AMP-Activated Protein Kinase in the Heart: Role During Health and Disease

Michael Arad, Christine E. Seidman, J.G. Seidman

Abstract—AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme that is expressed in most mammalian tissues including cardiac muscle. Among the multiple biological processes influenced by AMPK, regulation of fuel supply and energy-generating pathways in response to the metabolic needs of the organism is fundamental and likely accounts for the remarkable evolutionary conservation of this enzyme complex. By regulating the activity of acetyl–coenzyme A carboxylase, AMPK affects levels of malonyl–coenzyme A, a key energy regulator in the cell. AMPK is generally quiescent under normal conditions but is activated in response to hormonal signals and stresses sufficient to produce an increase in AMP/ATP ratio, such as hypoglycemia, strenuous exercise, anoxia, and ischemia. Once active, muscle AMPK enhances uptake and oxidative metabolism of fatty acids as well as increases glucose transport and glycolysis. Data from AMPK deficiency models suggest that AMPK activity might influence the pathophysiology and therapy of diabetes and increase heart tolerance to ischemia. Effects that are not as well understood include AMPK regulation of transcription. Different AMPK isoforms are found in distinct locations within the cell and have distinct functions in different tissues. A principal mode of AMPK activation is phosphorylation by upstream kinases (eg, LKB1). These kinases have a fundamental role in cell-cycle regulation and protein synthesis, suggesting involvement in a number of human disorders including cardiac hypertrophy, apoptosis, cancer, and atherosclerosis. The physiological role played by AMPK during health and disease is far from being clearly defined. Naturally occurring mutations affecting the nucleotide-sensing modules in the regulatory γ subunit of AMPK lead to enzyme dysregulation and inappropriate activation under resting conditions. Glycogen accumulation ensues, leading to human disease manifesting as cardiac hypertrophy, accessory atrioventricular connections, and degeneration of the physiological conduction system. Whether AMPK is a key participant or bystander in other disease states and whether its selective manipulation may significantly benefit these conditions remain important questions. (Circ Res. 2007;100:474-488.)

Key Words: AMPK ■ glycogen ■ metabolism ■ cardiomyopathy

AMP activated protein kinase (AMPK) is a highly conserved heterotrimeric enzyme that is expressed in most mammalian tissues, including heart muscle. Evolutionary conservation of its subunits in eukaryotes, from yeast to plants to humans, indicates fundamental biological functions that must confer significant benefits.1–3 Often described as an energy gauge, AMPK participates in regulation of fuel supply and energy-generating pathways in response to the metabolic needs of organ systems including liver, central nervous system, fat tissue and striated skeletal and cardiac muscles.4,5 Until recently, the roles of AMPK in physiology and human disease were underappreciated. The past decade has witnessed an exponential increase in studies of the roles of AMPK in normal stress response and pathophysiological
TABLE 1. Genetically Engineered Mice Carrying Mutant AMPK Alleles

<table>
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<tr>
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<td>Knockout mice</td>
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<td>γ3−/−</td>
<td>PRKAG3</td>
<td>γ3</td>
<td>K0</td>
<td>Endogenous</td>
<td>Decreased post-exercise glycogen resynthesis</td>
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<td>α1−/−</td>
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<td>α1</td>
<td>K0</td>
<td>Endogenous</td>
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<td>PRKAA2</td>
<td>α2</td>
<td>K0</td>
<td>Endogenous</td>
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<td>Transgenic mice (dominant negative)</td>
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<td>Tgα2 (PRKAA2)</td>
<td>α2</td>
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<td>Creatine Kinase*</td>
<td>Decreased glucose uptake and ischemic tolerance</td>
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<td>γ2</td>
<td>PRKAG2</td>
<td>R302Q</td>
<td>αMHC</td>
<td>PRKAG2 cardiomyopathy; glycogen storage</td>
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<td>Transgenic mice (skeletal muscle)</td>
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<td>MUTγ3</td>
<td>γ3</td>
<td>PRKAG3</td>
<td>R225Q</td>
<td>Creatine* kinase</td>
<td>Hampshire pig RN(−); increased muscle glycogen</td>
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<td>MUTγ3</td>
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<td>Myofibril protein</td>
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<td>MUTγ1</td>
<td>γ1</td>
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<td>Skeletal muscle; ACTA1</td>
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KO indicates knockout; RN, Rendement Napole. *Cardiac expression as well as skeletal muscle expression.

states. This expanding research field and multiple excellent reviews on AMPK4–8 are stimulated in part by the discovery of gene mutations in the subunits of AMPK that trigger animal and human diseases characterized by glycogen storage, evidence that AMPK plays can mitigate against ischemic damage, apoptosis and possibly atherosclerosis, and advances in understanding mammalian carbohydrate metabolism, which encourages speculation that AMPK regulation may provide new opportunities for diabetes therapies.

This review focuses on the important roles that AMPK plays in normal cardiac physiology and in cardiac disease states. Although cardiomyocytes contain large quantities of AMPK, the contribution of AMPK to normal cardiac function when ATP is abundant and the enzyme is inactive, or during aerobic exercise, remains uncertain. A clearer role has emerged for the function of AMPK in cardiac disease. AMPK is activated during clinically important pathological states such as cardiac hypertrophy and ischemia; whether the enzyme functions in protective processes or serves to exacerbate disease process is disputable and may be disease dependent. Discovery that human mutations of the γ subunit, encoded by PRKAG2, cause human cardiomyopathy, characterized by hypertrophy and glycogen accumulation, has stimulated investigations directed toward elaboration of the role of AMPK in cardiac metabolism by studies of genetically engineered mice (Table 1). In addition, considerable information has accrued from analyses of knockout mice lacking α1, α2, or γ3 AMPK subunit genes (Table 1). Although initial data regarding AMPK function in these model organisms led to conflicting mechanisms for cardiac disease, these discrepancies have now been largely resolved.

Here, we attempt to define the role of AMPK in the heart using insights drawn from clinical phenotype of patients with mutant AMPK and genetically engineered mice carrying AMPK mutations. Using experimental data from both cardiac and skeletal muscle, we offer an integrated picture of cardiac AMPK in health and disease.

AMPK Structure and Function

AMPK regulates energy metabolism by phosphorylation of key enzymes in metabolic pathways, such as acetyl–coenzyme A carboxylase (ACC) or mTOR (mammalian target of rapamycin), by modulating their activities, and by regulating the activity of transcription factors and transcriptional cofactors. Elucidation of the physiological role of AMPK began nearly 25 years ago, when Kim and coworkers9 described the regulation of ACC by phosphorylation and AMP. Experiments conducted by Hardie, Carling, and colleagues10–13 defined the specific activity of AMPK, substrate specificity, and the role of the enzyme in hepatic lipid metabolism. AMPK regulates carbohydrate and lipid metabolism in muscle tissue, by controlling uptake and catabolism for energy generation.14–16 These studies were complemented by extensive analyses of the yeast AMPK homolog, the sucrose nonfermenting protein 1 (SNF1)/SNF4 complex, which plays a critical role in cell adaptation to glucose-free environment. In yeast, SNF1/SNF4 mediates its activity through modulation of gene transcription, whereas, in mammalian cells, AMPK regulates metabolism both by regulating gene transcription and direct control of metabolic enzymes.

AMPK is composed of a catalytic α and regulatory β and γ subunits. Each α subunit contains a phosphorylation site (threonine 172) that plays a critical role in regulating AMPK function. The β subunit has scaffold-like properties, as well myristoylation, phosphorylation, and glycogen-binding sites17–19 (Figure 1). Each γ subunit comprises 4 CBS sequences, a motif named after cystathionine β-synthase, which is found in a wide variety of proteins. Each pair of CBS sequences forms a nucleotide-binding module called the Bateman domain, modules that bind adenosyl compounds. Each γ subunit contains 2 nucleotide-binding motifs capable of cooperative binding of 2 molecules of either AMP or ATP, thereby governing the interaction between γ and α subunits.20–22 AMPK is inhibited at high concentrations of ATP.
AMPK both protects the heart and exacerbates disease processes (discussed by Dyck and Lopaschuk). Activation by AMP mimetic, the AICA riboside (AICAR) or its intracellular metabolite ZPM (5-aminimidazole-4-carboxamide 1-beta-D-ribofuranoside), has been used to study AMPK activity in cultured myocytes, tissue preparations, and isolated perfused hearts. However, issues with AICAR penetration into cardiomyocytes and absence of stable cardiac cell lines required extrapolation from skeletal muscle studies, confounding efforts to understand cardiac AMPK function.

Because AMPK stimulation increases glucose uptake, AICAR administration to model organisms has produced hypoglycemia, evoking a neurohumoral response. AICAR, previously known as acadesine, was originally designed to increase intracellular adenosine. In addition to its cardioprotective effects, stimulation of adenosine has other consequences on cardiac functions. Collectively, these issues have complicated the interpretation of pharmacological manipulation of AMPK by AICAR.

Genetic studies have provided a complementary approach to biochemical studies of AMPK. Human polymorphisms in genes encoding AMPK subunits have been analyzed in populations with selected metabolic phenotypes. Case-control studies suggested an association between polymorphisms in the alpha2 subunit gene (PRKAA2) and glucose tolerance and blood cholesterol levels. Whether these polymorphisms alter AMPK activities, or indicate the consequences of another gene, is unclear. In contrast, Mendelian disorders have been characterized that reflect mutations only in the gamma2 subunit gene (PRKAG2). In humans, dominant PRKAG2 mutations cause cardiac hypertrophy associated with abnormal glycogen accumulation and conduction system disease. The clinical features of these mutations (Table 2) have suggested unexpected cardiac functions for AMPK. The development and study of genetically engineered animal models has further indicated that physiological activity of AMPK is critical for life-long normal heart functions.

### AMPK in Cardiac Metabolism

Although generally recognized as a cellular energy gauge (Figure 2), the precise role that AMPK plays in cardiac metabolism remain incompletely understood. Important questions include whether the various AMPK isoforms have unique roles that are relevant to particular metabolic state, identification of the full repertoire of cardiac protein targets of AMPK activity, and definition of the stimuli (such as increased AMP/ATP ratios) and kinases that activate AMPK. Studies of skeletal muscle AMPK have addressed some of these questions. Exercise, hypoxia, ischemia, and neurohumoral factors (leptin, adiponectin, and alpha-adrenoreceptor agonists) stimulate AMPK. Whether these factors mediate AMPK activity in the heart is under active investigation.
Two AMPK kinases have been identified in the heart, LKB1 and calmodulin-dependent protein kinase kinase.\textsuperscript{35,36} The serine/threonine kinase LKB1 appears to function in a myriad of cell processes including AMPK activation. LKB1 responds to anoxic stress by phosphorylating the AMPK/H\textsuperscript{9251} subunit at the Thr172 residue. Notably, this response is independent of AMP concentration.\textsuperscript{25} LKB1 activity as an AMPK kinase is also dependent on a number of associated proteins.\textsuperscript{35} During ischemia, LKB1 selectively phosphorylates the H\textsuperscript{9251} but not the H\textsuperscript{161} subunit, suggesting differential regulation and distinct physiological roles of various catalytic complexes and their upstream kinases.\textsuperscript{37} Calmodulin-dependent kinase kinase appears to be part of an independent regulatory pathway.

Nucleotides released from damaged tissue activate AMPK in endothelial cells through Ca\textsuperscript{2+}/calmodulin-dependent kinase kinase pathway but independently of AMP/ATP ratio.\textsuperscript{38}

Activation of AMPK can influence cardiac metabolism by regulating uptake and oxidative phosphorylation of fatty acids (reviewed by Dyck and Lopaschuk\textsuperscript{7}), the primary source of ATP in normal myocardium. AMPK phosphorylates acetyl-CoA carboxylase (ACC), which adds a carboxyl moiety to acetyl-CoA to yield the 3-carbon molecule malonyl-CoA. AMPK-mediated phosphorylation of ACC at serine residues (Ser79 in the hepatic isoform and Ser221 in muscle isoform) inactivates ACC. In the liver, malonyl-CoA is required for fatty acid synthesis. In the heart, malonyl-CoA as an inhibitor of carnitine palmitoyl transferase (CPT1), which is located at the outer mitochondrial membrane and is the rate-limiting step for internalizing and rendering fatty acids as substrates for oxidative phosphorylation. Hence activation of AMPK inhibits ACC and triggers a cascade of regulatory events that ultimately activate CPT1 and provide cardiac mitochondria with a preferred substrate for energy production: acyl carnitine.

This pathway is also regulated by malonyl-CoA decarboxylase (MCD), which degrades malonyl-CoA. There is controversy over whether or not MCD is activated during muscle contraction by AMPK.\textsuperscript{39,40} In vitro transfection studies by Sambandam et al\textsuperscript{41} suggest that this regulation occurs within mitochondria, rather than in the cytosol. In addition to preventing malonyl-CoA formation, AMPK appears to inhibit triglyceride synthesis in liver and muscle by interacting with glycerol-3-phosphate acyl transferase.\textsuperscript{42} Further evidence for the role of AMPK in stimulating cardiac fatty acid metabolism comes from analyses of individual cardiomyocytes. Addition of fatty acids to culture medium increases AMPK activity by several mechanisms. Targets of AMPK phosphorylation include PFK and ACC and may also include glucose transporter 4 (GLUT4).

**Physiological AMPK activation**

![Image of Physiological AMPK activation](https://example.com/ampkactivation_diagram.png)

**Figure 2.** AMPK increases ATP production in response to energetic stress via several mechanisms. Targets of AMPK phosphorylation include PFK and ACC and may also include glucose transporter 4 (GLUT4).
Stimulation of glucose is another established activity of AMPK, although the mechanism and physiological relevance of this activity are not well understood. AMPK stimulation of glucose uptake occurs via an insulin-independent mechanism. Early studies demonstrating that activated AMPK increased expression of glucose transporters, GLUT1 and GLUT4, suggested that GLUT4 is translocated from intracellular reservoirs to the sarcolemma to mediate AMPK effect on glucose uptake. Yang and Holman suggested that AMPK increases the sarcolemmal GLUT4 transporter number by decreasing endocytosis. The molecular mediators of these events and the specific phosphorylation targets of AMPK may include protein kinase C, endothelial nitric oxide synthase, and p38 mitogen-activated protein kinase/transforming growth factor-β–activated protein kinase 1–binding protein 1 (TAB1) complex. This AMPK-dependent pathway does not cross-communicate with the insulin–glycogen synthase kinase pathway and in fact may be mutually antagonistic. Insulin inactivates AMPK through Akt, and AMPK may inhibit insulin signaling. Alternatively, under pharmacological stimuli, AMPK can potentiate insulin signaling downstream protein kinase B, thereby helping to overcome insulin resistance.

The physiological importance of AMPK-mediated glucose uptake at rest and aerobic activity is not well defined. AMPK appears to control a different intracellular pool of transporters from that mobilized by insulin. The effect of insulin predominates at rest or during exercise, whereas the contribution of AMPK to glucose uptake and metabolism becomes central during oxidative stress, ischemia, and pathological hypertrophy.

AMPK stimulates glycolysis directly by activating phosphofructokinases, which provide the primary regulatory step in the glycolytic pathway. AMPK indirectly activates phosphofructokinase (PFK1) through its phosphorylation of phosphofructokinase 2 (PFK2), which converts fructose 6-phosphate to fructose-2,6-diphosphate, an allosteric stimulator of PFK1. The capacity of AMPK to activate PFK and increase glycolysis makes it an essential component in mounting the cellular response to energetic stress like anaerobic exercise, anoxia, and low-flow ischemia.

Finally, AMPK plays an important role in regulating glycogen metabolism. AMPK mutations affect glycogen metabolism, whereas primary defects in glycogen metabolism (eg, muscle phosphorylase [GP] deficiency, glycogen storage disease type II, or McArdle disease) activate AMPK while decreasing glycogen synthase (GS) activity. Carling and Hardie suggested that GS and phosphorylase kinase might be phosphorylated by AMPK. AMPK phosphorylates GS at Ser7 and inhibits it in skeletal myoblasts, rendering it glucose-6-phosphate (G-6-P) dependent. Paradoxically, repeat AICAR administration resulted in increased glycogen. The issue could be settled if AICAR increased the level of G-6-P, thereby overcoming GS inhibition. In another study, AICAR inhibited GS and activated GP in white but not red gastrocnemius in vivo. These effects could not be reproduced in vitro, which led these authors to conclude that the observed increase in glycogen was attributable to increased glucose uptake but not altered GS or GP. In isolated rat hearts, AMPK-activator AICAR increased glycogen breakdown but did not affect GS or GP activity, leaving the mechanism unknown. Hence, the pathways by which AMPK controls glycogen synthesis and breakdown and the precise phosphorylation targets remain to be elucidated. Whether AMPK mediates this action by controlling transcription or by controlling glycogen metabolism directly is also not known.

Although many studies have focused on the role of AMPK as a direct mediator of intermediary metabolism, AMPK also mediates transcription of genes involved in lipid and glucose metabolism. Recurrent muscle AMPK stimulation reproduces the effect of exercise training by increasing the expression of Glut-4 transporter, hexokinase II, mitochondrial biogenesis, and other mitochondrial enzymes (reviewed by Jorgensen et al). An important mediator of many of these effects is peroxisome proliferator–activated receptor-γ (PPARγ) coactivator 1α (PGC-1α), which increases expression of transcription factors: nuclear respiratory factors 1 and 2, mitochondrial transcription factor A. and PPARα. Importantly, each of these has its own downstream targets. For instance, the PPARα controls the genes involved in lipid metabolism.

The target genes regulated by AMPK in cardiac muscle are undefined at present. Recent evidence indicates that AMPK-mediated modification of gene expression leads to cardiac protection from anoxia and ischemia. Decrease in protein synthesis through eEF2 phosphorylation affords better tolerance to hypoxia-induced stress. The late phase of ischemic preconditioning involves increased expression and translocation of Glut-4, which appears to be related to PKC-dependent AMPK activation. Presumably α2-associated AMPK mediates this activity because the predilection of the α2 subunit for nuclear localization. Therefore, the role of AMPK as transcriptional mediator may be particularly important in the human heart, where α2 is much more abundant than α1 subunit. Future studies will be required to distinguish the roles of AMPK as a direct regulator of cardiac metabolism and as a mediator of cardiac transcription.

### Mutations in the Human AMPK Regulatory γ Subunit Cause Glycogen Storage Cardiomyopathy

For many years, individuals with unexplained left ventricular hypertrophy have been diagnosed with familial hypertrophic cardiomyopathy (HCM). HCM is typically caused by autosomal dominantly inherited mutations in the proteins participating in sarcomere assembly. This inherited cardiac disease is characterized by unexplained left ventricular hypertrophy, small left ventricular cavity, hyperdynamic contraction, and systolic anterior motion of mitral valve causing dynamic outflow obstruction. Patients may be asymptomatic or experience chest pain, shortness of breath, congestive heart failure, syncope, and malignant ventricular arrhythmia. The classic histopathologic features are hypertrophied and disarrayed myofibers separated by islets of replacement fibrosis. Approximately 5% to 10% of HCM patients, diagnosed by increased left ventricular wall thickness on their echocardiogram, have an unusual ECG pattern suggestive of preexista-
tion (abnormal activation of the ventricles bypassing the atrioventricular node). Although a few investigators suggested that these individuals had Wolf–Parkinson–White (WPW) syndrome, many electrophysiologists believed that individuals with left ventricular hypertrophy and QRS complexes reminiscent of delta waves on their ECG had no real cardiac preexcitation caused by accessory atrioventricular connections.78

Familial association between WPW pattern and left ventricular hypertrophy was described in 1995 in a large family in which the disease was inherited as an autosomal dominant trait and linked to a locus on chromosome 7q3.79 Many of the affected family members had delta waves on ECG and/or preexcitation exposed by adenosine test or experienced symptomatic conduction system disease: sick sinus syndrome or atrioventricular block. A separate study of another family with the same disease80 determined the electrophysiological characteristics of conduction system abnormalities. Delta waves correlated with distinct accessory pathways, which allowed for bidirectional conduction, inducibility of orthodromic atrioventricular reentry tachycardia. The accessory pathways could be treated by catheter ablation. Most individuals with the new syndrome had multiple accessory pathways, a unique feature rarely observed in sporadic WPW. Sick sinus and atrioventricular block often necessitated pacemaker implantation.

The molecular pathogenesis of the new type of cardiomyopathy remained an enigma until 2001, when 2 groups81–83 reported 4 mutations in the PRKAG2 gene, responsible for disease in 5 different families (Table 2). Subsequently, 6 additional families harboring 3 different mutations were reported,63 including a novel Asn488Ile mutation in the family in which this syndrome was originally defined.79 Distinct clinical features are associated with PRKAG2 mutations (summarized in Table 2); however, considerable phenotypic variability exists within and between different mutations. For example, the most common cardiac manifestation is left ventricular hypertrophy, which is found in up to 80% of genetically affected individuals. Hypertrophy may be concentric or asymmetric, may cause outflow obstruction, usually progresses over time,84 and may evolve into left ventricular dysfunction and dilatation81,84 (M.A., C.E.S., unpublished data, 2004). Associated right ventricular hypertrophy may also be found.85 The principal electrophysiological abnormalities in young individuals is ventricular preexcitation, found in approximately two-thirds of genetically affected individuals, is often associated with supraventricular arrhythmias (atrial fibrillation and, less commonly, atrioventricular reentry tachycardia). Conduction-system abnormalities develop in ~50% of older mutation carriers, often necessitating pacemaker implantation. Sudden cardiac death is not uncommon. Extra cardiac manifestations are usually less pronounced, except in neonatal disease66 but may include seizures, skeletal myopathy,84,87 and hypertension.83

PRKAG2 cardiomyopathy is often misdiagnosed as the more common disease, HCM.63,84 However, the mechanisms by which sarcomere protein gene mutations cause cardiac hypertrophy and PRKAG2 mutations cause cardiac hypertrophy are likely to be distinct. Initially, several models were proposed to explain how PRKAG2 mutations might cause cardiac hypertrophy. Some suggested that PRKAG2 mutations caused energy deficiency, whereas others suggested that abnormal membrane currents or abnormal gene expression/heart development65 caused cardiomyopathy with conduction abnormalities.

However, subsequent studies indicated that PRKAG2 cardiomyopathy is a unique type of glycogen storage disease primarily involving the heart.60 This idea was supported by:

- The known association between WPW pattern and cardiomyopathy in glycogen storage disorders (Danon disease, Pompe disease, and other glycogenoses).
- The finding of increased glycogen content in skeletal muscle of Hampshire pigs harboring a R200Q variant in PRKAG3, which is homologous to a R302Q PRKAG2 mutation in man.88

Confirmation of this model came from histologic analyses of formalin-embedded tissue samples, endomyocardial biopsies, and autopsy samples collected from individuals having either R302Q, Thr400Asn, or Asn488Ile mutation.63 All tissue samples displayed a similar pattern: absence of myofiber disarray and less fibrosis than usually found in HCM but, instead, vacuolated myocytes, which remained unexplained in the original pathological reports but were compatible with a storage-process disorder. Residual inclusion bodies in some of the vacuoles consisted of periodic acid–Schiff (PAS)-positive material compatible with nonsoluble polysaccharide (Figure 3). Collectively, these data strongly suggested a novel form of glycogen-storage disease, although the precise structures of the accumulated sugars were unclear. More recently, analyses of accumulated carbohydrates in murine models and in the most severe forms of human disease demonstrated that glycogen accumulates in this cardiomyopathy.13,86,89,101 There is a 30-fold increase in cardiac glycogen in mice expressing AMPK carrying the PRKAG2 N488I missense mutation. Subsequent studies of mutationally altered AMPK activity explained the dominant mode of disease inheritance and the phenotypes of cardiac glycogen accumulation and preexcitation in the conduction system.

Mice overexpressing PRKAG2 carrying human disease-causing mutations in their hearts have provided useful models for studying the consequences of these mutations. Transgenic mice overexpressing mutant N488I, R302Q, or R531G PRKAG2 cDNA under the control of α-myosin heavy chain (αMHC) cardiac-specific promoter perfectly recapitulate the human PRKAG2 cardiomyopathy.13,89,91 Because the αMHC promoter becomes activated in the hearts of newborn mice at the end of the first week of life, glycogen accumulation becomes pronounced in heart tissue during the second week of life, peaking at 5 weeks of age.13,89 Pronounced cardiac hypertrophy is observed by 2 weeks, peaks at 8 weeks age, and progresses to dilatation and contractile dysfunction by 20 weeks. N488I Transgenic mice display normal ECGs at birth, but, by 2 to 4 weeks, their ECGs exhibit delta waves. Delta waves on the ECG generally correspond to electrophysiological evidence of accessory pathways, which may be exposed by adenosine (blocking the atrioventricular node to expose...
conduction through accessory connection, if present) and suppressed by procainamide (a sodium channel blocker that slows conduction through the myocardium). PRKAG2 mutant mice experienced sinus bradycardia, atrial tachycardia, and atrioventricular tachycardia. Classically, preexcitation is considered to result from electrical transmission through well-defined bypass tracts (“bundles of Kent”) comprised of bundles of myocardium often in the junctions that form a physical connection between atria and ventricle, by passing the fibrous insulating layer, termed the annulus fibrosis. These discrete tracks can be observed on physical or histologic examination of hearts from WPW patients. However, preexcitation in transgenic mice expressing mutant AMPK appears to result from a different type of bypass track. In transgenic mice expressing mutant AMPK, and presumably in PRKAG2 cardiomyopathy, disruption of the annulus fibrosis by swollen, glycogen-filled cardiomyocytes produces abnormal tracks by which electrical impulses in the atria can pass directly to the ventricle, bypassing the atrioventricular node (Figure 4). This anatomy is consistent with multiple accessory pathways found in humans and mice with this disease. These disruptions of the annulus fibrosis disappear if glycogen levels are reduced in the cardiac myocytes (C. Wolf, C. Berul, M.A., J.G.S., and C.E.S., unpublished results, 2006).

**Effect of PRKAG2 Mutations on AMPK Function**

Several approaches have been taken to define the molecular consequences of human mutations on AMPK function. Because γ2 subunit containing complexes account for the minority of AMPK activity in the heart and elsewhere, one would imagine that haploinsufficiency (ie, mutations that cause inactivation of one allele) would not cause autosomal dominant disease. Further, because AMPK is largely inactive at rest, a gain of abnormal function was likely to account for disease pathogenesis. One approach to study the effect of PRKAG2 mutations on protein function was to introduce mutations, Thr400Asn and Asn488Ile, into the ancient yeast homolog snf4. Saccharomyces cerevisiae responds to lack of glucose by inducing expression of genes capable of metabolizing alternative sources of sugar through the action...
of the Snf1/4 complex. An interaction between Snf4 and Snf1 (the homolog of γ and α subunit, respectively) is required for yeast survival on media containing galactose, raffinose, or glycerol but is not required for growth in glucose-containing media.94 Snf4 harboring human mutations increased interaction with snf1 when yeast are grown in glucose-containing media, suggesting inappropriate basal activation of the enzyme complex.

Two groups studied the activity of 2 variants—R70Q γ1 and R225Q γ3 (which are homologous to human γ2 R302Q)—in pulmonary fibroblasts and COS cells transfected with α1β1γ1 or α2β2γ3.95,96 Their results were consistent with the model that the mutations increased basal AMPK activity but impaired sensitivity to AMP. By contrast, 2 other groups assessed AMPK activity in CCL13 cells transfected with a larger series of mutant PRKAG2 variants.22,108 PRKAG2 alleles containing R302Q, H383R, T400N, and R531G mutations, which are within CBS sequences, decreased AMPK activity, whereas Ins351Leu, which does not reside within a CBS, had no effect on AMP sensitivity and enzyme activity. The authors concluded that γ2 mutations do not cause constitutive AMPK activation and proposed that alternative mechanisms explain the cardiac pathogenesis of these mutations. More recently, Burwinkel et al86 analyzed the effects of R531Q mutation, which causes a fatal neonatal glycogenosis, on AMPK function. Biochemical characterization of mutationally altered AMPK demonstrated a striking impairment of AMP and ATP binding to the γ subunit, exceeding the effect of other PRKAG2 mutations. Even the R531G mutation, involving the same residue, has less effect on AMPK function. Functional analyses of R531Q and R531G AMPK, in HEK-293 cells, showed that both mutations increase the basal AMPK activity, concomitant with increased α subunit and ACC phosphorylation, but also abolish the responsiveness of AMPK to AMP.86 Kilimann and colleagues86 suggested that the apparent discrepant effects of human mutations on AMPK activity reflected the use of different cell lines, which contained variable amounts of the main upstream kinase LKB1 in CCL13 cells.35 The current consensus appears to be that in the presence of adequate upstream kinase (LKB1), human mutations increase the basal activity of AMPK and reduce the sensitivity of the protein to AMP.

The consequences of human PRKAG2 mutations on AMPK activity have also been assessed in the myocardium using tissue obtained from transgenic mice expressing mutationally altered PRKAG2. Studies in whole animals are complicated by 2 factors. First, multiple isoforms of AMPK subunits are expressed in cardiac tissue. Overexpression of wild-type or mutant PRKAG2 might lead to altered levels of different AMPK isoforms. Second, glycogen accumulation, which is a signature feature of PRKAG2 cardiomyopathy might be expected to alter AMPK activity. That is, AMPK was reported to be negatively regulated by glycogen.97,98 Although increased expression of γ2 protein might have altered the amount of either α1 or α2 in PRKAG2N488I transgenic hearts compared with levels observed in wild-type hearts, no changes in the amounts of these subunits were observed.89 These studies demonstrated that the amount of γ2 subunit is not limiting in the myocardium. Then AMPK activity in transgenic hearts was measured in complexes immunoprecipitated by α1 and α2 antibodies in 5-week-old mice with a fully developed phenotype. No differences in AMPK activities were observed between wild-type and transgenic hearts. Because glycogen could inhibit AMPK, we repeated the activity measurements in young (1-week-old) mice, which have little accumulated glycogen (because the transgene is not activated until shortly after birth). In young mutant PRKAG2N488I mice, significant increases in AMPK activity are found, and this increased activity is associated primarily with the α2 subunit. Recent studies13,91 using PRKAG2R302Q and PRKAG2R531G transgenic mouse demonstrated consistent findings. AMPK activity was reduced in enzyme complexes from older transgenic mice but not in 1-week-old mice.

Because wild-type AMPK was activated by AMP, the ability of AMP to activate mutationally altered AMPK was assessed under different conditions. Tian and colleagues59,60,99 used instantaneous freeze-clamped Langendorff-perfused hearts to achieve complete control of the metabolic environment and avoid any anoxia/ischemia associated with in vivo heart extraction. Under these conditions, PRKAG2N488I hearts had increased AMPK activity across a range of AMP concentrations.99 However, AMPK with the PRKAG2 N488I mutations demonstrated reduced activation in response to AMP. These measurements were consistent with earlier studies in cell culture showing decreased nucleotide binding to Bateman domains in mutationally altered γ2 subunits.22

These biochemical studies suggest a mechanism by which PRKAG2 missense mutations determine AMPK activity. Under normal physiological conditions, the heart has abundant ATP and nearly absent AMP. Under these conditions, high ATP levels inhibit AMPK. AMPK carrying PRKAG2 mutations has decreased affinity for ATP, causing inappropriate baseline activation of the enzyme. During times of extreme oxidative stress, such as ischemia, when ATP goes down, AMP levels increase and upregulate the enzyme activity. Mutationally altered subunits lack the capacity to sense and respond to this metabolic challenge. PRKAG2 missense mutations thus have a binary effect on enzyme function. Their predominant pathophysiological effect manifests at rest as reducing the affinity for ATP and increasing baseline AMPK activity.

Two other lines of evidence confirm that increased baseline AMPK activity is responsible for PRKAG2 cardiomyopathy. First, PRKAG2 mutations leading to reduced AMPK activity are predicted to produce different phenotypes from mutations that lead to increased AMPK activity100 (Table 1). Several models of genetically engineered mice with altered cardiac AMPK activity have been produced. These models help fill the gap left by absence of suitable pharmacological agents for long-term activation or inhibition of AMPK in vivo. Abrogation of α2 subunit activity leads to decreased AMPK activity, contraction-induced glucose uptake, and skeletal muscle glycolysis.101 Finally, α2 dominant-negative transgenic animals had unchanged or decreased cardiac weight and glycogen content. These data strongly suggest that
loss of AMPK function does not lead to glycogen-storage cardiomyopathy but, rather, to decreased glycogen levels. Second, a model that PRKAG2 mutations cause disease by increasing baseline activity of AMPK would imply that downregulation of AMPK activity in transgenic mice overexpressing mutant PRKAG2 should prevent cardiomyopathy. Xing et al. created transgenic mice, designated Tgα2DN, that express a dominant-negative form of the α2 subunit under control of the αMHC promoter in the heart. Tgα2DN mice have reduced cardiac AMPK activity. Compound heterozygous Tgα2DN×PRKAG2N488I mice have reduced AMPK activity and minimal cardiomyopathy, demonstrating that the PRKAG2 missense mutation mediates its effects by activating AMPK. Further analyses of these compound heterozygous mice demonstrated that α2 subunit–containing AMPK, rather than α1 subunit–associated AMPK, is responsible for the cardiac phenotype.

The Mechanism of Glycogen Storage
Pharmacological stimulation of AMPK with AICAR, an AMPK activator, causes mild and model-dependent changes in glycogen levels. Genetic manipulation of AMPK subunits significantly alters muscle and cardiac glycogen. The effects of 3 different types of genetic models have been described. α2-AMPK–deficient and α2 dominant-negative transgenic mice have reduced glycogen synthesis capacity and less glycogen. Mice lacking γ3 have impaired skeletal muscle glycogen resynthesis after exercise, suggesting a role in regulating glycogen synthesis. y2 mutations cause severe cardiac glycogen storage (30X) in association with increased AMPK activity and glucose uptake. Interestingly, wild-type γ2 transgenic mice, in which wild-type γ1 subunits are replaced with γ2 subunits, display moderate (5X) glycogen accumulation without measurable change in AMPK activity. Likewise, mice overexpressing either wild-type γ3 or R225Q have 2-fold increased muscle glycogen, which is unrelated to AMPK, GS, or GP activity.

Several groups have now addressed the question of whether the glycogen that accumulates during PRKAG2 cardiomyopathy is available for energy production. Recent studies have shown that PRKAG2 transgenic mice, either mutant or wild type, can use their accumulated cardiac glycogen during exercise or anoxia as an energy source. Similarly, increased muscle glycogen in R70Q γ1 transgenic mice served to improve exercise tolerance. These findings suggest that therapeutic approaches to decrease glycogen accumulation in these patients may well “cure” the disease. They also indicate that excess glycogen arises from altered regulation, which does not match synthesis with consumption rather than a defect leading to irreversible deposition of glycogen in cytoplasm (Figure 5). At rest, mutant hearts have increased AMPK activity, increased resting glucose uptake, normal levels of high-energy phosphates, and no substantial increase in tissue lactate, indicating no major excess of glycolysis over the oxidative phosphorylation. We assume that because heart normally “prefers” fatty acids as fuel but has no physiological capacity/enzymatic machinery to store fat; fatty acids undergo oxidation, whereas excess glucose may be directed to storage in the form of glycogen (Figure 5). A possible mechanism of fuel selection may involve inhibition of pyruvate dehydrogenase by free fatty acids, although the role of AMPK in regulating this critical enzyme needs further clarification.

Recent spectrophotometric studies of accumulated glycogen in PRKAG2 transgenic hearts showed increased absorbance consistent with less branching of carbohydrate chains than found in normal cardiac glycogen. Amylopectin (polyglucosan), a nonsoluble glucose polymer, accumulates in glycogen storage disease (GSD) type IV caused by brancher enzyme deficiency. In human PRKAG2 cardiomyopathy, similar deposits of nonsoluble cytoplasmic glycogen are found in formalin-preserved cardiac tissue samples. The relationship between PRKAG2 mutations and altered glycogen branching remains uncertain.

Integrating these data suggests a complex interaction between metabolic status, level of AMPK subunits, enzyme activity, and glycogen level. All of the myocardial and electrophysiological features of PRKAG2 cardiomyopathy can be explained by inappropriate glycogen accumulation. Although PRKAG2 mutations create activated AMPK, which leads to glycogen accumulation, the target proteins phosphorylated by the activated AMPK and the precise mechanism(s) that lead to glycogen accumulation are not yet identified. Despite that, we suggest that PRKAG2 cardiomyopathy has many features similar to other classic glycogen storage diseases and should be classified as such (Figure 6). We propose a negative feedback loop in which AMPK is involved in setting the level of cellular glycogen stores (Figure 5). Alterations in AMPK function, for example, γ2 mutations or replacing γ1 with γ2, interfere with metabolic regulation and cause glycogen levels to rise until a new
steady state is achieved. In principle, these feedback loops would be appropriately regulated if AMPK were inhibited by glycogen. Although glycogen does bind AMPK via the β subunit, glycogen binding does not appear to inhibit AMPK.\textsuperscript{18,19} Perhaps physical association of glycogen with AMPK exposes the enzyme to inhibitory modulation by phosphatases or other mediators. Similarly, the mechanism by which replacing γ1 with its γ2 homolog upregulates glycogen is unclear but may involve subtle AMPK activation not detectable by conventional assays or different subcellular localization of γ2-containing complexes.

The Role of AMPK in Physiological and Other Forms of Cardiac Myocyte Hypertrophy

Physiological hypertrophy, or pressure-overload hypertrophy, is the cardiac response to hypertension. This hypertrophy response has a significant effect on cardiac energetics. In the mouse heart, AMPK is activated during pressure overload–induced hypertrophy,\textsuperscript{60} and reduced activity in adiponectin-deficient mice has been associated with worse hypertrophy and outcome after aortic banding.\textsuperscript{61,112} In addition, AMPK appears to have a role in regulating Akt1-induced hypertro-
phy in neonatal cardiomyocytes. Hypertrophic hearts undergo relative oxygen deficit and switch from using fatty acids as the primary energy source to glucose. The role of AMPK may be to compensate for an increased AMP/ATP ratio secondary to pressure overload rather than being the cause of the pathogenic response. Alternatively, AMPK has been implicated as a regulator of the Akt-mTOR pathway, which leads to decreased protein synthesis through inactivation of eukaryote elongation factor 2, eEF-2, and inhibition of mTOR complex.

Because of the wide range of AMPK targets, defining its role in the hypertrophic process has proven difficult. For example, AMPK can be either pro- or antiapoptotic, depending on experimental conditions. The ability of AMPK to upregulate p53 and mediate Bax translocation to mitochondria suggests an antiproliferative function. By contrast, AMPK activity also protects cardiomyocytes from palmitate load or anoxic damage associated with antiapoptotic activity. Transgenic mice associated with constitutive AMPK activity had a net increase in heart muscle mass (after correcting for glycogen and water) and increased expression of hypertrophy associated genes. These are most probably secondary phenomena resulting from the evolving pathological process and not directly from changes in AMPK activity. Because various models associated with decreased AMPK activity did not show any hypertrophy, AMPK may be more of a bystander than a key player in the hypertrophic process. In conclusion, the role of AMPK in cardiac hypertrophy remains unknown.

AMPK may play other roles in altering cardiac myocyte morphology and proliferation. The main AMPK activator, LKB1, is in fact a tumor suppressor gene. AMPK activates the tuberous sclerosis tumor suppressor protein complex TSC1-TSC2 to inhibit mTOR signaling and cell growth and proliferation (recently reviewed Motoshima et al). Interestingly, inactivating mutations in TSC1-TSC2 cause tuberous sclerosis, a syndrome associated with benign tumors including multiple hamartomas and cardiac rhabdomyomas. Whether these regulatory functions are related to cardiac disorders should be answered in the near future. One recent exciting study addressed the potential of AMPK stimulation to inhibit coronary atherosclerosis. AMPK activation suppressed vascular smooth muscle proliferation through a p53-dependent mechanism. This action is expected to work in synergy with lipid lowering effect and improved endothelial function to attenuate the leading cause of morbidity and mortality in the western hemisphere.

AMPK and Cardiac Electrophysiology
PRKAG2 cardiomyopathy patients often have significant conduction-system disease, including WPW syndrome (Table 2). Whereas 1 pathophysiological mechanism appears to be physical disruption of conduction cells by glycogen engorgement, other parallel mechanisms are also possible. For example, AMPK phosphorylates and inhibits the cystic fibrosis transmembrane conductance regulator in lung epithelium, an ATP-gated chloride channel responsible for cystic fibrosis. Increased AMPK activity is also associated with inhibition of the amiloride-sensitive Na+ channel in epithelial cells of lung and kidney origin. More recently, Wang et al suggested that the effect of AICAR on glucose-mediated insulin secretion involves inhibition of ATP-dependent K channel. Suspecting that modulation of membrane channels is involved in the mechanism of arrhythmia in PRKAG2 cardiomyopathy, Light et al transfected cardiomyocytes with the constitutively active T172D mutant of the α1 subunit. The action potential duration was significantly prolonged in α1T172D-transfected myocytes, causing early afterdepolarization. The mutant slowed the open-state inactivation of sodium current similar to the pathophysiology observed in long QT syndrome caused by SCN5a mutations. The relationship of this model to human disease remains unclear, because neither QT prolongation nor torsades de pointes has been reported in PRKAG2 cardiomyopathy.

The effect of AMPK mutations on cardiac ion channels requires further study. Hallows et al hypothesized that inhibiting transmembrane conductance, by preventing waste of high energy phosphates, might fall within the general role of AMPK. If this rule applies to the heart, one could expect inhibition of ion currents to contribute to bradyarrhythmia and conduction system disease in PRKAG2 cardiomyopathy.

Although PRKAG2 mutations may affect ion channels, studies of murine models strongly suggest that the primary mechanism leading to conduction disease is an abnormal anatomical substrate for electrical coupling between atria and ventricles. Either electrical connections, which normally exist during embryonic life, do not involute or they develop postnatally by muscle tissue penetrating through annulus fibrosis. Transgenic mice develop glycogen storage, multiple atrioventricular communications, and preexcitation several weeks after birth (Figure 4). Other glycogen storage disorders, such as Danon disease, also cause preexcitation, probably by a similar mechanism. Most likely, there are 2 mechanisms leading to cardiac preexcitation: some forms are caused by glycogen accumulation, whereas others are caused by the formation of accessory bypass tracts formed from abnormal cell proliferation or growth. This may explain relatively low prevalence of typical WPW arrhythmia (atrioventricular reentrant tachycardia) in PRKAG2 cardiomyopathy despite multiple accessory pathways in the affected individuals (Table 2).

Conclusions
In summary, AMPK plays a critical role in cardiac metabolism, as it does in a variety of cell types. Seven different genes encode the various α, β, and γ subunit isoforms, which appear to be able to assort in all combinations producing as many as 12 different AMPK proteins. The expression of these different subunits varies considerably from tissue to tissue. One approach to defining the role of these different AMPKs is to identify naturally occurring mutations that alter physiology.

Two different types of naturally occurring mutations in AMPK subunits have been described. In pigs, missense mutations in the γ3 subunit cause muscle glycogen accumulation. In humans, γ2 (or PRKAG2) missense mutations cause glycogen-storage cardiomyopathy. Studies in both humans and animal models have demonstrated that these
mutations in the γ subunit lead to constitutive activation of PRKAG2. In mouse heart, the α2γ2 AMPK isozyme appears to be responsible for most of the disease manifestations. The mutationally altered PRKAG2 leads to altered glycogen metabolism and to dramatic accumulation of cardiac glycogen, which is responsible for both cardiac hypotropy and preexcitation. Whether activated AMPK also triggers other pathways that may contribute to the increase in cardiac mass or electrophysiological abnormalities is unknown. Similarly, accumulated glycogen in the cardiac myocytes leads to disruption of the annulus fibrosis and bypass tracts, which contribute to preexcitation.

In this review, we examine the role of cardiac AMPK using the concepts derived from assessing phenotypes of human patients bearing AMPK mutations. Investigators have speculated on the multiple pros and cons of pharmacological AMPK manipulation for treatment of cardiovascular disease. To date, we have no sufficient understanding of the role of AMPK in cardiac health and disease to guide pharmacological manipulations for patients. AMPK may eventually emerge as a major regulator of muscle/heart glycogen stores. However, glycogen storage that occurs in response to constitutive PRKAG2 mutations may not reflect normal cardiac physiology, and more research is required to understand the contribution of AMPK to normal glycogen metabolism.

Over the next several years, we expect that the precise molecular mechanisms, both direct regulation of metabolism and indirectly by mediating transcription, by which AMPK mutations lead to glycogen accumulation will be dissected. Identification of these mechanisms will lead to better understanding of the role of AMPK in regulating cardiac energy metabolism as well as energy metabolism in other cells. In addition, we anticipate that definition of these mechanisms will lead to therapies directed toward reducing glycogen accumulation in PRKAG2 cardiomyopathy.

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