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Review

AMPK in cardiovascular health and disease

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Adenosine Monophosphate-activated Protein Kinase (AMPK), a serine/threonine kinase and a member of the Snf1/AMPK protein kinase family, consists of three protein subunits that together make a functional enzyme. AMPK, which is expressed in a number of tissues, including the liver, brain, and skeletal muscle, is allosterically activated by a rise in the AMP: ATP ratio (*ie* in a low ATP or energy depleted state). The net effect of AMPK activation is to halt energy consuming (anabolic) pathways but to promote energy conserving (catabolic) cellular pathways. AMPK has therefore often been dubbed the "metabolic master switch". AMPK also plays a critical physiological role in the cardiovascular system. Increasing evidence suggest that AMPK might also function as a sensor by responding to oxidative stress. Mostly importantly, AMPK modulates endogenous antioxidant gene expression and/or suppress the production of oxidants. AMPK promotes cardiovascular homeostasis by ensuring an optimum redox balance on the heart and vascular tissues. Dysfunctional AMPK is thought to underlie several cardiovascular pathologies. Here we review this kinase from its structure and discovery to current knowledge of its adaptive and maladaptive role in the cardiovascular system.

Keywords: AMPK; cardiovascular physiology; cardiovascular system; oxidative stress; atherosclerosis

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Introduction

One of the many important cellular control systems is the AMP-activated protein kinase (AMPK), which is the putative metabolic or energy sensor of the cell. AMPK signaling appears to have broad implications in cardiovascular health and disease. This review will focus on AMPK's emerging role as an integrating metabolic sensor in the cardiovascular system.

The discovery of AMPK is not recent. Its existence was uncovered by two independent observations reported in 1973 with the discovery that the same kinase inactivates 3-hydroxy-3-methylglutaryl coenzyme A (CoA) reductase and acetyl-CoA carboxylase (ACC) in hepatic fat metabolism[1, 2]. Several years after these seminal reports, Munday *et al*, in the process of studying the V_{max} of ACC, proposed the name AMP-activated protein kinase as the primary enzyme responsible for attenuating this parameter[3]. Finally, with Carling et al reporting that the HMG-CoA reductase (HMGR) and ACC kinases were one and the same enzyme, the name was formally adopted in 1989[4, 5]. Subsequently, AMPK was purified and its subunit structure was analyzed by Grahame Hardie's group at the University of Dundee[6] with detailed analysis of its all important catalytic subunit published by Bruce Kemp's laboratory at the St Vincent's Medical Research Institute in Victoria, Australia[7]. Based on these ground breaking studies and work by various other investigators, it has been revealed that AMPK is a heterotrimer with α, β, and γ subunits. The α subunit has catalytic activity while the other two have a regulatory role. Overall, multiple AMPK subunit isoform combinations have been identified and these subunits are encoded by distinct genes. Thus far, two α subunits, two β subunits and three γ subunits have been identified[8–10].

AMPK physiology

AMPK subunits

The standardized nomenclature of AMPK subunit genes utilizes a prefix PRKA followed by the subunit identifier A1, A2, B1, B2, G1, G2, and G3 (*eg*, PRKAG3)[11]. The gene loci for the subunits are located on 5 different chromosomes: α1 (5p12), α2 (1q31), β1 (12q24.1), β2 (1q21.1), γ1 (12q12–14), γ2 (7q35–36), and γ3 (2q35). With this plethora of subunits it is not surprising that gene expression and variant splicing can give rise to twelve possible heterotrimeric combinations of AMPK[12] (Figure 1).

Both α subunits are similar in that they have about 550 residues (Figure 1) as well as conserved NH_2-terminal catalytic domains. The β subunits differ in the first 65 residues but in all other respects are highly conserved. The γ subunits on the

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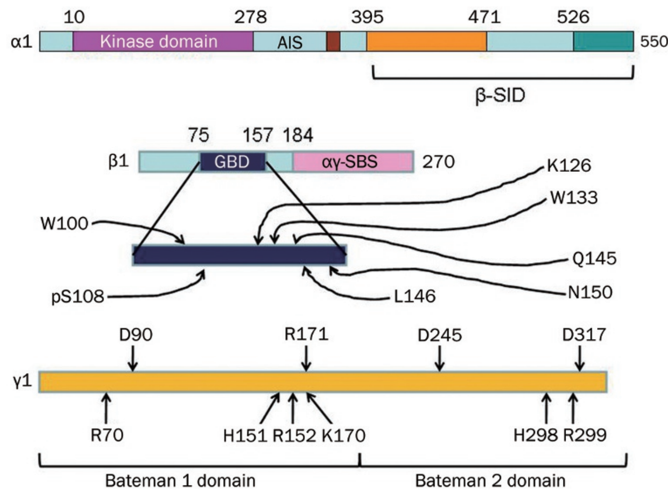

Figure 1. Features of the AMPK subunits (modified from Steinberg and Kemp, Physiol Rev 2009 89: 1025–78). Colored regions are ones whose structure is known. Numbers associated with α and β subunits are N- and C- terminal residues from the crystal structure. AIS: Auto inhibitory sequence. β-SID: β-subunit interacting domain. GBD: Glycogen binding domain. αγ-SBS: α and γ subunit interacting sequence. In the expanded glycogen binding domain schematic of the β subunits, letters with numbers are sites of sugar binding (pS108 is the site at Serine 108 where phosphorylation occurs). In the γ subunit, D90, R171, D245 and D317 are residues that form H-bonds with 2′,3′-ribose hydroxyl groups, while R70, H151, R152, K170, H298 and R299 represent basic residues that occupy the solvent accessible core of the subunit which makes contact with the nucleotide phosphates.

other hand (and in contrast to the other two), differ in length (γ1 being the shortest at 331, γ3 intermediate at 489 and γ2 the longest at 569)[11]. However, all three share a COOH-terminal having about 300 residues. Significant differences exist in AMPK subunit structure and genetic sequence between mammals and yeast, for example (multiple α and γ subunits in mammals and 2 rather than three β subunits in yeast).

Evidence suggests that variance of the α subunits determines subcellular localization of the molecule with the α1 isoform being largely cytosolic as well as being associated with the plasma membrane in carotid body type 1 cells and airway epithelial cells[13, 14]. In contrast α2 appears to be concentrated in the nuclei of several cell types such as pancreatic β cells, neurons and skeletal muscle[15–17].

The β subunits feature the glycogen binding domain (GBD) which occupies a position on the central conserved region of the subunit. The crystal structure of the GBD was reported in 2005[18]. Another conserved region on this subunit is in the C-terminal region and there is compelling evidence that the C-terminal domain is all that is needed to form a functional αβγ unit that can be regulated by AMP[19].

The three γ subunits have variable N-terminal regions followed by four tandem repeats of a 60-aa sequence named as a CBS (cystathionine β-synthase) motif by Bateman *et al*[20]. It has since been discovered that these are actually two domains on the subunit (Figure 1; Bateman 1 and 2 domains), each with the capacity to bind AMP with a 1:1 stoichiometry[21]. The critical nature of these domains was revealed when investigators reported attenuated AMP binding and activation when mutations were induced in these regions[21]. The Bateman domains also bind ATP antagonistically, but with a lower affinity than that for AMP[21] and this is consistent with the fact that ATP antagonizes activation of AMPK by AMP[22]. Interestingly, the two Bateman domains also act cooperatively in that the second site remains inaccessible to AMP until the nucleotide has bound to the first[21]. This synergy between the two domains is a potential mechanism by which AMPK activation can respond to even small changes in cellular AMP levels[12].

In summary, the α subunit provides catalytic activity while the β and γ subunits are regulatory. The α subunit has a Thr172 phosphorylation site. The β subunit has scaffold-like properties and also possesses myristoylation, phosphorylation and glycogen-binding sites. The γ subunit features the nucleotide-binding module. Thus, for correct function all three units act in concert and are necessary for normal function.

AMPK activation

Mammalian AMPK is sensitive to the AMP: ATP ratio. It is therefore activated as a consequence of any cellular process, normal or anomalous, that either decreases ATP levels, or increases AMP concentrations. For example, mechanisms such as hypoxia, glucose deprivation or metabolic inhibition of ATP synthesis, will all activate AMPK[23]. If ATP production remains unaltered but consumption is increased, the same result will ensue. Examples of increased ATP synthesis include activation of motor proteins, activity of ion channels/pumps and utilization by biosynthetic pathways. In addition, less well understood yet empirically established modulators of AMPK activity have appeared in literature and the list is ever growing. Such modulators include cytokines [leptin, adiponectin, ghrelin, cannabinoids, IL-6[24], ciliary neutrotrophic factor (CNTF; [25])], certain drugs (metformin[26], thiazolidinediones[27]) and some plant derived compounds (berberine[28], resveratrol[29]), to name a few.

Activation of AMPK complexes that contain the α1 subunit isoform are reported to be localized in the cytosol. In contrast, AMPKα2 activation results in translocation to the nucleus and is thought to facilitate modulation of gene expression[30, 31]. The β subunit also appears to be involved in determining the localization fate of the molecule in that myristoylation of the β subunit targets the complex to the Golgi while phosphorylation on various residues promotes nuclear translocation[32].

Once AMPK is activated, it switches on (the concept of the "metabolic master switch"; most likely first proposed by Prof HARDIE of the University of Dundee) catabolic pathways that can generate ATP while at the same time, terminates processes that consume ATP. The rapid "switching" required to closely and quickly regulate and balance cellular energy resources is achieved by brisk phosphorylation of metabolic enzymes as well as that of various transcription factors and co-activators which control gene expression[23]. Analysis has shown that the nucleotide AMP, allosterically binds to and activates the

γ subunit of AMPK which triggers phosphorylation of the α subunit at Thr172.

Upstream AMPK kinases

By the early 2000s, it had become clear that a critical phosphorylation event took place on the α subunit (on Thr172) in the process of AMPK activation. However, the identities of upstream phosphotransferases that were responsible for this had remained elusive. In 2003, breakthrough discoveries in the yeast system (Schizosaccharomyces pombe) identified Sak1 (Snf1-activating kinase-1), Elm-1 (elongated morphology-1) and Tos3 (Target of Sbf3) as kinases upstream of the Snf1 complex (homolog of the serine/threonine protein kinase found in S cerevisiae)[33–35]. Although unequivocal human orthologs of these three kinases have not been found in the human genome, the two protein kinases closest in sequence to these are LKB1 (a serine/threonine tumor suppressor kinase) and the Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ)[30]. Evidence now demonstrates phosphorylation of the AMPK α subunit can either be dependent on, or independent of, its LKB1 activity. Specifically, LKB1 appears to be critically involved in the activation of AMPKα2 but not AMPKα1[30, 36]. AICAR (5-Aminoimidazole-4-carboxyamide ribonucleoside; an important activator of AMPK) is an adenosine analog taken up by muscle and phosphorylated to form 5-aminoimidazole-4-carboxamide-1-D-ribofuranosyl-5′-monophosphate (ZMP), which stimulates AMPK activity and glucose transport in skeletal muscle. LKB1 is essential for AICAR induced activation of AMPK. Empirically, it has been reported that deletion of LKB1 will prevent activation of AMPKα2 in cardiac and skeletal muscle cells[36].

Unlike LKB1, CaMKKβ is regulated within the cell and its levels increase in response to elevations in intracellular Ca^{2+} ($[Ca^{2+}]_i$). Therefore, stimuli that amplify ($[Ca^{2+}]_i$ (such as bradykinin and thrombin), also activate AMPKα1 consequent upon increased CaMKKβ activity[37, 38].

Downstream targets of AMPK

Once activated, AMPK can influence several downstream targets in the cell. Many of these targets are currently recognized[20], however more are being discovered and it has been speculated that this number may eventually rise into the hundreds. These downstream effectors of AMPK influence diverse cellular processes and include lipid metabolism [*eg*, acetyl-CoA carboxylase (ACC); HMG-CoA reductase], carbohydrate metabolism (*eg*, glycogen synthase; 6-phosphofructo-2-kinase); cell signaling [*eg*, endothelial NO synthase (eNOS); insulin receptor substrate-1 (IRS-1)], ion transport [cystic fibrosis transmembrane conductance regulator (CFTR)] and transcription [*eg*, p300; hepatocyte nuclear factor-4α (HNF4-α); transducer of regulated CREB activity 2 (TORC2)][14, 39–45]. One of the most intriguing questions has been: How does AMPK recognize its downstream targets? AMPK has been found to phosphorylate a serine residue in these targets. Further, phosphorylation sites appear to have conserved motifs where hydrophobic residues are found 5 residues from the N terminal and 4 from the C terminal (P-5 and P+4). This motif is designated Φ-[β.X]-X-X-S/T-X-X-X-Φ, where Φ is hydrophobic and β is basic. This motif has been confirmed using variant synthetic peptide substrates[46, 47].

AMPK in cardiovascular physiology

Homeostatic mechanisms in the heart and the vascular endothelium are critical in maintaining cardiovascular health. These mechanisms are involved in diverse cardiovascular functions like regulation of vascular tone, maintenance of tissue perfusion, vascular permeability, myocardial function, anticoagulant activity and inflammatory responses[48–50].

AMPK in the heart

While AMPK signaling has a specific physiological role in the heart, its importance is accentuated under conditions that place a stress on this organ. Such stressors include excess hemodynamic load, myocardial ischemia and hypoxia[51]. Specifically, AMPK activates the glycolytic pathway by phosphorylating phosphofructokinase-2, enhances fatty acid β-oxidation and enhances ATP availability[52, 53]. It also promotes the translocation of glucose transporter 4 (GLUT-4) to the plasma membrane in the cardiomyocyte thus increasing the uptake of glucose to serve as the primary energy substrate[51].

AMPK in endothelial cells

Endothelial cells in the CVS can sense changes in hemodynamic forces, ambient pO_2 and local blood-borne signals. They can then respond with appropriate control and regulatory processes to maintain homeostasis. Such responses can include release of paracrine mediators such as nitric oxide (NO), prostacyclin and endothelin-1 (ET-1)[48]. In addition, activity of cell surface enzymes can be modulated such as angiotensin converting enzyme (ACE) which regulates the bioactivity of vasculoactive molecules like angiotensin II and bradykinin[48]. Furthermore cell surface adhesion molecules are also modulated in addition to promotion of immune cell recruitment and migration in case of vascular injury along with increased vascular permeability and intravascular thrombosis[48].

The central signaling molecule involved in endothelial function is nitric oxide (NO) which is synthesized from L-arginine by NO synthase (NOS) in endothelial cells. NOS exists as three isoforms: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). All three isoforms share a carboxyl terminal domain homologous to cytochrome P-450 reductase and has binding sites for nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), Flavin mononucleotide (FMN) and tetrahydrobiopterin (BH4)[54]. eNOS is typically activated by an increase in $[Ca^{2+}]_i$. A strong physiological stimulus for NO synthesis vie eNOS activation is the shear stress caused by increases in blood flow[54]. It has also been demonstrated that other physiological stimuli such as insulin, estrogen and vascular endothelial growth factor (VEGF) can activate the phosphatidyl inositol-3 kinase (PI3K)/Akt system and in turn this phosphorylates NOS et Ser1177[55]. AMPK is the only kinase

recognized to date that can also potentially phosphorylate eNOS on more than one site. These sites have been reported as Ser1177 and Ser633[41, 56] (activating sites on the reductase domain of eNOS), and at Thr495 (inhibitory at the CaM-binding domain of the enzyme)[41]. Many reports have identified that AMPK-dependent eNOS activation (at Ser1177) can occur following endothelial cell stimulation by diverse agents and these include VEGF[57], peroxisome proliferator-activated receptors γ (PPAR-γ) agonists[58], AICAR[59], and metformin[60]. In 2004, it was reported that the biguanide drug, metformin activated AMPK mediated by mitochondrial RNS (reactive nitrogen species) and the PI3K pathway[61]. Specifically, this report demonstrated that metformin activated AMPK and increased the phosphorylation of ACC (its downstream effector) at Ser79 in cultured BAEC (bovine aortic endothelial cells), that this was mediated through c-Src and was PI3K-dependent, was $ONOO^-$-dependent, that the peroxynitrile oxidant was sourced to the mitochondrion and that inhibition of mitochondrial complex I activated AMPK. Further, the paper also validated these findings *in vivo*. Further studies have also established that metformin-induced AMPK activation is beneficial to endothelial function via Heat Shock Protein 90 (hsp90) mediated activation of eNOS[60]. These investigations have revealed that metformin can increase the conversion of arginine into citrulline in BAECs in a dose-dependent fashion, suggesting that NO synthesis was occurring via activation of eNOS. This study also revealed that eNOS activation by metformin was PI3K-dependent, was clearly mediated through the activation of AMPK and that it enhanced the association of hsp90 (an important stress response marker) with eNOS. Recently, evidence has also emerged that NO itself might act as an endogenous activator of AMPK[62]. In this report, we have shown that NO activates AMPK in endothelial cells through a Ca^{2+}-dependent mechanism involving CaMKKβ and that AMPK activation can itself increase NO release through AMPK-dependent phosphorylation of eNOS at Ser1177. These data imply that a positive feedback relationship might exist between eNOS and AMPK activation. However, further investigation of this possibility is warranted.

Once NO is synthesized via the activation of eNOS it diffuses to the surrounding tissue and exerts its multiple physiological effects which include vascular smooth muscle relaxation and proliferation, and inhibiting leukocyte adhesion and migration, platelet adhesion and aggregation and expression of adhesion molecules[63, 64]. In disease conditions, the endothelium undergoes complex changes and loses its protective and pro-homeostatic function and acquires pro-atherosclerotic properties. Collectively these abnormalities are referred to as "endothelial dysfunction" and are typified by reduced bioavailability of NO[65]. In these alterations, AMPK also appears to play an important role.

AMPK and vascular smooth muscle

AMPK activation in the endothelium is also linked to endothelial control of vascular smooth muscle function, particularly vasorelaxation by NO which appears to be regulated, at least in part by AMPK activity. Vasorelaxation is a critical arm of vascular tone and tone is a central determinant of blood pressure regulation. Sustained high blood pressure is responsible for a variety of serious disease states such as hypertrophic cardiomyopathy, coronary artery insufficiency, myocardial infarction, hypertensive encephalopathy, cerebrovascular disease, hypertensive retinopathy and hypertensive nephropathy being important examples. It has been established that metformin therapy is beneficial for the cardiovascular system by virtue of its ability to improve vasodilatory function[66, 67]. It is possible that these effects of metformin are dependent on eNOS activation as demonstrated by various investigators. Another possibility that merits consideration is the fact that endothelium-dependent vasorelaxation is not exclusively regulated by NO. It is provocative to speculate that AMPK might be linked to vasorelaxation via the generation of epoxyeicosatrienoic acids by the cytochrome P450 epoxygenases[30]. In fact LKB1 and AMPK can be activated by stimulation of the constitutive androstane receptor and pregnane X receptor (both of the nuclear receptor superfamily that are thought to be involved in the detoxification of xenobiotics), using Phenobarbital (a classic P450 inducer)[68]. In mice that lack hepatic α1 and α2 isoforms of AMPK, this response is not detectable[69]. In keeping with the vasorelaxative role of AMPK, it is now fairly well established that AICAR and metformin can both relax arterial preparations *ex vivo*[60, 61, 70]. Interestingly, there seems to be a species- and vessel-dependent difference in published literature in terms of chemical sensitivity of AMPK activation. For example, unlike rat and mouse aortae, porcine carotid artery smooth muscle is reportedly insensitive to AICAR and metformin, even though this tissue can have AMPK activation by other means, such as by hypoxia and 2-deoxyglucose (which causes a metabolic block in the glycolytic pathway)[71].

AMPK as a redox sensor and modulator

Oxidative stress and a shift in the cellular redox balance are critical underpinnings of endothelial dysfunction. In turn, endothelial disturbances underlie cardiovascular pathology[30]. The decreased bioavailability of NO is also associated with the generation of reactive oxygen species (ROS) in the vessel wall such as peroxynitrite[72]. Many studies have now established that there is an intricate balance between AMPK signaling and the redox balance in the vascular milieu. For example, AMPK has been shown to inhibit the formation of reactive oxygen species (ROS) by NADPH oxidase and stimulate NO production be eNOS[30]. Furthermore, AMPK has also been implicated in JNK activation, NF-κB-mediated transcription, E-selectin expression and vascular cell adhesion molecule-1 (VCAM-1) expression, in endothelial cells that have been exposed to H_2O_2, TNF-α or fatty acids. As a consequence, these signaling events lead to attenuated monocyte adhesion to the endothelial surface[73–76]. Silencing AMPKα1 has also been reported to decrease the expression of Manganese Superoxide Dismutase (MnSOD), catalase, γ-glutamylcysteine synthase and thioredoxin, in endothelial cells[77]. AMPK also appears to have direct links to NADPH oxidase, a membrane

bound enzyme complex which is normally latent in neutrophils and is activated to assemble in the membranes during the respiratory burst. It generates $O_2^{\cdot-}$ transferring electrons from NADPH inside the cell across the membrane and coupling these to molecular oxygen to produce $O_2^{\cdot-}$. It has been shown that AICAR or AMP can suppress the production of superoxide anion stimulated by phorbol esters or fMLP (Formyl-Methionyl-Leucyl-Phenylalanine)[78]. In neutrophils, AICAR also has the ability to reduce PMA-dependent (phorbol 12-myristate 13-acetate; a potent activator of PKC) H_2O_2 release and induction of phosphorylation of JNK, p38, MAPK, and ERK1/2[30]. The exposure of cultured human endothelial cells (HEC) to high glucose concentration (10 mmol/L) can generate ROS and this can effectively be attenuated by AMPK-activating drugs, such as rosiglitazone[79]. AMPK can also influence the cellular redox state via prevention of tyrosine nitration and inhibition of prostacyclin synthase in endothelial cells that have been exposed to high glucose. This has been reported with the demonstration that AICAR could inhibit high glucose-induced nitration and inactivation of prostacyclin synthase in HUVECs, that AMPK activation was necessary for AICAR to reduce oxidative stress, that prostacyclin synthase nitration by AMPK was mediated through an upregulation of UCP-2 (mitochondrial uncoupling protein-2) and that this was dependent on activation of p38 kinase as well[80]. These findings are especially of note given that the basic role of uncoupling proteins is to prevent oxidative injury or minimize oxidative stress. In fact, such a mechanism has also been validated in *in vivo* experiments where AICAR-mediated AMPK activation markedly increases UCP-2 expression and reduction of $O_2^{\cdot-}$ and prostacyclin synthase nitration in diabetic wild-type but not in AMPKα-deleted mice[80]. Unpublished recent data (Xu *et al*) from our laboratory has also underscored the role of UCP-2 in mitigating mitochondrial ROS synthesis in an AMPK-dependent manner under ischemic conditions in skeletal vasculature. In this study we have shown that abrogation of ROS production via UCP-2 is critical for the compensatory neovascularization that is an important element in tissue response to ischemia/hypoxia.

Recently, novel findings demonstrating that hypoxia-reperfusion via $ONOO^-$ can activate AMPK in a c-Src mediated, PI3K-dependent manner, have been reported[81]. These data also show that the thromboxane receptor (TPr), once stimulated, can trigger ROS-mediated LKB1-dependent AMPK activation in vascular smooth muscle resulting in inhibition of cellular protein synthesis[82], that PKCζ can regulate AMPK activity by increasing phosphorylation at Ser428 of LKB1 (resulting in association of LKB1 with AMPK and consequent phosphorylation of LKB1 itself at Thr172)[83], that the PKCζ phosphorylation of LKB1 results in nuclear export of the kinase and prompts AMPK activation in endothelial cells[84], and most recently, ROS are required in the process of AMPK activation by statin drugs as well as PKCζ[85]. A new mechanism has also been proposed through which H_2O_2 can mediate AMPK activation independent of changes in the AMP:ATP ratio in endothelial cells. This has been observed in this cell type under nutritional and oxidative stress with free radical species appearing to be derived from the mitochondria. The most interesting aspect of this recent study is that under nutritive challenge (utilizing 2-deoxyglucose to induce a biochemical block by preventing glycolytic breakdown at the hexokinase step), AMPK activation led to autophagy rather than cell death. Further, under conditions of hypoxia, endothelial cell death was prevented in an AMPK and ROS dependent manner. Overall, several recent studies reinforce the idea that AMPK activation is acutely sensitive to cellular redox balance and this relationship appears to be independent of its putative function of responding to ATP depletion (or more precisely elevated AMP:ATP) and triggering energy conserving pathways in the cell. AMPK's response to the redox balance also appears to favor cell survival pathways.

AMPK and cardiovascular diseases

Myocardial hypertrophy

Cardiac hypertrophy can develop under physiological conditions as is seen in trained athletes as a compensatory response to intense exercise. It can also occur pathologically in response to left ventricular pressure or volume overload as seen in hypertension or valvular heart disease[86]. In recent years, evidence has begun to emerge that AMPK can modulate the development of cardiac hypertrophy. For example, Tian *et al* have reported that following aortic banding in rats that led to cardiac hypertrophy, concomitantly also activated AMPK in the myocardium[87] . Allard *et al* have also reported that hypertrophied rodent hearts exhibit increased glycolysis in conjunction with AMPK activation[88]. However, contrary data has also been published that suggests that cardiac hypertrophy may or may not be associated with a change in AMPK signaling. For example, phenylepherine treated hypertrophic cardiomyocytes have been shown to have unchanged AMPK activity[89]. More specifically, data from Dyck's group at the University of Alberta has shown depressed AMPK activity in a spontaneously hypertensive rat model[86]. Furthermore, mice lacking adiponectin that have reduced AMPK signaling are more susceptible to afterload-induced hypertrophy[90]. Interestingly, reports suggest that pharmacological activation of AMPK can mitigate the hypertrophy associated with hemodynamic pressure overload. For example, treatment with the AMPK activator AICAR has been shown to reverse cardiac hypertrophy in rats following aortic banding[91]. Resveratrol (a phytoalexin that can potently activate AMPK) is also reported to inhibit hypertrophy in the heart following aortic banding in the rat[92]. However, it has to be pointed out that AMPK does not appear to have a physiological role in limiting the hypertrophic response of cardiac myocytes based on the findings of Russell *et al* and Xing *et al*. These investigators demonstrated that mouse hearts with impaired AMPK signaling are not hypertrophied[93, 94].

Ischemic injury and ischemic pre-conditioning

Myocardial ischemia-reperfusion injury (inflammatory and oxidative damage to the cardiac muscle caused by reperfusion

following a relief of ischemia), is an important cardiopathic mechanism where AMPK is also thought to play a role. It is known for example, that AMPKα1 and α2 activity is elevated during cardiac ischemia[5]. Increased AMPK activity likely compensates for the ischemic condition by upregulating GLUT4 translocation and enhancing fatty acid oxidation[53, 95]. However, it remains unclear if AMPK activity contributes positively or negatively to reperfusion in that reports have suggested both scenarios being dominant[93, 94].

Short periods of cardiac ischemia can protect the heart against injury from subsequent and more sustained ischemia. This phenomenon is called ischemia pre-conditioning and has been known since the 1980s following a seminal description by Keith Reimer's group at Duke University[96]. It has been shown that AMPK can be activated during these short periods of ischemia in the heart[97, 98]. AICAR has been shown to potentiate pre-conditioning as well as prolonging the period over which the beneficial and protective effects of this phenomenon can last. This was demonstrated by Tsuchida *et al* and Burckhartt *et al* in rabbits undergoing coronary ligation[99, 100]. Furthermore, AICAR has also been shown to contribute to pre-conditioning in the rat liver against ischemic injury *in vivo*[101]. AMPK has also been noted to mediate pre-conditioning in the isolated cardiomyocyte under hypoxic conditions[102].

AMPK genetic mutations in heart disease

Naturally occurring mutations in the γ2 subunit of AMPK have been identified that appear to be involved in the pathogenesis of the Wolf-Parkinson-White syndrome (WPWS), a ventricular pre-excitement anomaly that leads to cardiac arrhythmias. This condition is quite rare affecting 0.9 to 3% of the general population[103]. Mechanistically, the γ subunit mutation in WPWS appears to cause excessive glycogen storage and deposition in cardiomyocytes and these cells contribute to the formation of accessory excitation pathways (called the Bundle of Kent) resulting in dysrhythmias[104]. Several transgenic mouse models of AMPK γ mutations have been studied in recent years that demonstrate various disturbances in their cardiac phenotype. These transgenic models have not clarified the exact relationship of γ subunit mutations with glycogen storage anomalies, AMPK signaling and heart rhythm disturbances and further work is merited. For example, transgenic mice harboring two different mutations in the γ2 subunit appear to have opposing effects on AMPK activity *in vivo*. The N4881 mutation is reported to increase AMPK activity compared with wild type animals, whereas the R302Q mutation attenuates AMPK activity[105, 106].

AMPK and vascular smooth muscle proliferation, angiogenesis and neointimal hyperplasia

In terms of the consequences of endothelial activation of AMPK, several aspects need to be borne in mind. Overall, the activation of this molecule by FGF (fibroblast growth factor), adiponectin, hypoxia and VEGF, seems to be critical for angiogenesis[30, 107–109]. For example, dominant negative AMPK (DN-AMPK) is able to suppress both endothelial cell migration in response to VEGF as well as *in vitro* differentiation into tube-like structures under hypoxic conditions[107]. Similarly, increased VEGF expression has been shown in muscle cells treated with AICAR leading to enhanced angiogenic repair in response to ischemic injury in the mouse hind limb. This phenomenon reportedly involves AMPK-dependent activation of p38 MAPK[110]. Quite recently, Leick, and colleagues have reported that an AMPK/PGC-1α-dependent mechanism is likely responsible for exercise-induced VEGF expression in skeletal muscle as well as exercise-training-induced prevention of senescent decline in VEGF protein content[111]. Intriguingly, AICAR has also been shown to decrease neointimal hyperplasia in the rat femoral artery denudation model linked to ERK1/2 inhibition (in part)[112].

AMPK, ER stress, and atherosclerosis

The role of AMPK activation in anti-atherosclerotic processes in the vasculature is also currently under intense study and several fascinating insights have been revealed. For example, the activation of mammalian target of Rapamycin (mTOR) by oxidized low density lipoprotein (LDL) has been shown to be involved in smooth muscle cell proliferation, and AMPK activation by resveratrol can block the activation of the PI3K/Akt/mTOR/p70S6K pathways with consequent inhibition of DNA synthesis and proliferation of smooth muscle cells[113]. Research has recently revealed that AMPK acts as a suppressor of endoplasmic reticulum (ER) stress and that this is dependent on the α2 subunit expression of AMPK[114]. It has been demonstrated that reduction in AMPKα expression resulted in increased ER stress in human umbilical vein endothelial cells (HUVEC). When ER stress markers were measured in mouse aortic endothelial cells (MAEC) derived from AMPKα2 knockout mice, these were seen to be significantly higher compared with α1 knockout cells or WT controls. This implied that that the α2 isoform of the catalytic subunit of AMPK was critical for suppressing ER stress. In this study the mechanism of heightened ER stress associated with reduced activity of AMPKα2 was identified to be increased oxidation of the sarcoplasmic/endoplasmic ER Ca^{2+} ATPase (SERCA) pump in the ER. SERCA oxidation hampered its activity and caused increased $[Ca^{2+}]_i$ levels. When Ca^{2+} chelators were used to treat endothelial cells in this study, ER stress was mitigated. Abrogation of ER stress *in vivo* was also achieved following long term treatment of LDL receptor/AMPKα2 double knockout mice with the potent chemical ER chaperone, Tauroursodeoxycholic acid (TUDCA)[114]. As part of this study it was also observed that LDLr/AMPKα2 double knockout mice that were fed a pro-atherosclerotic high fat diet, developed advanced aortic root atherosclerosis. Interestingly, histological analysis of these lesions revealed that they were significantly more severe compared with LDLr only knockout animals fed a similar diet. These findings compellingly demonstrated that AMPKα2 not only attenuated ER stress *in vitro* as well as *in vivo*, but also played a critical role in vascular pathology characterized by atherosclerosis. Thus, the role of AMPK in the development of this significant human pathology cannot be understated.

Summary and conclusions

It has become evident with continuing research that AMPK plays a pivotal role in the complex dynamics of bioenergetics, in both health and disease. The signaling system that has AMPK as its centerpiece is fairly complex. A fact that speaks to how far reaching are the effects of activation or inhibition of this kinase. In a physiologic sense, this molecule has layers of influence that it exerts on cellular processes. These include switching between anabolic and catabolic states and altering cellular dynamics by directly influencing genetic controls and protein expression. In conditions where cellular systems are under metabolic strain, AMPK's ability to quickly correct dynamic processes appears to be an evolutionarily conserved mechanism seen across a wide phylogenetic scale, indeed in all organisms studied thus far. Even when the cellular homeostatic machinery has been overrun by pathological forces, AMPK acquires a central position in buffeting these fluxes, frequently pointing them in a correcting direction and occasionally contributing to the mounting damage that leads to cell death.

Although AMPK is traditionally thought of as an intracellular energy switch or fuel gauge, recent research has shown that this molecule is also a key player in maintaining physiological processes in the cardiovascular system, both in the heart and the vasculature. In the heart, signaling by AMPK appears to regulate the bioenergetic status of the cardiomyocyte as well as maintain the heart muscle and its electrical pace makers in optimum condition. As described, mutations in the AMPK subunit structure can cause rare but serious heart rhythm anomalies. In the endothelium, AMPK appears to redress the disturbed redox balance associated with vascular pathology and also exerts anti-atherosclerotic effects via improving NO bioavailability, attenuation of ROS stress and activation of pro-angiogenic factors.

Some drugs and compounds that have become centerpieces of therapeutics as well as public health dogma, in disorders like diabetes (*eg*, metformin), cardiovascular disease (*eg*, polyphenols a resveratrol) and infectious diseases (*eg*, berberine), appear to deliver their protective/therapeutic effects via modulation of AMPK signaling (Figure 2).

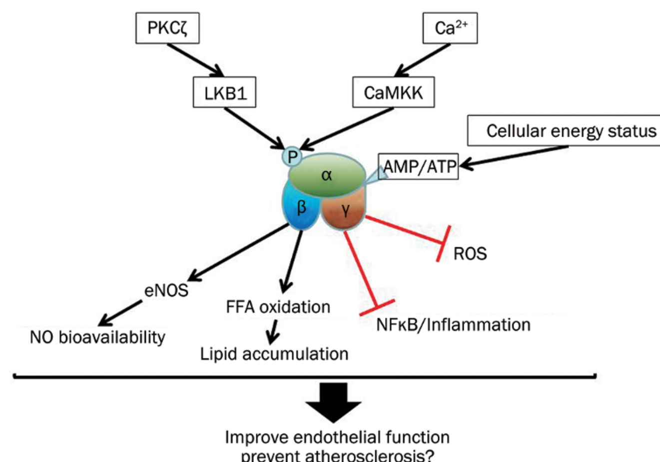

Figure 2. Summary of AMPK activation and its effects in the endothelium (modified from Zou and Wu, Clin Exp Pharmacol Physiol 2008 35 (5–6): 535–45). AMPK senses an increase in AMP:ATP by binding AMP to the γ subunit. This allosterically activation facilitates the activity of LKB1 of which AMPK is a substrate. Additional regulation is affected by CaMKKβ. Once activated, AMPK improves NO bioavailability, enhances FFA oxidation and inhibits ROS production in the endothelium via several different pathways. In addition, by blocking the NFκB signaling system, AMPK also inhibits inflammatory responses in the endothelium, possible contributing to its role as an anti-atherogenic regulator. Improvement in endothelial function by AMPK activation is well established in the literature. However, its direct role in preventing atherosclerosis is less well characterized.

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