-lowering effect via HMG-CoA reductase inhibition, statins have also been shown to activate AMPK in human and bovine endothelial cells [125]. Xenos et al. [126] have shown that AMPK protein levels in human endothelial cells are increased after being treated with fluvastatin for 2 days. Sun et al. [125] have also shown that atorvastatin and lovastatin cause a rapid activation of AMPK/eNOS/ACC in mouse myocardium and endothelial cells. The atorvastatin dose used in that study was 50 mg/kg of body weight in mice which is equivalent to 80 mg/day in human. That study has shown that atorvastatin did not alter the cellular AMP/ATP ratio, suggesting a different mechanism of AMPK activation. The beneficial effects of statins on endothelial function have been suggested to be the result of its ability to up-regulate eNOS [121,122,127–129] and its anti-inflammatory and anti-artherogenic effects [124,130]. These observations have all suggested that AMPK activation might be the key to the pleiotropic effects of statins on cardiovascular protection; however, further mechanistic and translational studies are required to show that AMPK activation is indeed the key to the effects of statin treatment, as well as examining the doses of different statins required to activate AMPK.

Compound A-769662

Cool et al. [131] have identified a thienopyridone family of AMPK activators, compound A-769662, which stimulates AMPK directly in partially purified rat liver and inhibits fatty acid synthesis in primary rat hepatocytes. Short-term treatment of normal Sprague–Dawley rats with A-769662 decreases liver malonyl-CoA levels and the respiratory exchange ratio, $\dot{V}_{CO_2}/\dot{V}_{O_2}$ (carbon dioxide production/oxygen consumption), indicating an increased rate of whole-body fatty acid oxidation. In *ob/ob* mice, treatment with compound A-769662 has been shown to decrease plasma glucose, reduce weight gain and significantly decrease both plasma and liver triacylglycerol (triglyceride) levels. These results demonstrate that small-molecule-mediated activation of AMPK *in vivo* is feasible and, therefore, represents a promising approach for the treatment of Type 2 diabetes mellitus and the metabolic syndrome. However, the compound has poor oral bioavailability, limiting its use in clinical settings.

An alternative small-molecule compound that is safe, potent, acts directly on AMPK and has good oral

bioavailability would be an attractive candidate to progress towards clinical development.

CONCLUSIONS

Activation of the AMPK pathway may be the key in treating and preventing various cardiometabolic diseases; however, it is still uncertain whether direct activation of the AMPK pathway in the absence of a physiological stress will be beneficial or deleterious overall in humans. It is hoped that chronic activation of AMPK will not result in 'over-compensatory' activation of other systems such as the RAAS (renin–angiotensin–aldosterone system) activation in HF. Alterations in cardiac AMPK activity are associated with a number of cardiovascular-related diseases, such as pathological cardiac hypertrophy [50], myocardial ischaemia [36], glycogen storage cardiomyopathy [52] and Wolff–Parkinson–White syndrome [51], suggesting a possible maladaptive role in such conditions. Andersson et al. [132] described antisatiety effects of AMPK, which may lead to weight gain. Furthermore, McCullough et al. [133] also demonstrated that activated AMPK may be harmful in stroke. All of these uncertainties will need to be clarified by further translational studies, and much effort is still required to define the roles of AMPK activation in various conditions that have been discussed above. Furthermore, it is also a great challenge for pharmaceutical companies to produce a specific AMPK activator that has predictable effects owing to its heterotrimeric structure and its complex interactions with various upstream and downstream kinases. The other approach in which many researchers have adopted is to develop a compound that targets the downstream kinases of AMPK [i.e. a malonyl-CoA activator or CPT-1 (carnitine palmitoyltransferase-1) activator]. The AMPK/malonyl-CoA/CPT-1 axis might represent an interesting pathway for further research in cardiac substrate utilization and fatty acid metabolism. The AMPK–adipocytokine interaction has also formed the rationale for the development of new treatment modalities for the treatment of obesity. Lastly, AMPK/mTOR/eEF2/p70^{RSK} axis modulation may be the key to understanding the pathogenesis of cardiac myocyte hypertrophy and mitochondrial biogenesis. A greater understanding of the biochemistry and physiology of AMPK and a better understanding of the mechanisms of action of existing agents have nonetheless opened up a new horizon for the treatment and prevention of cardiovascular and metabolic disease.

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