# The Role of AMPK in the Control of Cardiac Hypertrophy

**Nikole J. Byrne, Miranda M. Sung, and Jason R.B. Dyck**

**Abstract** The development of cardiac hypertrophy in response to sustained cardiac stress causes considerable structural and metabolic remodeling of the heart that can have profound detrimental consequences. AMP-activated protein kinase (AMPK), a well-studied mediator of cellular energy homeostasis, has been shown to be a regulator of cardiac hypertrophy via its influence on several key signaling pathways involved in cardiomyocyte growth control. Although the ability of activated AMPK to inhibit protein synthesis has been a major focus of the anti-hypertrophic effects of AMPK, alterations in other cellular processes such as cardiac energy metabolism and cytoskeletal remodeling have also emerged as complimentary pathways by which AMPK is thought to inhibit the development of cardiac hypertrophy. Consistent with this, increasing evidence supports the use of pharmacological activators of AMPK to prevent the progression of cardiac hypertrophy. Despite these findings, this concept is not universally accepted as AMPK has also been shown to be elevated in hypertrophic hearts, suggesting that AMPK plays a role in promoting rather than inhibiting cardiomyocyte growth. This chapter reviews some of the published literature that focuses on the role of AMPK in the control of cardiomyocyte growth and discusses the potential benefits and pitfalls that may accompany the approach of pharmacologically activating AMPK to control the pathogenesis of cardiac hypertrophy.

**Keywords** Cardiac hypertrophy • AMPK • Energy metabolism • Protein synthesis • prkag2 mutation

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G.D. Lopaschuk and N.S. Dhalla (eds.), *Cardiac Energy Metabolism in Health and Disease*, Advances in Biochemistry in Health and Disease 11,
DOI 10.1007/978-1-4939-1227-8_13, 

## 1 Introduction

In response to hemodynamic stress such as an increase in aortic pressure, the heart undergoes both structural and metabolic remodeling. Early structural remodeling often involves thickening of the walls of the left ventricle (LV), which leads to a physiological condition known as cardiac hypertrophy [1]. There are several processes necessary for cardiac hypertrophy including enhanced protein synthesis [2], cell enlargement, expansion of the cytoskeleton and a higher degree of sarcomeric organization [3]. Although early morphological changes involved in LV hypertrophy (LVH) are initially adaptive in order to normalize wall stress and maintain cardiac output [4], prolonged pathological stimuli such as hypertension or valvular heart disease often induce changes in the cardiomyocyte that become maladaptive. This condition can worsen over time and transition from a compensated hypertrophy to a decompensated hypertrophy and eventually heart failure [3, 5].

Although much of the pathogenesis to decompensated hypertrophy can be attributed to structural remodeling of the cardiomyocyte and the surrounding extracellular matrix, these changes in cardiac morphology also impact other parameters including cardiac energy metabolism. Indeed, it has been well documented that pathological LVH results in a genetic "reprograming" of the metabolic regulatory circuits of the cardiomyocyte such that rates of fatty acid oxidation (FAO) are reduced while rates of glycolysis are increased [6–11]. This reprograming to a more fetal metabolic profile ultimately results in impaired cardiac energetics and contributes to impaired cardiac performance [11–14]. As such, interventions that would lessen cardiomyocyte growth as well as improve cardiac energetics could be of great benefit in the treatment of pathological LVH. Importantly, the energy sensing kinase, AMP-activated protein kinase (AMPK) has emerged as a key player in these processes (i.e. cell growth control and cardiac energetics) providing a potential therapeutic target in the prevention and/or treatment of cardiac hypertrophy.

LVH is recognized as a significant independent risk factor for cardiac-related morbidity and mortality [15, 16], and a growing body of evidence shows that regression of LVH greatly improves outcomes [17, 18]. Therefore, there has been a great deal of research aimed at understanding the mechanisms involved in the development of cardiac hypertrophy and developing new therapeutic strategies for the treatment of LVH. Our current understanding of the pathophysiology of LVH with a focus on the role of AMPK in the hypertrophic process will be discussed herein, as well as the use of AMPK modulators as a potential anti-hypertrophic therapy.

## 2 AMP-Activated Protein Kinase (AMPK)

AMPK is known to be a major regulator of cellular substrate metabolism, which is necessary to maintain an adequate energy level for cell survival during both normal and pathological conditions [19–21]. AMPK is a serine-threonine kinase that responds to metabolic stresses by sensing changes in the intracellular AMP:ATP

ratio and turning on catabolic pathways to generate ATP while turning off anabolic processes, such as protein synthesis, that consume ATP [21–23]. This has led to AMPK being termed the "fuel gauge" of the cell and it plays an important role in the heart, which has the highest energy demand of any organ in the body.

AMPK is a heterotrimeric protein comprised of a catalytic α subunit as well as β and γ regulatory subunits. While the α and β subunits each have two isoforms [24–26], both of which are found in the mammalian heart, only two of three isoforms of the γ subunit are expressed in the heart [27]. Of the two isoforms of the α subunit, the α2 subunit is found predominantly in the murine heart [27, 28], whereas both α1 and α2 are expressed equally in the human heart [27]. Activating phosphorylation of Thr172 residue in the activation loop of the α subunit by upstream kinases (AMPKKs) is a key mechanism by which AMPK is activated [29]. The regulatory γ subunit senses the metabolic status of the cell upon allosteric binding of two molecules of either AMP or ATP in one of its two competing binding sites [27, 30], thereby regulating phosphorylation of Thr172 under varying energy conditions. Lastly, the regulatory β subunit has a central region that binds glycogen, as well as serves as a scaffolding protein that binds both the α- and γ-subunits at their C-terminal region to hold the holoenzyme together as one complex [28]. As mentioned earlier, activation of AMPK is dependent upon both allosteric activation by AMP and most importantly phosphorylation at Thr172 of the α1 and α2 subunit by upstream AMPKKs [31]. In addition, hormones such as adiponectin, insulin and leptin have also been shown to alter AMPK phosphorylation and activity [32]. Studies have also shown that AMPK can undergo inhibitory phosphorylation at AMPK α1 on its Ser485 residue and AMPK α2 on its Ser491 residue by Akt or protein kinase A, respectively [33, 34].

## 3 Activation of AMPK

To date, three upstream kinases of AMPK have been identified; including calcium/malmodulin-dependent protein kinase kinase (CaMKK) [35, 36], the tumor suppressor LKB1 [37] and transforming growth factor-beta-activating kinase 1 TAK1 (Fig. 1). However, LKB1 has been recognized as the major AMPKK in the heart [31] and more work on the role of LKB1 in the heart has been performed compared to the other two known AMPKKs. While LKB1 was originally thought to be constitutively active [37], studies in cancer cells have shown that LKB1 can be inhibited by covalent modification [38], suggesting that LKB1 activity may be regulated in a similar manner in other cell types such as the cardiomyocyte. Consistent with this, more recent studies have shown that inhibition of cardiac LKB1 occurs upon formation of covalent adducts with 4-hydroxy-2-nonenal (HNE) [39, 40], which subsequently decreases the activity of AMPK. As the LKB1/AMPK signaling axis has been shown to act to suppress cardiomyocyte cell growth [39, 41], decreased LKB1 activity by HNE adduct formation leads to a permissive environment for increased protein synthesis and hypertrophic growth [39]. In agreement with impaired LKB1

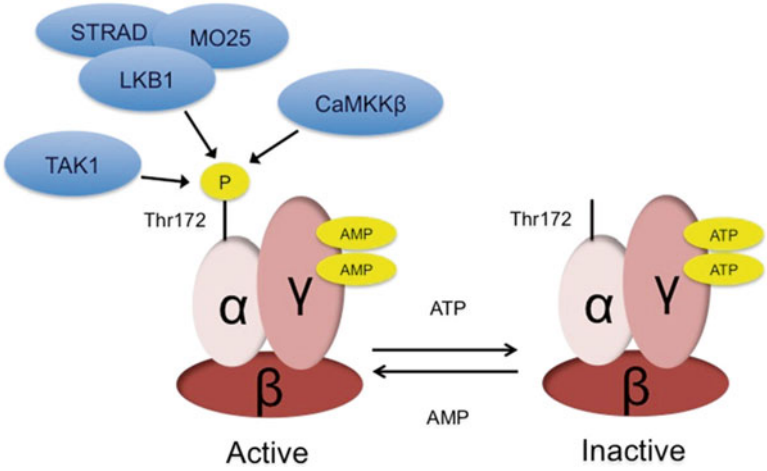


**Fig. 1** Structure and activation of AMPK. AMPK is made up of three subunits: α, β, and γ. Activation of AMPK requires binding of AMP to the γ subunit during times of cellular stress, which promotes phosphorylation of threonine 172 of the α subunit by upstream kinases (AMPKKs). Known upstream kinases include the calcium-dependent protein kinase kinase (CaMKK), transforming growth factor-β-activated kinase-1 (TAK1), and most importantly in the heart, LKB1 in a complex with two accessory subunits (STRAD and MO25). In the presence of low AMP:ATP ratio, ATP molecules bind to AMPK thus inhibiting the allosteric activation of the molecule

activity allowing increased cellular growth, mice with a cardiomyocyte-specific deletion of LKB1 have reduced AMPK activity and develop LVH [41]. Although LKB1 is known to regulate at least 13 different downstream kinases [37, 42], it has been shown that cardiomyocyte cell growth induced by LKB1 suppression can be rescued via activation of AMPK [41], demonstrating that AMPK and not another LKB1 target protein is the mediator of this specific cellular process. Together, these findings support the concept that an intact LKB1/AMPK signaling pathway is necessary to prevent abnormal cardiomyocyte cell growth.

## 4 The Role of AMPK in Cardiac Metabolism and Cell Growth

The healthy heart has a high energy demand and as a result must produce a considerable amount of energy in the form of ATP (estimated 3.5–5 kg of ATP in the human heart) in order to support normal contractile function and ionic homeostasis [43]. Under normal physiological conditions, the healthy adult heart derives >95 % of its ATP from mitochondrial oxidative phosphorylation, while the remainder is generated from glycolysis [44–47]. Although the heart can utilize a variety of substrates to produce energy, the normal healthy adult heart preferably uses fatty acids as a fuel substrate and obtains 50–70 % of its ATP from the oxidation of fatty acids.

Cardiac AMPK plays a pivotal role in regulating cardiac energy metabolism and increasing net ATP production [48]. Indeed, AMPK increases FA utilization in response to increased energy demand by enhancing the availability [49] and uptake of fatty acids [50, 51] as well as via direct effects on acetyl CoA carboxylase (ACC) to promote FAO [52]. In addition to its actions on the regulation of FAO, AMPK also regulates glucose metabolism by inducing expression and promoting translocation of the glucose-transporter GLUT4 to the plasma membrane, thus enhancing glucose uptake [53] and subsequently stimulating glycolysis [54]. Moreover, AMPK is partially responsible for turning off energy consuming processes, such as protein synthesis, in times of metabolic stress in order to help conserve ATP [55, 56]. More recent evidence supports the role of AMPK in regulating the expansion of microtubules [57], which is a key component of the cytoskeleton. This is especially important in modulating cell growth and proliferation in the context of preventing and/or treating cardiac hypertrophy. Therefore, it is now recognized that AMPK targets a wide variety of signaling pathways involved in controlling cardiac energy metabolism as well as cell growth, which will each be further discussed throughout this review.

As mentioned earlier, energy production from the oxidation of FAs is significantly reduced in the setting of pressure-overload induced cardiac hypertrophy [6, 58–60]. Reduced expression of both oxidative enzymes [9, 61] and fatty acid transport proteins [45, 62] following chronically increased cardiac load have been noted as possible explanations. Furthermore, hypertrophied hearts display a significant down-regulation of peroxisome proliferator-activated receptor-alpha (PPARα) and PPAR gamma coactivator 1alpha (PGC-1α), which are critical regulators of genes involved in cellular energy metabolism, in particular FAO [63, 64]. Deactivation of PPARα has previously been linked to the development of cardiac hypertrophy [63]. In fact, both transcription and protein levels of PPARα in the heart were found to be reduced in rats subjected to transverse aortic constriction (TAC) [65], an experimental model of pressure-overload induced hypertrophy.

Although the exact mechanism responsible for decreased PPARα levels remains unclear, it has been shown that AMPK has the ability to inhibit cardiac hypertrophy by enhancing the activity of PPARα [66] and that it mediates this effect by reducing the activity of extracellular signal regulated protein kinase (ERK1/2) [65]. This finding correlates with previous studies showing that inhibition of ERK1/2 may play a role in regulating hypertrophy [67, 68]. In fact, pharmacological activation of AMPK by 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR) prevented the development of cardiac hypertrophy and increased the transcriptional level and activity of PPARα *in vitro* and *in vivo* [65]. Importantly, this effect of AMPK activation on PPARα and ERK1/2 was abolished in the presence of EGF, an activator of ERK1/2 [65]. Thus, AMPK is thought to restore PPARα activity by reducing the phosphorylation of ERK1/2, thereby promoting FAO and leading to improved myocardial energy metabolism [66]. As well, these findings suggest that AMPK activation may be beneficial to alleviate pressure-overload induced cardiac hypertrophy. However, it should be noted that not all evidence supports PPARα as an effective target for treatment of cardiac hypertrophy as overexpression of PPARα can cause contractile dysfunction in the hypertrophied rat heart [69].

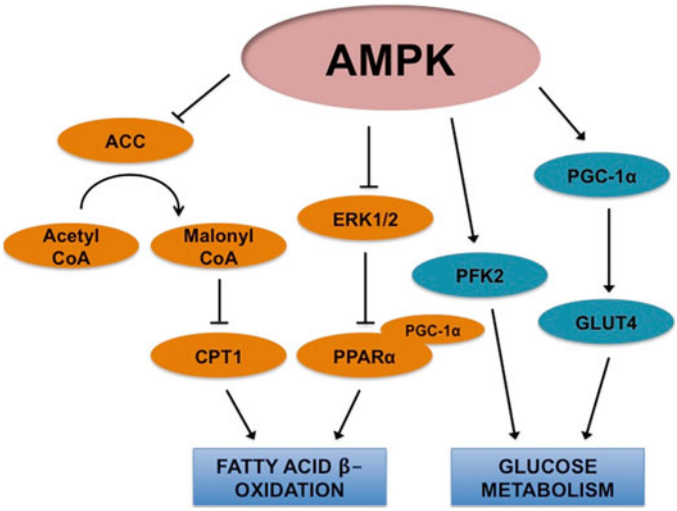


**Fig. 2** Regulation of cardiac metabolism by AMPK. Once activated, AMPK is shown to act on numerous signaling cascades that alter energy metabolism of the cardiomyocyte. For example, AMPK is thought to reduce phosphorylation of extracellular signal regulated protein kinase (ERK1/2), thus restoring activity of PPARα which then upregulates genes that encode oxidative enzymes involved in fatty acid β oxidation. PPAR gamma coactivator 1alpha (PGC-1α) acts as a coactivator with PPARα to promote mitochondrial biogenesis, thereby improving myocardial oxidative metabolism. FAO is also increased upon phosphorylation of acetyl-CoA carboxylase (ACC) by AMPK. This inactivation of ACC reduces the conversion of acetyl-CoA to malonyl-CoA, thus permitting carnitine palmitoyltransferase I (CPT1) to transport fatty acids into the mitochondria for subsequent oxidation. AMPK also enhances glucose metabolism by inducing expression of the glucose-transporter GLUT4 via PGC-1α as well as promoting translocation of vesicles containing GLUT4 to the plasma membrane. This together with the activation of phosphofructokinase 2 (PFK2) by AMPK ultimately stimulates glycolysis

Therefore it remains to be determined whether targeting this pathway as a potential treatment for LVH is an appropriate strategy.

The heart displays tremendous metabolic flexibility and the subsequent increase in glucose metabolism in the hypertrophied heart is thought to be a compensatory mechanism to generate ATP in response to decreased rates of FAO [6]. Indeed, both humans and animal models with cardiac hypertrophy demonstrate an increase in glucose utilization [70, 71]. AMPK acts on PGC-1α to promote expression of the glucose transporter GLUT4 [72] and has also been shown to indirectly promote the translocation of vesicles containing GLUT4 to the plasma membrane [53]. In addition, AMPK also accelerates rates of glycolysis via its effects on phosphofructokinase 2 (PFK2) [54] (Fig. 2). Together, these effects may provide benefit to the hypertrophying heart by increasing the production of ATP via glycolysis [73, 74] and the ability of AMPK to increase glycolytic flux may play a critical role in the heart's response to stress. However, this shift towards accelerated rates of glycolysis in the hypertrophied heart does not fully compensate for the reduced energy output resulting

from diminished FAO [6, 10, 75]. In fact, despite increased glucose uptake and accelerated rates of glycolysis, most studies show either no change or a reduction in mitochondrial glucose oxidation in the hypertrophied heart [6, 76–78]. As a result, by-products of incomplete glucose metabolism such as protons and lactate, are shown to accumulate in cardiomyocytes and may divert ATP towards clearance of these by-products, thereby reducing efficiency of myocardial contraction [79, 80].

## 5 AMPK Inhibits Protein Synthesis

Cell growth is a complex and energetically costly process that is highly regulated at several levels. Along with the energy status of the cell, gene transcription and protein synthesis are key requirements for cell enlargement. As mentioned earlier, there is increasing evidence showing that AMPK acts as a negative regulator of LVH by down-regulating protein synthesis in cardiomyocytes [81]. Indeed, AMPK has been shown to influence several pathways involved in protein synthesis through both direct and indirect control of multiple mediators. For example, eukaryotic elongation factor-2 (eEF2) functions in mediating the translocation of the ribosome along mRNA during peptide-chain elongation [82]. Phosphorylation of eEF2 (Thr56) by its upstream kinase eEF2 kinase (eEF2K) results in the inactivation of eEF2 [83]. Interestingly, AMPK is able to directly phosphorylate eEF2K at Ser398 (and subsequently activate eEF2K) [56], and thus has the ability to regulate the activity of eEF2 [2, 19, 84, 85]. Indeed, activation of AMPK by AICAR in adult rat ventricular myocytes results in increased phosphorylation of eEF2 (thus reducing its activity) and subsequent inhibition of protein synthesis [85]. Furthermore, pharmacological activation of AMPK by metformin and AICAR was shown to inhibit protein synthesis and cardiac hypertrophy induced by phenylephrine treatment or activated Akt, and this was mediated by an increase in phosphorylation of eEF2 [2, 84]. Therefore, increased AMPK activity is shown to negatively regulate protein synthesis and cardiac hypertrophy through the eEF2 kinase/eEF2 signaling pathway and may be a key pathway by which cell growth can be controlled.

In addition to controlling peptide-chain elongation, AMPK also regulates protein synthesis through indirect regulation of the pro-hypertrophic mammalian target of rapamycin (mTOR)/p70S6 kinase signaling cascade [55, 86]. mTOR is a key regulator of myocardial protein synthesis [87] and can regulate cell growth and proliferation by coordinating a response to availability of amino acids and nutritional requirements [87, 88]. Assembly of mTOR with numerous adaptor proteins forms a distinct complex named mTOR complex 1 (mTORC1), and its activation leads to increased cardiac growth [89]. Phosphorylation of mTOR at Ser2448 by Akt leads to activation of this kinase [90], which in turn activates p70S6K by phosphorylation of this protein at multiple sites [91]. Activation of p70S6K occurs via phosphorylation of Ser411, Ser418, Thr421 and Ser424 residues [91, 92]; which is then followed by phosphorylation of the catalytic domain [93] and the linker region [94] to promote activity of the kinase. Through phosphorylation of the 40S ribosomal

protein S6, p70S6K promotes translation of mRNAs specific for ribosomal proteins as well as initiation and elongation factors [95]. Importantly, AMPK is able to inhibit protein synthesis and cardiac hypertrophy by directly phosphorylating and inactivating mTOR at Thr 2446 [96]. Indeed, several studies suggest that mTOR signaling is involved in regulating cardiac hypertrophy [89, 97–99]. Rapamycin, a specific inhibitor of mTOR, attenuates pressure-overload induced hypertrophy and prevents the activation p70S6K, a target of mTOR [89, 99]. Furthermore, the AMPK activator metformin prevented the development of cardiac hypertrophy induced by pressure overload as well as blunted mTOR activation. However this effect of metformin was abolished in AMPKα2-deficient mice [99], suggesting that inhibition of mTOR by metformin is dependent upon activation of the AMPK pathway. Moreover, AMPKα2-deficient mice are shown to have increased phosphorylation of cardiac p70S6K and are more prone to developing cardiac hypertrophy in response to isoproterenol or TAC [100]. Lastly, spontaneously hypertensive rats with impaired cardiac LKB1/AMPK signaling display enhanced mTOR/p70S6K signaling, which is consistent with the profound cardiac hypertrophy observed in these animals [39]. Interestingly, restoration of the LKB1/AMPK signaling pathway using resveratrol, a known AMPK activator, decreases the activation of p70S6K and lessens the development of cardiac hypertrophy in these rats [39]. Similarly, pharmacological activation of AMPK in neonatal rat cardiomyocytes has been shown to result in a significant decrease in p70S6K activity and subsequently reduced rates of protein synthesis [56, 84]. Taken together, there is strong evidence supporting the role of activated AMPK in controlling mTOR/p70s6K activity and preventing protein synthesis and hypertrophic growth in the cardiomyocyte.

Another possible mechanism by which AMPK regulates protein synthesis is via TSC2 [55, 86], a tumor suppressor gene shown to inhibit mTORC1 activity [101, 102] and reduce cell growth [103]. The ability of AMPK to promote TSC2 activity, which is normally active under unstressed conditions [104, 105], was first observed in HEK293 cells where it was shown to phosphorylate the enzyme at two residues (Thr1227 and Ser1345), leading to increased activity [55]. The heterodimeric complex that forms between TSC1 and TSC2 [106] goes on to inhibit the mTOR/p70S6K signaling cascade described above [55]. Consistent with this, mice embryos carrying TSC1/2 homozygous mutations display excessive cardiac cell growth during maturation and die prematurely [107]. In addition, inhibition of AMPK in neonatal rat cardiomyocytes is thought to prevent activation of TSC2, thus allowing for stimulation of the mTOR/p70S6K pathway to up-regulate protein synthesis, resulting in increased cell size [108]. Taken together, these studies suggest that AMPK also down-regulates protein synthesis and the development of cardiac hypertrophy through TSC2, which lies upstream of the mTOR/p70S6K signaling axis (Fig. 3).

Although somewhat controversial, the serine/threonine protein kinase Akt appears to oppose the effects of AMPK on the mTOR/p70S6K pathway [109, 110]. In numerous studies, activation of Akt has been found to be involved in promoting cardiac growth [109, 111] as well as cardiac hypertrophy [101, 106, 112]. More specifically, overexpression of Akt in neonatal rat cardiomyocytes results in increased activity of p70S6K, increased protein synthesis and increased myocardial

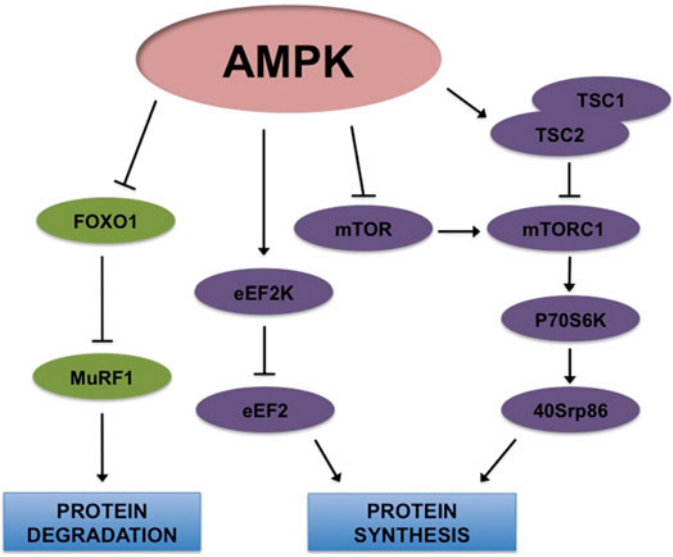


**Fig. 3** Regulation of cellular growth by AMPK. AMPK is also shown to act on several signaling cascades that limit cell growth. Primarily, AMPK activates the tuberous sclerosis complex 2-gene product (TSC2), which forms a complex with TSC1 to inhibit the mammalian target of rapamycin (mTOR)—p70S6 kinase (p70S6K) signaling cascade and thus reduces protein synthesis. AMPK also inhibits protein synthesis via activation of eEF2 kinase which phosphorylates/deactivates the eukaryotic elongation factor-2 (eEF2). In addition to these effects, AMPK may also contribute to increased degradation of unnecessary bulk proteins via inhibition of the FOXO/MuRF1 signaling pathway

cell size [84, 110]. Furthermore, Akt is known to reduce activation of AMPK by phosphorylating Ser485/491, which subsequently impedes phosphorylation of AMPK at Thr172 by LKB1 [106, 109, 111, 113, 114]. In agreement with this, LKB1 was unable to activate AMPK in cardiomyocytes expressing a constitutively active form of Akt1 [115], providing further evidence that Akt may promote the development of cardiac hypertrophy in part by preventing AMPK activation [84]. Furthermore, pharmacological activation of AMPK was shown to inhibit Akt-induced protein synthesis in neonatal rat cardiomyocytes, likely through its regulation of both p70S6K and eEF2 signaling pathways [84]. Therefore, pharmacological activation of AMPK may be an approach to counteract the pro-hypertrophic actions of Akt.

## 6 The Role of AMPK in Transcriptional Remodeling and Cell Growth

In addition to the role of AMPK in regulating protein synthesis, AMPK has also been shown to regulate the calcineurin/nuclear factor of activated T cells (NFAT) pathway that is responsible for mediating transcription of several pro-hypertrophic

genes [116]. Calcineurin is a calcium-calmodulin-dependent protein phosphatase that dephosphorylates the transcription factor NFAT causing it to translocate into the nucleus and promote transcription of its target genes [117]. Of importance, calcineurin/NFAT signaling is thought to play an important role in pathologic hypertrophic signaling. Indeed, calcineurin transgenic mice display significant enlargement of the LV when compared to their non-transgenic littermates [118]. However, activation of AMPK has been shown to reduce the degree of NFAT translocation [2, 119] and thus may contribute to the anti-hypertrophic effect of AMPK in the heart. Indeed, pharmacological activation of AMPK using resveratrol was associated with suppression and inhibition of calcineurin [2], as well as its downstream target NFAT [119]. Although the precise molecular signaling events involved in this regulatory circuit have not yet been fully investigated, blocking the calcineurin/NFAT pathway clearly reduces the hypertrophic response, providing some insight into signaling mechanisms involved in pathological hypertrophy as opposed to physiological hypertrophy.

As cardiac hypertrophy is characterized by cardiomyocyte enlargement in the absence of cellular division [57], cytoskeletal remodeling is a very important aspect of this process. An imbalance between protein synthesis and turnover can lead to enhanced accumulation of contractile myofibers and other proteins that is also characteristic of cell hypertrophy [120]. As such, preventing the accumulation of contractile myofibers may be another approach that could be used to lessen the development of LVH [113]. Importantly, it has been hypothesized that part of AMPK's anti-hypertrophic effects also involves inhibiting the atrophy-related FOXO/MuRF1 signaling pathway [113]. Muscle RING finger 1 (MuRF1), a ubiquitin ligase, is thought to both degrade unnecessary bulk proteins (that would otherwise augment cell growth) [121] and impede pro-hypertrophic stimuli (i.e. ERK1/2) [122]. Interestingly, activation of AMPK by AICAR in neonatal rat cardiac myocytes prevents phenylephrine-induced hypertrophy and upregulates MuRF1 via the FOXO1 transcription factor [113]. Although the exact mechanism through which this AMPK-mediated activation occurs is unknown, MuRF1 is thought to regulate pressure-overload induced cardiac hypertrophy via interaction with several proteins and transcription factors [123]. Therefore, a better of understanding of how/if AMPK regulates MuRF1 to prevent cardiac hypertrophy is needed.

Independent of protein synthesis and transcription events, AMPK has also been shown to limit cellular expansion of cultured cells by reducing the proliferation of microtubules [57]. Microtubules play a key role in determining cellular size and organization and contribute to both structure and transport within the cell. As microtubules accumulate they are known to contribute to the development of pressure-overload induced hypertrophy leading to contractile dysfunction and thus may play a significant role in the development of heart failure [124, 125]. Interestingly, AMPK has been shown to change the binding of the microtubule-associated protein tau to microtubules in neurons [126]. However, whether or not this occurs in the cardiomyocyte has not been firmly established. That said, AMPK is closely related to the MAP-microtubule affinity-regulating kinases (MARK) subfamily, which is responsible for phosphorylating microtubule-associated proteins (MAPs) [127].

In fact, AMPK is thought to be able to phosphorylate and thus deactivate MAP4 [57], which would otherwise promote assembly and stabilization of microtubules [128, 129] in response to pressure overload [130]. Following pharmacological activation of AMPK, phosphorylation of MAP4 was increased and this was associated with reduced stability of microtubules and more importantly limited cell expansion and microtubule growth [57]. However, MAP4-deficient cells demonstrate similar reduction in microtubule stability [57], thus it has yet to be shown to what extent this microtubule instability depends on MAP4 alone as opposed to in conjunction with other kinases. Furthermore AMPK deficient mice showed increased levels of total tubulin in the heart following TAC [57]. As increased levels of total tubulin is known to be required for microtubule growth, these findings strongly suggest that AMPK may also play a role in the regulation of microtubule levels and may represent an alternative mechanism whereby AMPK regulates the development of cardiac hypertrophy [57].

## 7 PRKAG2 Mutations

In addition to changes in AMPK activity via phosphorylation and/or pharmacological activation, perturbations in the AMPK γ subunits have also been associated with the development of cardiac hypertrophy. Indeed, mutations in the *prkag2* gene that encodes for the γ2 subunit result in the decreased ability of AMPK to bind ATP [131]. This disruption of the ability to sense AMP:ATP homeostasis has been shown to result in changes in AMPK activity [131] and may be responsible for excessive cellular glycogen storage that is characteristic of these mutations [58]. In addition to glycogen accumulation in hearts of humans with *prkag2* mutations, ventricular pre-excitation can develop in these patients and they display symptoms similar to Wolff-Parkinson-White syndrome (WPW) [132]. Ventricular pre-excitation is caused by glycogen accumulation in cardiomyocytes that leads to functional bypass tracts which connect the atria and ventricles [131]. These abnormal conduction pathways allow electrical impulses to bypass the atrioventricular node, resulting in a defective cardiac conduction system [132–134]. In addition, up to 80 % of individuals affected with this naturally occurring mutation also exhibit left ventricular hypertrophy [132], supporting the concept that alterations in AMPK signalling may be causative in the development of ventricular pre-excitation and/or LVH.

In order to further elucidate the mechanisms by which *prkag2* mutations produce LVH and electrophysiological abnormalities characteristic of this condition, several transgenic mouse models have been generated. Data from these *in vivo* models as well as *in vitro* models have suggested that the PRKAG2 cardiac syndrome arises as a result of alterations in AMPK activity [135, 136]. However, it is unclear if PRKAG2 mutations are AMPK activating or inactivating mutations. While N488I and T400N mutations of the *prkag2* gene have been reported to increase activity of AMPK [136, 137], both R302Q and R531G mutations result in inhibition of AMPK activity [138]. In the past, these variations in the activity of AMPK have made it

difficult to distinguish between compensatory alterations and changes which are a direct product of the mutation itself [131]. Nonetheless, the cardiac phenotype of glycogen accumulation is consistent in all murine models exhibiting a *prkag2* missense mutation [136, 139].

Comparing the activity of cardiac AMPK in early and later stages of the disease in the various transgenic mouse models with *prkag2* mutations has allowed for a better understanding of the role of AMPK in this disease. Transgenic mouse models with heart-specific R302Q mutations revealed significantly higher activity of AMPK at 7 days than at 2–5 months of age [139]. Although not shown directly, it is speculated that this latter decrease in AMPK activity is due to a feedback mechanism in which the accumulation of glycogen found as a result of AMPK activation eventually inhibits AMPK [139]. Contrary to this, the N488I mutation has been shown to be an AMPK activating mutation in both early development and in adulthood of the mice [136, 140, 141]. Therefore, regardless of the mechanism responsible for glycogen accumulation, the development of ventricular pre-excitation commonly observed in patients with *prkag2* mutations is now known to be a result of excessive deposition of glycogen and not directly attributed to alterations in AMPK activity [142]. This condition highlights the important role that AMPK has in normal cellular physiology and how alterations in AMPK activity can contribute to glycogen storage cardiomyopathy and cardiac hypertrophy. In addition, the phenotype induced by the activating *prkag2* mutations also raise concerns about whether pharmacological activation of AMPK for the treatment of LVH would cause additional cellular growth or glycogen deposition.

## 8 AMPK in the Hypertrophied Heart

The precise role of AMPK in the hypertrophied heart remains somewhat controversial, as various studies have implicated AMPK as both contributing to and inhibiting the development of cardiac hypertrophy. For instance, an early study by Tian et al. [59] showed that both expression and activity of AMPK are elevated in hearts subjected to chronic pressure overload. While this finding appears to be in direct contrast with the concept that AMPK activation prevents LVH, it is also possible that AMPK activation occurs at a much later stage of LVH development when growth has already occurred and energetic deficiency is driving the activation of AMPK. In agreement with this, decreased AMPK signaling in adiponectin-deficient mice was found to permit hypertrophic growth in response to pressure overload [143, 144], thus supporting the concept that AMPK activation may, indeed, be beneficial in the treatment of LVH.

Although the question of whether harmful or beneficial results arise from the activation of AMPK in cardiac hypertrophy remains, these seemingly opposite results may, in fact, be explained by the stage of hypertrophy studied. The aforementioned studies showing increased activity of AMPK in the hypertrophied heart

were performed in animal models with advanced stages of hypertrophy [59], at a point when activation of AMPK may be an adaptive response of the heart in response to ATP depletion. Contrary to this, numerous pharmacological activators of AMPK have been shown to prevent cardiac hypertrophy in a number of models, which support the idea that AMPK is a negative regulator of cardiac hypertrophy. Therefore, it is likely that AMPK activation early on during the development of hypertrophy may be able to prevent cardiac hypertrophy. However, activation of AMPK during later stages of pathological hypertrophy may be an adaptive response to an energetic deficiency and thus pharmacological activation at this stage may also be of benefit, albeit for ATP supply and not cellular growth control.

## 9 AMPK as a Pharmacological Target to Prevent Cardiac Hypertrophy

The discovery of an association between AMPK and the development of LVH has led to numerous studies investigating pharmacological agents that may be able to activate AMPK in order to clinically treat this pathological condition. Due to its role in maintenance of glucose and lipid homeostasis, AMPK is a well-known target in the treatment of type 2 diabetes and has more recently emerged as a potential target in the treatment of the metabolic syndrome [145]. Similarly, the antidiabetic drug, metformin, was found capable of activating AMPK [146] and is under investigation to determine its potential role in attenuating LVH [147]. However, more recent evidence also supports the potential effectiveness of the polyphenol resveratrol, an active ingredient of green tea and red wine, in preventing LVH [2, 39, 148, 149]. Lastly, the AMP-analog AICAR has long been used as an AMPK activator following the finding of its ability to stimulate glucose uptake in skeletal muscle [150]. Studies providing evidence that indirect pharmacological activators of AMPK (such as metformin, resveratrol and AICAR) as well as specific AMPK activators (such as A-769662) may prevent and/or reverse the development of cardiac hypertrophy will be further outlined below.

Metformin has been found to lessen the hypertrophic effects of pressure overload in TAC mice [99] through indirect activation of AMPK [22, 151]. Moreover, administration of metformin following occlusion of the left coronary artery of a mouse model was associated with increased phosphorylation of AMPK but also a reduced heart to body weight ratio [152]. Metformin has been shown to not only reduce protein synthesis in the cardiomyocyte [84], but is also capable of suppressing oxidative stress and associated cardiac hypertrophy [22]. The ability of metformin to attenuate cardiac hypertrophy appears to rely on activation of AMPK as this effect of metformin is lost in AMPKα2–/– mice [99]. Lastly, long-term metformin treatment shows promise in preventing pressure overload-induced LVH via activation of AMPK and downstream endothelial nitric oxide synthase (eNOS) [147]. Although the exact mechanism through which metformin activates AMPK is not

fully understood, there is speculation that it may influence the AMP:ATP ratio or perhaps activate an upstream kinase (AMPKK) [153].

The naturally-occurring polyphenol resveratrol has also been used to activate AMPK in hopes of developing a strategy to treat LVH. Evidence from studies with resveratrol suggests that resveratrol-mediated AMPK activation attenuates cardiac hypertrophy via a direct effect on pathways controlling protein synthesis and cell growth including eEF2 and p70S6 kinase [154]. Resveratrol treatment was also found to reduce hypertrophy in the spontaneously hypertensive rat upon reactivation of the LKB1/AMPK signaling pathway [39]. Resveratrol treatment prevented LVH in hypertensive rodents in both the presence or absence of changes in systolic blood pressure, suggesting that resveratrol may have direct effects on the heart to prevent LVH independent of changes in cardiac load [39, 149, 155–157]. Although preliminary data supports the use of resveratrol as a potential adjunct therapy in the treatment of LVH, future studies are required to show whether its anti-hypertrophic effects do, in fact, depend on AMPK.

Another activator of AMPK is AICAR, an analog of adenosine that mimics the effects of AMP by allosterically activating AMPK [158, 159]. Following treatment with AICAR, rats subjected to TAC demonstrated both elevated AMPK activation and reduced cardiac hypertrophy [119]. Pharmacological activation of AMPK via AICAR is also associated with regression of cardiac hypertrophy [2] as a result of reduced protein synthesis and growth of cardiac fibroblasts [2, 19, 139]. Neonatal cardiomyocytes treated with AICAR to activate AMPK demonstrated reduced free tubulin and therefore a significant decrease in stability of microtubules, as well as a weakened hypertrophic response to phenylephrine [57]. However, it should be noted that mimicking the stimulatory effects of adenosine is reported to have other consequences on cardiac function, and thus requires further understanding prior to any pharmacological use of AICAR [160, 161]. While these studies provide evidence that the use of AICAR can reduce or eliminate the development of cardiac hypertrophy, a better understanding of AICAR's mode of action, both in activating AMPK in the heart as well as potential side effects is necessary.

Since the compounds discussed above are indirect AMPK activators, treatment with these compounds may cause undesirable off-target effects resulting from activation of non-AMPK signaling cascades. Therefore, many arguments favor the use of specific AMPK activators such as A-769662, a thienopyridone compound developed by Abbott Laboratories [162]. The compound has shown to be effective in activating and maintaining phosphorylation of AMPK in cell-free assays [163, 164], likely through binding to a novel site on the β subunit of AMPK [163, 164]. Recent studies have demonstrated the cardioprotective effect of the small molecule A-769662 on the ischemic heart [164]. Although A-769662 activates AMPK independent of alterations in the AMP:ATP ratio, due to its poor oral availability the drug may not be ideal for pharmacological use in patients [163]. Nonetheless, this small molecule is a useful experimental tool that offers insight into the effectiveness of targeted AMPK activation as a therapeutic approach to treat different cardiac diseases.

## 10 Conclusions

AMPK is known to phosphorylate multiple downstream targets and has a critical role in modulating metabolic activities such as glucose transport and fatty acid oxidation. In addition, AMPK has emerged as a key player in protein synthesis and cell remodeling. Since alterations in both metabolism and cell growth occur in the development and pathogenesis of LVH, AMPK agonists may prove to be useful in the treatment of cardiac hypertrophy. However, genetic mutations in the AMPK γ2 subunit gene (*prkag2*) cause inappropriate activation of AMPK and a glycogen storage myopathy that ultimately leads to ventricular pre-excitation. Therefore, whether or not pharmacological activation of AMPK by compounds such as metformin, resveratrol, AICAR, and A-769662 (or newly developed AMPK agonists) is beneficial or harmful in the setting of LVH is still being investigated. Thus, despite exciting preclinical findings, additional research in this area is necessary before AMPK agonism can be considered for further development with the aim of treating LVH in humans.

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