Role of AMP-activated protein kinase in healthy and diseased hearts

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THE HEART is capable of utilizing a variety of substrates to meet its immense demand for energy. Under normal physiological circumstances, cardiac function is dependent on the production of intracellular ATP derived primarily by the utilization of fatty acids, glucose, and lactate (123). A variety of transport proteins and regulatory enzymes control the uptake and subsequent metabolism of these energy substrates. Whereas many of these enzymes are regulated through substrate supply and/or energy demand, covalent modification of a number of these enzymes is also important. These regulatory mechanisms mediate substrate utilization and are essential for proper cardiac function.

Fatty acids provide a concentrated supply of energy accounting for ~50–75% of the myocardial acetyl CoA-derived ATP (84). Upon entering the cardiac myocyte, fatty acids are transported into the mitochondria and undergo β-oxidation to produce acetyl CoA that is eventually used to produce ATP. Glucose is transported into the cell via glucose transporters (GLUT) and can undergo glycolytic metabolism to generate pyruvate and ATP. This can occur in the absence of oxygen while still producing ATP. However, in the normally functioning aerobic myocardium, ATP produced via glycolysis often contributes <10% of the overall ATP. Indeed, the majority of carbohydrate-derived ATP is derived when the pyruvate generated from glycolysis is further metabolized within the mitochondria. When the energy demands of the heart are fulfilled, excess glucose can be stored in the form of glycogen. Endogenously stored glycogen may be used as a source of ATP when the supply of other fuel substrates is limiting. Together, myocardial fuel selection, utilization, and storage are complex processes that require precise regulation to coordinate substrate metabolism with myocyte energy demand.

Under normal circumstances, glucose and fatty acid metabolism is a tightly regulated process. However, during various physiological and pathophysiological conditions, fatty acid and glucose uptake and subsequent metabolism can be dramatically altered. For example, pathophysiological conditions such as diabetes, hypertrophy, chronic ischemia, and other forms of cardiomyopathies are associated with alterations in fatty acid and glucose metabolism (84). These changes may be due, in part, to alterations in the activities of regulatory proteins that control fatty acid and glucose uptake and metabolism. In the heart, chronic alteration in the utilization of energy substrates has been termed “metabolic remodeling” and involves alterations in the expression of genes that encode enzymes involved in the regulation of fatty acid and glucose uptake, storage, and metabolism (122). Whereas many of these enzymes are subject to chronic regulation via gene expression, multiple acute control mechanisms are also involved, including phosphorylation/dephosphorylation. The AMP-activated protein kinase (AMPK) has emerged as being centrally involved in the regulation of a number of these enzymes and is thus a major regulator of cardiac energy metabolism.
AMPK is a heterotrimeric protein that regulates both whole body and cellular energy utilization (64). Early reports showed that AMPK is activated by metabolic stresses that deplete cellular ATP (22, 40, 143). In healthy cells, ATP is maintained at concentrations 10-fold above that of ADP (10 to 1 ratio). However, the AMP-to-ATP ratio (AMP/ATP) is a much more sensitive indicator of cellular energy status and represents the signaling system that is monitored by AMPK. As initially postulated by the adenylate charge hypothesis (8), small changes in AMP concentrations indicate larger changes in cellular energy balance, and these changes regulate AMPK activity to maintain ATP concentrations within a very narrow range (105). Therefore, despite minor decreases in ATP levels, there is a more dramatic increase in intracellular AMP levels, which activates AMPK by a number of different mechanisms.

AMPK is activated allosterically by AMP, which accounts for approximately a fivefold increase in kinase activity (1, 25, 113). In addition, AMP binding makes AMPK a better substrate for its upstream kinase (AMPKK) and subsequently activates AMPK by a number of different mechanisms. There is a more dramatic increase in intracellular AMP levels, activity to maintain ATP concentrations within a very narrow range (105). Therefore, despite minor decreases in ATP levels, there is a more dramatic increase in intracellular AMP levels, which activates AMPK by a number of different mechanisms.

AMPK is activated allosterically by AMP, which accounts for approximately a fivefold increase in kinase activity (1, 25, 113). In addition, AMP binding makes AMPK a better substrate for its upstream kinase (AMPKK) and subsequently results in an increase in AMPK phosphorylation (at Thr-172 of the α-catalytic subunit, discussed in PHOSPHORYLATION CONTROL OF MAMMALIAN AMPK) (50). Whereas this phosphorylation increases AMPK activity 50- to 100-fold in the liver (50), this level of activation is not generally observed in the heart, possibly due to a higher baseline phosphorylation level of AMPK. In addition to stimulating AMPK phosphorylation, AMP binding to the kinase inhibits dephosphorylation of AMPK by protein phosphatases (34). Whereas the AMP/ATP seems to be primarily responsible for the activation of AMPK, it has also been shown that AMPK can be activated by a fall in the phospho-creatine-to-creatine ratio (PCr/Cr) in skeletal muscle (105). During times of energy shortage, PCr is depleted to replenish ATP and because PCr can directly inhibit AMPK activity, lower levels of PCr can activate AMPK (105). However, PCr is not generally thought to directly regulate AMPK activation in the heart, even during ischemia when PCr and ATP are depleted (4). Although PCr/Cr may not be involved in regulating AMPK activity in the heart, it is clear that AMP is involved in controlling cardiac AMPK activity and thus contributes to re-establishing ATP content, which is essential for proper cellular function.

In addition to the classical mechanisms responsible for AMPK activation, there are a number of conditions/treatments that activate AMPK in a manner that are not dependent on changes in AMP/ATP or PCr/Cr. For example, leptin (94), adiponectin (140), hyperosmotic stress (42), metformin (42), and long-chain fatty acids (26, 41, 133) have been reported to activate AMPK in an AMP-independent fashion. This latter finding is particularly interesting given that the majority of cardiac energy is derived from fatty acid oxidation. During times of appropriate energy supply when, for example, fatty acids are in excess, one would not predict an activation of AMPK. However, ex vivo hearts perfused in the presence of physiological concentrations of long-chain fatty acids result in increased phosphorylation and activation of AMPK (26, 41). These findings suggest that AMPK may be involved in sensing fatty acid availability and can then direct cardiac substrate utilization accordingly. Whereas these studies may have identified a unique “substrate-sensing” role for cardiac AMPK, it is also possible that elevated levels of free fatty acids simply activate AMPK via oxidative stress. This alternative interpretation would not necessarily support a direct “substrate-sensing” role for AMPK but may confirm the findings of Yang and Holman (141).

Mammalian AMPK is activated allosterically by AMP, which accounts for approximately a fivefold increase in kinase activity (1, 25, 113). In addition, AMP binding makes AMPK a better substrate for its upstream kinase (AMPKK) and subsequently results in an increase in AMPK phosphorylation (at Thr-172 of the α-catalytic subunit, discussed in PHOSPHORYLATION CONTROL OF MAMMALIAN AMPK) (50). Whereas this phosphorylation increases AMPK activity 50- to 100-fold in the liver (50), this level of activation is not generally observed in the heart, possibly due to a higher baseline phosphorylation level of AMPK. In addition to stimulating AMPK phosphorylation, AMP binding to the kinase inhibits dephosphorylation of AMPK by protein phosphatases (34). Whereas the AMP/ATP seems to be primarily responsible for the activation of AMPK, it has also been shown that AMPK can be activated by a fall in the phospho-creatine-to-creatine ratio (PCr/Cr) in skeletal muscle (105). During times of energy shortage, PCr is depleted to replenish ATP and because PCr can directly inhibit AMPK activity, lower levels of PCr can activate AMPK (105). However, PCr is not generally thought to directly regulate AMPK activation in the heart, even during ischemia when PCr and ATP are depleted (4). Although PCr/Cr may not be involved in regulating AMPK activity in the heart, it is clear that AMP is involved in controlling cardiac AMPK activity and thus contributes to re-establishing ATP content, which is essential for proper cellular function.

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Additional isoforms of each AMPK subunit have been identified and are encoded by separate genes. Two cDNAs encoding distinct isoforms of the α-subunit termed α1 (119) and α2 (19) were isolated. The α1 gene encodes a 548-amino acid protein, whereas the α2-subunit encodes a 552-amino acid protein (119). The two α isoforms are 90% identical over their NH2-terminal domain but only 60% identical over the COOH-terminal domain. The NH2-terminal region contains the kinase domain, as well as several potential regulatory sites that are phosphorylated by upstream kinases (138). The COOH-terminal region is responsible for mediating protein-protein interactions between the α-subunit and β-subunit (30). The α1-subunit is ubiquitously expressed, whereas the α2-subunit is expressed mainly in the heart, skeletal muscle, and liver (119, 129). In the heart, data suggest that the α2-associated AMPK activity predominates over the α1-activity (78, 110).

Invited Review

Invited Review

AMPK in Healthy and Diseased Hearts

Mammalian AMPK is a heterotrimeric complex consisting of a 63-kDa catalytic α-subunit, a 43-kDa regulatory β-subunit, and a 38-kDa γ-subunit. AMPK was initially purified from rat liver (20) and subsequently purified to heterogeneity from porcine liver as a heterotrimer (33, 95). Expression of the α-subunit together with the β- and γ-subunits confers a 50- to 100-fold increase in kinase activity compared with expression of the α-subunit alone (37). This demonstrates that the majority of AMPK activity is dependent on the formation of the heterotrimeric complex.

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Two cDNAs encoding distinct β-subunits referred to as β1 and β2 have been cloned. The β1 cDNA encodes a 270-amino acid protein, whereas β2 encodes a 271-amino acid protein. These proteins share 71% amino acid identity and contain similar domains. For example, the β-subunits contain a glycogen-binding domain (amino acids 72–151) that is common in enzymes that metabolize glycogen and starch (58). This region may be important for AMPK localization and/or substrate targeting, which is supported by the observation that AMPK has been found localized to glycogen granules (111). This is particularly important given that the fact that cardiac AMPK appears to play a role in the regulation of glycogen accumulation (21) and may be responsive to glycogen levels (48) (discussed in Myocardial Glucose Utilization). The β-subunits also possess a COOH-terminal (Association with the SNF1 Complex) domain that is required for interacting with the α- and γ-subunits (61, 125). The β1-subunit is ubiquitously expressed, whereas the β2-subunit is highly expressed only in heart and skeletal muscle with lower expression in the lung and kidney (24, 125).
Three different isoforms of the γ-subunit have been cloned. The γ1-isoform is ubiquitously expressed, and its transcript encodes a protein of 331 amino acids (43). In the heart, the majority of AMPK activity is associated with complexes containing the γ1-subunit (78). The γ2 gene has two transcriptional start sites that produce transcripts of differing length and encoding two proteins of different size (γ2α, γ2β). The γ2α 3.8-kb transcript is expressed primarily in the brain, kidney, pancreas, and liver, but not the heart, whereas the 3.0-kb γ2α transcript is expressed at a very high level in the heart with lower expression in the muscle, kidney, spleen, and testis (25). Both of the γ2α transcripts encode a protein of 569 amino acids that contains an additional 241 amino acids at its NH2-terminus that are not present in the other γ-subunit genes. The γ2b transcript is 2.4 kb in length and encodes a protein of 328 amino acids that is expressed in the heart, testis, and placenta (75). The γ3-subunit is expressed exclusively in skeletal muscle (25, 144). Currently, information regarding the function of the NH2-terminus of the γ-subunits is not available, although the additional 241 amino acids at the NH2-terminus of γ2 suggest the potential for unique targeting, regulation, and/or substrate identification. All of the γ-subunit isoforms of AMPK also contain four tandem repeats of a “Bateman domain” (113). The domains likely represent the AMP/ATP nucleotide binding sites within the AMPK heterotrimERIC complex (25). Evidence that this domain is important for AMPK function is derived from a number of naturally occurring point mutations in the Bateman domain that interfere with AMP binding and consequently alter AMPK activity (31, 113). Although roles for these isoforms have not yet been fully defined, it is apparent that the γ-subunits can be major regulators of AMPK activity. Indeed, the γ2-subunit has gained considerable attention due to the existence of mutations that result in specific cardiomyopathies (discussed in Regulation of Glycogen Storage and Wolff-Parkinson-White Syndrome).

The existence of several AMPK subunit isoforms encoded by separate genes implies that a functional AMPK heterotrimer may be essential to cellular survival. This redundancy suggests that additional subunits may be able to compensate for a mutation and loss of function of a similar subunit. In support of this concept, no overt cardiac phenotypes are apparent in the mutation and loss of function of a similar subunit. In support of this concept, no overt cardiac phenotypes are apparent in the mutation and loss of function of a similar subunit. This redundancy suggests that these isoforms could have functions unique to certain organs. It is possible that particular combinations of different AMPK subunits target specific metabolic pathways or respond to different upstream kinases. In the heart, specific isoforms may confer specialized roles for AMPK in the cardiac myocyte that need to be fully investigated. In this regard, consideration should be given to the reports demonstrating specific isoform expression in the heart and the tendency to consider these findings as representing the isoforms of AMPK expressed in the cardiac myocyte. Indeed, the heart contains multiple cell types, and these cells have different AMPK subunit content, which may not necessarily reflect the distribution of the different AMPK subunits within the cardiac myocyte.

PHOSPHORYLATION CONTROL OF MAMMALIAN AMPK

For many years, the kinases responsible for phosphorylating AMPK were elusive. Only recently, through genetic approaches in Saccharomyces cerevisiae have the identity of the upstream kinases of the yeast AMPK orthologue Snf1 been determined. Phosphorylation and activation of Snf1 in vitro were catalyzed by Elm3, Pak1, and Tos3, and deletion of all three kinases is required to abolish Snf1 activity (55, 121). The mammalian kinase LKB1 shares significant sequence homology to the yeast AMPKKs, and recent work demonstrated LKB1 to be an AMPKK that phosphorylates AMPK at its activating phosphorylation site (Thr-172) (49, 55). LKB1 was originally identified as a tumor suppressor that was mutated in patients with Peutz-Jeghers syndrome (52). LKB1 is a serine/threonine kinase that complexes with the MO25 and STRAD regulatory proteins (10, 17). STRAD is considered to be a pseudokinase, whereas MO25 is a scaffolding protein. Together, these three proteins form an active AMPKK (49). LKB1 is abundantly expressed in rat and mouse hearts (4, 110), but its precise role in this organ is only beginning to emerge. Recently, mice having a cardiac-specific LKB1 null genotype were generated. Interestingly, LKB1-deficient hearts and cardiac myocytes failed to stimulate phosphorylation of AMPK α3 at Thr-172 in response to conditions known to activate AMPK (110). Conversely, the AMPK α1-subunit was phosphorylated at Thr-172 under the same conditions even in the absence of LKB1 expression (110) (discussed in Role of AMPKKs in myocardial ischemia-reperfusion).

Whereas LKB1 may be one of the major upstream AMPKKs, other AMPKKs have been identified. For example, the Ca2+/-calmodulin-dependent protein kinase kinases (CaMkKα and CaMkKβ) have both been shown to phosphorylate AMPK at its activating phosphorylation site (51, 59, 137). Additional sites on AMPK are also targets for phosphorylation by other kinases that potentially regulate AMPK activity. Indeed, Woods et al. (138) showed that the AMPK α-subunit was phosphorylated at Thr-258 and Ser 485/491, although mutation of these sites to alanine indicated these were not necessary for kinase activity in vitro. It has been shown by our laboratory (69, 118) and others (57) that activated Akt directly phosphorylates AMPK on a site separate from Thr-172. This alternative phosphorylation site appears to prevent subsequent phosphorylation at Thr-172 by AMPKKs under steady-state conditions (69, 118). Since a number of kinases have been shown to phosphorylate AMPK directly, defining the role that these upstream kinases play in the heart will contribute to an increased understanding of cardiac AMPK regulation.

USE OF AMPK TRANSGENIC AND KNOCKOUT MICE TO STUDY FUNCTIONS OF CARDIAC AMPK

Several mouse strains expressing genetically modified forms of AMPK subunits have been created (Table 1). Under normal conditions and normal diets, mice that have a global deletion of either the AMPK α1 gene or the AMPK α2 gene have similar body compositions as wild-type mice, are fertile, and have normal life spans (63, 131). The metabolic phenotype of AMPK α2-knockout mice is characterized by glucose intolerance, insulin resistance, and lower insulin secretion (131). Interestingly, these metabolic defects were not altered by a high-fat diet even though the AMPK α2-null mice increased...
Table 1. Cardiac phenotypes of transgenic and knockout mouse models that alter AMPK activity

<table>
<thead>
<tr>
<th>Model</th>
<th>Description of Cardiac Phenotype</th>
<th>Refs.</th>
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<tbody>
<tr>
<td>Total α1 KO</td>
<td>No cardiac phenotype reported.</td>
<td>63</td>
</tr>
<tr>
<td>Total α2 KO</td>
<td>Insulin resistance, no overt cardiac phenotype.</td>
<td>131</td>
</tr>
<tr>
<td>DN α2 D157A cardiac-specific expression</td>
<td>During ischemia, decreased glucose uptake, increased ATP depletion, decreased left ventricular dysfunction during ischemia. Normal cardiac function during exercise, without ATP depletion.</td>
<td>99, 139</td>
</tr>
<tr>
<td>KD α2 K54R cardiac-specific expression</td>
<td>No cardiac hypertrophy. During ischemia-reperfusion, decreased glucose uptake, glycolysis and failure to increase fatty acid oxidation. Impaired cardiac function and increased cardiac injury following reperfusion.</td>
<td>107</td>
</tr>
<tr>
<td>γ2-WT cardiac-specific expression</td>
<td>Increased glycogen content.</td>
<td>6, 32</td>
</tr>
<tr>
<td>γ2-N488I cardiac-specific expression</td>
<td>Increased AMPK activity and cardiac hypertrophy associated with glycogen accumulation and ventricular pre-excitation similar to WPW syndrome after 8 weeks of age.</td>
<td>6</td>
</tr>
<tr>
<td>γ2-R302Q cardiac-specific expression</td>
<td>Decreased AMPK activity and cardiac hypertrophy associated with glycogen accumulation and ventricular pre-excitation similar to WPW syndrome after 3 weeks of age.</td>
<td>117</td>
</tr>
<tr>
<td>γ2-R531G cardiac-specific expression</td>
<td>Decreased AMPK activity and cardiac hypertrophy associated with glycogen accumulation and ventricular pre-excitation similar to WPW syndrome after 4 weeks of age.</td>
<td>32</td>
</tr>
<tr>
<td>Cardiac-specific LKB1 knockout</td>
<td>Slightly lower heart weight and enlarged atria. Ischemia-induced decrease in ATP and AMPKα2 activity but a substantial AMPKα1 activity still present.</td>
<td>110</td>
</tr>
</tbody>
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AMPK, AMP-activated protein kinase; KO, knockout; WT, wild-type; WPW, Wolff-Parkinson-White.

ROLE OF AMPK IN HEALTHY HEARTS

Regulation of Cardiac AMPK in Normoxic Heart During Exercise

In the rat, it has been shown that AMPK phosphorylation and AMPK activity is increased following contraction of skeletal muscle either ex vivo or during exercise (128, 134). Similar to skeletal muscle, cardiac AMPK activity is also increased in proportion to the level of exercise intensity (29) or due to acute increases in workload (12). In addition to this, Musi et al. (99) have shown that AMPKα2 activity increased to a greater extent than AMPKα1 activity in response to acute exercise (99). These acute increases in workload appear to activate AMPK due to increases in AMP levels (99). However, the mechanism by which continuously contracting cardiac muscle adapts to chronic increased workload induced by exercise is not currently known. Studies in skeletal muscle have demonstrated that pharmacological- or exercise-induced activation of AMPK increased PGC1-α to promote mitochondrial biogenesis (7, 124, 145). Whether this also occurs in the heart has not been investigated. Nevertheless, these studies support the concept that cardiac AMPK has a role in the adaptation of the heart to physiological stresses induced by acute and chronic increases in workload.

Regulation of Myocardial Energy Utilization

In essence, AMPK activation is beneficial to the heart through the activation of energy-generating pathways and inhibition of energy-consuming pathways. Because the maintenance of constant ATP levels is paramount for proper cardiac function, activated AMPK increases energy production several ways (Fig. 1). These include 1) increasing fatty acid uptake and oxidation (72, 89), 2) accelerating glucose uptake (106), and 3) stimulating glycolysis (92). At the same time that energy production is increased, cardiac AMPK switches off energy-consuming pathways such as protein synthesis (18, 23, 56, 60) and, as demonstrated in skeletal muscle, possibly lipid synthesis (27, 98) in an attempt to conserve intracellular ATP.
Whereas the relative importance of the inhibition of energy-consuming pathways in a metabolically stressed healthy heart likely plays only a minor role in maintaining mechanical function, inhibition of these energy-consuming pathways may be more relevant in chronically or severely stressed hearts (discussed in Regulation of Cardiac Myocyte Protein Synthesis). The major roles of AMPK in myocardial energy utilization are discussed below.

**Myocardial fatty acid utilization.** When the heart requires fuel, fatty acids are imported into the cardiac myocyte and oxidized in the mitochondria to produce ATP. AMPK appears to mediate the uptake and utilization of fatty acids by the heart via a number of processes (Fig. 1). For example, activation of AMPK has been shown to be involved in the recruitment of cardiac lipoprotein lipase to the coronary lumen thus promoting fatty acid availability to the heart (5). Moreover, recent evidence has shown that AMPK activation induced by myocyte contraction can mediate CD36 (a fatty acid transport protein) translocation from intracellular compartments to the plasma membrane, suggesting that AMPK is also involved in the regulation of fatty acid transport (89). In addition to increasing fatty acid levels within the cardiac myocyte, AMPK activation also increases fatty acid oxidation for the generation of ATP (72, 73, 91). This occurs through the phosphorylation and subsequent inhibition of acetyl CoA carboxylase (ACC) (38, 72, 73, 135). ACC produces malonyl CoA, which is an inhibitor of carnitine palmitoyl transferase (CPT)-1, the enzyme responsible for the import of fatty acyl CoA into the mitochondria. As a consequence of AMPK activation, a lower malonyl CoA level relieves inhibition of CPT-1, accelerates fatty acyl CoA transport into the mitochondria, and results in enhanced β-oxidation of fatty acids and subsequent ATP generation (9, 46). Therefore, by promoting fatty acid availability, fatty acid transport, and fatty acid oxidation, AMPK is a central mediator of fatty acid utilization in the heart.

**Myocardial glucose utilization.** AMPK regulates cardiac glucose utilization at several levels (Fig. 1). AMPK stimulates glucose uptake (106), glycolysis (92), and possibly glycogen metabolism (21). Similar to what has been observed in skeletal muscle, activation of cardiac AMPK promotes the translocation of GLUT4 to the sarcolemma of the myocyte in an insulin-independent manner (13, 106, 142). However, it is also possible that AMPK activation causes GLUT4 to be retained in the sarcolemma, thus increasing overall GLUT4 levels in the plasma membrane (142). Although the mechanism by which AMPK activation promotes increased GLUT4 levels in the plasma membrane is not clearly defined, there is evidence that this may be mediated, at least in part, by nitric oxide (79). That is, pharmacological inhibition of endothelial nitric oxide synthase and the downstream cGMP pathway partially inhibits 5-amino-4-imidazole-1-carboxamide ribofuranoside (AICAR)-induced GLUT4 translocation to the plasma membrane (79). In contrast to this study, nitric oxide donors have been shown to inhibit AMPK activity and GLUT4 translocation to the plasma membrane in ischemic canine myocardium (77). Whereas the involvement of nitric oxide in the regulation of AMPK-mediated GLUT4 translocation to the plasma membrane is controversial, there are likely additional pathways involved that have yet to be identified.

Regardless of the exact mechanism, elevated levels of GLUT4 in the sarcolemma is likely to increase glucose uptake, and this increased intracellular glucose has the potential to provide elevated substrate for glycolysis. In addition to simply providing additional substrate for glycolysis, AMPK activation promotes the production of ATP by glycolysis (93) through the direct phosphorylation and activation of phosphofructokinase (PFK) 2 (92). This enzyme produces fructose 2,6-bisphosphate, which is a potent stimulator of glycolysis. Together, both direct and indirect activation of glycolysis by AMPK further increases the supply of pyruvate for glucose oxidation (36).

As previously mentioned, when the energy demands of the heart are fulfilled, excess glucose can be stored in the form of glycogen. This process is regulated primarily by the enzyme glycogen synthase. AMPK has been shown to inhibit glycogen synthase activity in vitro (21, 136), and it is expected that
during times of diminished ATP levels there is a reduction in intracellular glycogen content in vivo. The effect AMPK may have on glycogen synthase would ultimately increase ATP supply by directing glucose to energy-producing pathways. Despite this rationale and in vitro evidence showing that AMPK activation may inhibit glycogen synthesis, in vivo data do not necessarily support this function for AMPK in the heart (6, 14, 83). Indeed, glycogen levels are lower or not changed in the transgenic hearts possessing impaired AMPK activity (the K54R α2 or the D157A α2 transgenic mice, respectively) compared with hearts from wild-type mice (96, 107). In addition, both gain- and loss-of-function mutations associated with Wolff-Parkinson-White syndrome increase the glycogen content of the heart (discussed in Regulation of Glycogen Storage and Wolff-Parkinson-White Syndrome), providing further evidence that the precise role of AMPK in glycogen metabolism is unknown.

**Regulation of Cardiac Myocyte Protein Synthesis**

Another mechanism by which AMPK can compensate for reduced ATP levels in the cardiac myocyte is the repression of energy-consuming metabolic processes that are not required for immediate cell survival. The inhibition of nonessential energy-consuming pathways in an energetically compromised heart would have beneficial effects since ATP could be directed toward essential cellular processes such as contractile function and ion homeostasis (65). One such nonessential energy-consuming process, at least in the short term, is protein synthesis. Early reports showed that oxygen deprivation and the resulting decrease in intracellular ATP inhibits protein synthesis (62). This now has been linked to the activation of AMPK and the ability of AMPK to regulate the activity of key enzymes controlling protein synthesis such as the tuberous sclerosis complex-2 (60), and eukaryotic elongation factor-2 kinase (eEF2k) (18). Because of the direct regulation of AMPK to these master regulators of cell growth and protein synthesis, AMPK activation has also been shown to indirectly regulate this process through downstream effects on several important molecules involved in protein synthesis, including mammalian target of rapamycin (mTOR) (16, 70), p70 S6 kinase (p70S6K) (23), eIF4E-binding proteins (4E-BP1) (67), and eukaryotic elongation factor-2 (eEF2) (18, 23, 56). Together, these data support the concept that AMPK activation inhibits protein synthesis and thus may be one mechanism by which cellular ATP is conserved for more essential cellular processes. Whereas the relative importance of inhibiting protein synthesis in the healthy heart is unlikely to play a major role in maintaining mechanical function, AMPK-mediated control of protein synthesis may be more relevant in chronically/severely stressed hearts.

**ROLE OF AMPK IN DISEASED HEARTS**

**Regulation of Myocardial Energy Utilization**

Many cardiovascular-related disorders, such as pathological cardiac hypertrophy, heart failure, myocardial ischemia, diabetic cardiomyopathy, and lipotoxic heart disease (see Refs. 84, 88, and 122 for reviews) are associated with alterations in cardiac energy metabolism. Since AMPK is central to the regulation of cardiac energy metabolism, the regulation of AMPK may be important in these various pathological settings. For example, AMPK activation may be essential for adaptation of cardiac energy metabolism to certain disease states and/or extreme (or less physiological) energetic stresses. However, it is also possible that this adaptive process may become maladaptive in certain situations or that in some circumstances AMPK activation may actually contribute to cardiac damage (Fig. 2). Furthermore, the dysregulation of AMPK associated with mutations in the γ2-subunit may contribute to the progression of Wolf-Parkinson-White syndrome (Fig. 3). These concepts are reviewed below.

**Role of AMPK in myocardial ischemia-reperfusion injury.** Both AMPK α1- and α2-subunits are activated during myocardial ischemia, though α2 appears to be activated to a greater extent (78, 107, 110). Interestingly, the activation of AMPK during ischemia is not dependent on which γ-subunit is contained within the AMPK heterotrimeric complex since both γ1- and γ2-containing complexes are activated to a similar extent (78). Clues to the role of AMPK activation during ischemia-reperfusion have been provided by the analysis of transgenic mouse lines (summarized in Table 1). Previous reports of the targeted deletion of either the AMPK α1- or α2-subunits did not reveal an obvious cardiac phenotype under a normal cardiac workload (63, 131). However, ex vivo perfused hearts from the AMPK α2 K45R transgenic mouse failed to increase glucose uptake in response to ischemia-reperfusion (107). This likely occurs due to insufficient GLUT4 translocation to the plasma membrane. A similar finding using AMPK α2 D157A mutant transgenic mice also showed that decreased AMPK α2 activity resulted in a partial inhibition of cardiac glucose uptake following ischemia (139). In support of the beneficial role of AMPK in ischemia-reperfusion, AMPK has been suggested to be necessary for adiponectin to mediate a component of its protective effect against ischemia-reperfusion injury (115). In addition, AMPK may activate downstream pathways that have cardioprotective roles during ischemia-reperfusion. Indeed, AMPK has been shown to activate p38-MAPK in the cardiac myocyte, which may also contribute to increased glucose uptake (80, 104, 127). These observations are consistent with an AMPK-mediated increase in glucose utilization being beneficial during and following ischemia.

In contrast to the evidence demonstrating that AMPK activation is beneficial during ischemia, it has been suggested that AMPK activation could be detrimental to postischemic recovery (39). During ischemia, oxidative metabolism is inhibited while glucose uptake is increased and glycolysis (nonoxidative ATP production) is accelerated (106). Upon reperfusion following mild ischemia or short periods of severe ischemia, contractile function can recover once energy production has been restored. Although many mechanisms contribute to ischemic injury, the extent of recovery is dependent, at least in part, on the type of substrate metabolized by the heart upon reperfusion (Fig. 2). Circulating fatty acid levels are elevated during ischemia (74, 85, 97), and it has been shown that high plasma fatty acid levels are detrimental to both the ischemic and the reperfused ischemic heart (53, 86, 88, 103). During myocardial ischemia, AMPK is activated, which leads to the phosphorylation and inhibition of ACC (72, 73), increased GLUT4 levels in the sarcolemmal membrane (142), and the activation of PFK-2 (73, 106, 108, 109). The overall effect of activated AMPK is to increase ATP supply by accelerating the rates of
fatty acid oxidation and glycolysis. However, high rates of fatty acid oxidation dramatically inhibit glucose oxidation rates via the Randle cycle (102). That is, fatty acid-derived acetyl CoA is able to decrease the production of glucose-derived acetyl CoA via inhibition of the pyruvate dehydrogenase complex (66). This ultimately causes an imbalance between glucose oxidation and glycolysis and, as a result, lactate as well as protons can accumulate (35, 54). On the basis of this correlation, it was originally proposed by the group from Gary Lopaschuk’s laboratory (73) that ischemia-induced activation of AMPK sets in motion a series of metabolic events that are detrimental to recovery of contractile function during reperfusion.

Whereas the evidence from transgenic animals appears to disprove the notion that AMPK activation during ischemia is detrimental to functional recovery postischemia, it is important to note that the AMPK/H92512 D157A transgenic mouse hearts were perfused in the absence of fatty acids (139) or, in the case of the AMPK/H92512 K45R transgenic mice, with significantly lower concentrations of fatty acids than are observed in vivo.

Fig. 2. Cardiac metabolism during ischemia-reperfusion and the beneficial and potential detrimental effects of AMPK. Circulating fatty acids are elevated in the ischemic setting (1). AMPK is activated during ischemia and promotes the uptake of fatty acids via increased translocation of CD36 to the plasma membrane (2). Increased AMPK activity also increases fatty acid oxidation by phosphorylating and inhibiting ACC (3), which relieves the inhibition of CPT1 by malonyl CoA. Since fatty acid oxidation is intimately linked to glucose oxidation, these high rates of fatty acid oxidation reduce glucose oxidation by inhibiting the pyruvate dehydrogenase complex (4). Although the role of AMPK activation during ischemia has not been clearly defined, AMPK activation may be beneficial in ischemia due to increased GLUT4 translocation to the plasma membrane (5) and the potential inhibition of glycogen synthesis (6), which increases glucose supply for ATP production via glycolysis (7). However, the hydrolysis of glycolytically derived ATP in the absence of glucose oxidation results in an accumulation of protons and lactate that may cause cardiac dysfunction during reperfusion (8). This suggests that AMPK activation may potentially be harmful to the reperfused, ischemic heart.

Fig. 3. Proposed effects of the γ2-subunit mutations on AMPK activity and glycogen accumulation. A: γ2-subunit mutations that inactivate AMPK could increase glycogen synthase activity (1) and suppress glycolytic rates (2). However, because of other mechanisms, GLUT4 localization at the plasma membrane may not be affected. Together, this would result in the inhibition of glucose utilization and the redirection of glucose to glycogen synthesis (3). B: γ2-subunit mutations that activate AMPK could promote excessive GLUT4-mediated glucose uptake (1) that exceeds elevated glycolytic rates (2). The resulting increased level of glucose-6-phosphate may lead to the excess glucose-6-phosphate being stored as glycogen despite diminished glycogen synthase activity (3). The net effect would be increased glycogen accumulation (4).
during ischemia (107). Therefore, with the use of these experimen-
tal conditions, the heart relies more heavily on glucose as a 
source of energy than it would normally following ischemia 
(87). This may provide one explanation for why the putative 
detrimental effects of AMPK-mediated increased fatty acid 
oxidation rates following ischemia were not observed in these 
studies. However, irrespective of the fatty acid content of the 
perfusate in the study by Russell et al. (107), the AMPK α2 
K45R transgenic mice did display greater tissue injury and 
increased apoptosis following ischemia (107). As mentioned, 
this model it is possible that the ability of AMPK to prevent 
cardiac injury is related to the AMPK-mediated glucose uptake 
and glycolytic flux following ischemia. This suggests that 
enhanced glucose uptake by AMPK may protect against ische-
mia-reperfusion injury if the potential negative consequences 
of increased fatty acid oxidation are not present. Overall, from 
these studies, it is obvious that more research in the area is 
needed before we fully understand whether AMPK activation 
is beneficial or detrimental to the ischemia-reperfusion heart.

Role of AMPKKs in myocardial ischemia-reperfusion. 
ALTHOUGH the consequences of ischemia-induced activation of 
AMPK are still unclear, there is consensus that AMPK is 
significantly phosphorylated and activated during myocardial 
ischemia (11, 72, 78). Once LKB1 was identified as an 
AMPKK, it was hypothesized that LKB1 would also be activ-
vated during ischemia. However, in one report it was shown 
that myocardial ischemia regulates AMPK independently of 
phosphorylation by LKB1 (4). Consistent with these data, 
others found that LKB1 was not activated by ischemia yet both 
AMPKα1 and α2 activities were increased (110). Moreover, in 
the skeletal muscle, activation of AMPK by phenformin, 
AICAR, and contraction does not involve stimulation of LKB1 
activity (49). Because LKB1 activity is not activated by ische-
mia or several other stimuli that activate AMPK, it is likely that 
LKB1 is constitutively active. It has been suggested that under 
conditions of depleted ATP and increased AMP, binding of 
AMP to the AMPK γ-subunit causes a conformational change 
that permits enhanced phosphorylation of the AMPK α-subunit 
by the constitutively active LKB1 (110). Interestingly, it has 
recently been shown in LKB1 null hearts that ischemia failed 
to induce AMPK α2 activity (via Thr-172 phosphorylation) but 
did increase AMPK α1 activity and Thr-172 phosphorylation 
(110). Similar regulation was observed in anoxic LKB1-defi-
cient mouse hearts (110). Therefore, LKB1 appears to be a 
necessary in vivo kinase of AMPK α2 but not AMPK α1 in the 
heart during myocardial ischemia. Whether this finding was 
specific to the cardiac myocyte was also examined. That is, the 
absence of LKB1 in hearts from these mice expressing Cre 
recombinase from the muscle creatine kinase promoter would 
be restricted to cardiac myocytes. Since the heart contains 
multiple cell types including endothelial cells that express only 
the α1-subunit (112), the contribution of LKB1 to AMPK α1 
activity in endothelial cells during ischemia may be responsible 
for the observed activation of the α1-subunit in the whole heart 
homogenates. However, α1 phosphorylation persists in hy-
poxic myocytes isolated from LKB1 null hearts, thereby ex-
cluding endothelial contribution (110). Taken together, these 
data suggest that LKB1 is the major regulator of AMPKα2 
activity during myocardial ischemia and another AMPKK may 
be responsible for the activation of AMPKα1 during cardiac 
ischemia. One likely candidate is the CaMKK, though at 

present little is known about the role of CaMKK in the heart 
during myocardial ischemia (51, 59, 137). Future studies should 
help reveal the potential roles of alternative AMPKKs.

Regulation of Cardiac Myocyte Protein Synthesis 
CHRONICALLY increased cardiac workload can lead to cardiac 
hypertrophy (68). Cardiac hypertrophy is associated with sev-
eral molecular alterations, including changes in gene expres-
sion, increased mRNA translation to promote protein synthesis, 
increased myocyte size, and altered energy metabolism (120). 
During the development of pressure or volume overload-
induced hypertrophy, cardiac energy metabolism is often dra-

matically altered such that overall oxidative metabolism (glu-
cose and fatty acid oxidation) becomes reduced and glycolysis 
is increased (2, 3, 82, 132). These metabolic changes have been 
attributed to a downregulation of metabolic genes involved in 
fatty acid utilization and an upregulation of certain genes 
involved in glucose uptake and the glycolytic pathway. As 
mentioned, AMPK has also been proposed to be responsible 
for increased glycolysis, directly via PFK-2 regulation (101). 
This switch to a more glycolytic metabolism appears to be an 
adaptive response to chronic reduction in myocardial perfusion 
resulting from increased myocyte cell size (76). However, as 
hypertrophy progresses, a reduction in fatty acid oxidation may 
eventually deprive the heart of energy by decreasing ATP 
generation from a major source of energy, namely fatty acids. 
Whether AMPK activation is involved in an adaptive or a 
maladaptive switch in myocardial energy metabolism during 
hypertrophy is currently unknown.

Whereas AMPK appears to play a role in the regulation of 
cardiac energy metabolism in the hypertrophic heart, AMPK 
also has been suggested to regulate other mediators of hyper-
trophy such as protein synthesis (16, 18, 23). However, the 
precise role of AMPK in regulating cardiac myocyte growth 
during hypertrophy remains controversial. For example, some 
studies have presented a correlation between AMPK activation 
and the development of cardiac hypertrophy (6, 126), whereas 
other studies show that AMPK activation can inhibit hypertro-
phic growth (23, 114). One mechanism we have explored is the 
role of AMPK in Akt-induced hypertrophy. Chronic activation 
of Akt in transgenic mice promotes the development of cardiac 
hypertrophy (100, 116), and it is thought that Akt promotes 
cardiac hypertrophy through several mechanisms, including 
activation of the mTOR pathway and protein synthesis (60). 
This is particularly interesting since we (23) and others (18) 
have shown that AMPK inhibits proteins synthesis and Akt 
activation can negatively regulate AMPK activity in the heart 
(69). Based on this, we hypothesize that one mechanism by 
which Akt could promote hypertrophic growth is to inactivate 
AMPK to promote protein synthesis (23). In support of this 
concept, we found that pharmacological activation of AMPK 
attenuates cardiac hypertrophic growth and protein synthesis 
induced by constitutively active Akt (23). Despite the ability of 
AMPK activation to prevent cardiac hypertrophy, our previous 
data do not support the concept that AMPK is a necessary 
component of the hypertrophic signaling mechanisms. In fact, 
the various mouse models with impaired AMPK activity have 
normal or smaller heart weights compared with wild-type 
hearts (63, 107, 110), which argues against inactivation of 
AMPK being essential for hypertrophic growth. However, it is
not known whether a decrease in AMPK activity makes the heart more susceptible to hypertrophic growth stimuli. Indeed, recent work has shown that AMPK may play a more direct role in the hypertrophic process, at least in the adiponectin-mediated effects on cardiac hypertrophy. Shibata et al. (114) have shown that adiponectin-deficient mice have diminished AMPK signaling and are more susceptible to pressure-overload-induced cardiac hypertrophy (114). Moreover, administration of adiponectin reversed the severity of cardiac hypertrophy and restored AMPK signaling, whereas expression of a dominant negative mutant of AMPK reversed the beneficial effects of adiponectin (114). Therefore, it appears that AMPK is necessary for adiponectin to inhibit cardiac hypertrophy. Whether this apparent direct role of AMPK in the hypertrophic process in adiponectin-deficient mice is consistent in all models of cardiac hypertrophy is as yet unknown. Finally, it is possible that either adiponectin and/or other activators of AMPK such as metformin or thiazolidinediones may be used to prevent hypertrophic growth. However, in the case of metformin, the doses used to activate AMPK in cell culture are significantly higher than those seen clinically. To fully explore the utility of AMPK activators for the prevention of cardiac hypertrophy, more specific activators of AMPK are necessary. A recent report (28) has identified a small molecule activator of AMPK and has characterized some of its effects. This molecule may be useful in testing the role of AMPK activation in the setting of cardiac hypertrophy.

**Regulation of Glycogen Storage and Wolff-Parkinson-White Syndrome**

Naturally occurring mutations in the human γ2-subunit of AMPK have been identified and appear to be involved in the development of a hypertrophic cardiomyopathy associated with excessive glycogen storage that results in the development of Wolff-Parkinson-White syndrome (15, 45, 90). Indeed, patients with a familial form of Wolff-Parkinson-White syndrome resulting from mutations in the AMPK γ2 gene display characteristic conduction system abnormalities that have been proposed to develop due to altered AMPK activity. That is, perturbations in AMPK activity appears to be responsible for excessive glycogen deposition within the cardiac myocyte and ostensibly, it is these glycogen-filled myocytes that form bypass tracts that lead to the development Wolff-Parkinson-White syndrome (44). Although this appears to be the most likely mechanism by which conduction system abnormalities arise in these patients, one cannot rule out the potential effects that alterations in AMPK activity may have on ion channel activity (47). For example, we have shown that AMPK activation increases hH1 sodium channel activity in cardiac myocytes (81), suggesting that other ion channels may also play a role in the development Wolff-Parkinson-White syndrome in patients that possess mutations in the AMPK γ2 gene.

The phenotypes of transgenic mouse lines expressing these mutations in the γ2-subunits of AMPK in a heart-specific manner were consistent with the cardiac hypertrophy and glycogen accumulation associated with Wolff-Parkinson-White syndrome in humans with mutations in the γ2-subunit (6, 32, 117). Interestingly, control transgenic mice expressing the nonmutated γ2-subunit with no changes in AMPK activity also developed increased glycogen deposition, suggesting that the presence of higher levels of the γ2-subunit alone has effects on the cardiac myocyte (6, 32). Considerable controversy regarding the role of AMPK activity in the progression of glycogen storage and the development of Wolff-Parkinson-White syndrome in these transgenic mice remains. In vitro analysis showed that a number of these naturally occurring mutations in the human γ2-subunits (R302Q, H383R, R531G, and an insertion of a leucine residue following amino acid 350) do not create constitutively active AMPK enzymes but do alter the ability of AMP to stimulate AMPK activity (31). Adding further confusion, transgenic mice expressing two different mutations in the γ2-subunit appear to have opposing effects on AMPK activity in vivo. That is, the N488I mutation was reported to increase AMPK activity compared with wild-type transgenic or nontransgenic mice (6), whereas the R302Q mutation reduced AMPK activity (117). Furthermore, transgenic mice expressing the N488I γ2 mutation have diminished AMPK activation in response to ischemic stress (146). Whereas it has not yet been reconciled how opposite effects on AMPK activity can produce almost identical phenotypes in the various transgenic mouse models, it is likely that alterations in the activity of AMPK complexes containing γ2 mutations lead to compensatory changes in glucose handling. Theoretically, AMPK-activating mutations could promote glycogen accumulation through increased glucose uptake that exceeds glucose utilization (Fig. 3), whereas AMPK-inactivating mutations may increase glycogen deposition by directly increasing glycogen synthesis activity (Fig. 3). Experimental evidence for these hypotheses will require further in vitro and in vivo experimentation.

Recently, a third transgenic mouse line harboring the R531G mutation in the γ2-subunit of AMPK was created. These mice develop a progressive cardiac phenotype whereby cardiac function appears normal at 1 wk of age, but by 4 wk of age, cardiac hypertrophy and significant glycogen accumulation is present (32). Compared with wild-type and nontransgenic mice, the R531G mutant mice had similar AMPK activities at 1 wk of age, but the AMPK activity was substantially reduced by 4 wk of age due to decreased phosphorylation at Thr-172 of AMPK (32). Taken together, these results suggest that the pathogenesis of Wolff-Parkinson-White syndrome is not a simple loss or gain of function mutation since the altered AMPK activity in these mice develops gradually and likely occurs subsequent to glycogen accumulation (32). Therefore, dysregulation of AMPK activity appears to contribute to the development of Wolff-Parkinson-White syndrome, though detailed studies will be required to fully assess what effects the γ2 mutations and glycogen have on AMPK activity in the other transgenic models involving mutations in the γ2-subunit of AMPK.

**Conclusions**

Since the heart works even when the body is at rest, an efficient and highly sensitive signaling system that regulates the conversion of metabolic substrates to ATP must be present so that sufficient energy is constantly produced. AMPK signaling coordinates multiple metabolic pathways, such as fatty acid and glucose utilization (Fig. 1). Because of this role, AMPK allows the heart to maintain proper ATP supply during times of metabolic stress. Whereas the beneficial effects of
AMPK activation during mild and/or acute energetic stresses are not in question, it is unclear whether AMPK activation is beneficial or detrimental to the heart during situations of severe and/or chronic energetic stress such as that observed during myocardial ischemia. Furthermore, the precise role of AMPK in certain cardiopathological conditions such as cardiac hypertrophy is also unknown. That is, there is conflicting data as to whether AMPK causes or prevents cardiac hypertrophy. Finally, though it is clear that AMPK α2-subunit mutations contribute to Wolff-Parkinson-White syndrome, it remains to be established how these mutants alter AMPK activity and the mechanism by which this leads to glycolysis accumulation. Additional characterization of mouse models that alter AMPK activity (Table 1) in the ischemic and hypertrophic settings and further studies involving the transgenic mice expressing the γ2 mutations should help to resolve these issues. It is anticipated that future studies will refine the metabolic signals that control AMPK activity, identify novel AMPK targets, fully characterize the role of the existing AMPKKs in the regulation of AMPK (and possibly identify new AMPKKs), as well as fully characterize the mechanisms underlying glycolysis accumulation in hearts of the mice expressing the various γ2 mutations. Whereas considerable research progress has been made in the understanding of the metabolic roles and regulation of cardiac AMPK, much effort is still required to fully elucidate its roles in ischemia-reperfusion injury, cardiac hypertrophy, glycolytic storage-cardiomyopathy, and Wolff-Parkinson-White syndrome.

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