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Heart metabolism

AMPK – A pivotal rheostat in the control of cardiac metabolism

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AMP-activated protein kinase (AMPK) is emerging as a central regulator of the myocardial stress response. AMPK exerts important metabolic and non-metabolic actions and lies at the intersection of potential novel approaches to the treatment of coronary artery disease and type 2 diabetes. This review focuses on emerging data regarding the regulation of AMPK, the metabolic actions of AMPK in the heart and highlights the possible role of AMPK in novel cardioprotective strategies.

Introduction

Despite progress in cardiovascular disease treatment with anti-thrombotic therapy, statins and neurohormonal blockade, myocardial-based approaches can provide additional opportunities for unique therapeutic advances. The extraordinary increase in the prevalence of obesity, aging and type 2 diabetes has led to a surge in cardiovascular disease (CVD) rates, underscoring the important links between metabolism and cardiovascular disease. Treatment strategies focused at this intersection such as AMP-activated protein kinase (AMPK) can be crucial in addressing the anticipated CVD epidemic. AMPK plays a central role in the metabolic response of the heart to stress. AMPK regulates glucose, fatty acid and glycogen metabolism in the heart and also exerts beneficial non-metabolic effects. AMPK is activated during cellular stress, both by an increase in the cellular AMP concentration as well as by an AMP-independent pathway via its

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upstream kinase. Thus, the paradigm of AMPK regulation and function in the heart is expanding.

AMPK background

The heart requires ATP to maintain contraction, membrane ion gradients and calcium homeostasis. The heart is a metabolic omnivore, utilizing substrates such as fatty acids, glucose, lactate and ketones for energy generation, depending upon their availability and the regulatory status of its metabolic pathways. At rest, the heart generates approximately 70% of its energy from fatty acids, yet during periods of ischemia (such as during acute myocardial infarction) aerobic metabolism greatly diminishes and anaerobic pathways such as glycolysis increase in importance (Table 1). Utilization of glucose during ischemia and into post-ischemic reperfusion is energetically beneficial (see Opie and Lopaschuk for review [1]), and an increase in myocardial glucose uptake is a crucial metabolic response of the heart to ischemia [2].

AMPK is a protein kinase crucial in the regulation of myocardial glucose uptake [3] and fatty acid oxidation [4]. In general, AMPK stimulates energy-generating pathways and inhibits energy-consuming pathways, and this overall protection of cellular energy state is beneficial to the ischemic heart. Although excessive fatty acid oxidation can be detrimental to the post-ischemic heart by inhibiting glucose oxidation via classic Randle Cycle mechanisms (see Glossary), AMPK promotes ATP production and has protective effects in the ischemic and post-ischemic heart. We recently showed that AMPK deficiency increases myocardial injury and apop-

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Glossary

AICAR: 5-aminoimidazole-4-carboxamide riboside; a non-specific, cell-permeable AMPK activator which is converted to ZMP, an AMP analogue

eNOS: endothelial (or type III) nitric oxide synthase; catalyzes the production of NO from L-arginine

GLUTs: cell-membrane glucose transporters; in the heart, GLUT1 regulates basal glucose uptake whereas GLUT4 is the inducible glucose transporter

LKBI: an upstream kinase of AMPK; mutations in LKBI lead to the Peutz-Jegher intestinal polyposis syndrome

p38/MAPK: a member of the mitogen-activated protein kinase family; participates in numerous cellular stress responses by phosphorylating downstream substrates including transcription factors and enzymatic cascades

Randle cycle: fatty acid oxidation-produced citrate and ATP lead to blockade of glycolysis (PFK-1)

Thiazolidinediones: TZDs; anti-diabetic agents (rosiglitazone, pioglitazone) which stimulate the PPAR- γ pathway

TUNEL staining: TdT-mediated dUTP-biotin nick end-labeling; a method of detecting apoptotic cells by labeling the ends of broken DNA strands

tosis in perfused mouse hearts during moderate ischemia and reperfusion [5]. AMPK-deficient hearts had decreased glucose uptake during and after ischemia and reduced fatty acid oxidation after ischemia. The cardioprotective effects of AMPK can also extend beyond its metabolic effects. In addition, recent studies also demonstrate that stimulation of AMPK increases skeletal muscle glucose uptake and might improve insulin resistance [6]. Thus, the AMPK pathway can be at the convergence of potential treatment strategies for both myocardial ischemia and type 2 diabetes.

Initially identified as an inhibitor of liver HMG-CoA reductase and acetyl-CoA carboxylase, AMPK is an evolutionarily conserved serine-threonine protein kinase which coordinates cellular metabolic stress responses [7]. AMPK is a heterotrimeric enzyme, composed of a catalytic alpha subunit (α_1 or α_2) isoform and a regulatory gamma subunit (γ_1 , γ_2 , or γ_3) tethered together by a structural beta subunit (β_1 or β_2) (Fig. 1). The alpha subunit contains an N-terminal catalytic domain including the Thr¹⁷² residue which is phosphorylated leading to activation of the enzyme. The beta subunit contains both a glycogen-binding domain, whose functional

role is unclear, and specific residues crucial to alpha and gamma subunit binding [8]. The gamma subunit contains four cystathionine beta-synthase (CBS) domains which mediate AMP binding and sensitivity. In the heart, the alpha 2 isoform is the most abundant [5,9]. AMPK conserves cellular energy by upregulating energy-producing pathways such as fatty acid oxidation and glucose uptake, although switching off anabolic pathways such as protein and fatty acid biosynthesis (Fig. 1). AMPK also regulates ion channel activity, gene transcription, protein synthesis, cell survival and mitochondrial biogenesis [7]. Investigators are currently studying many aspects of the AMPK pathway and there is substantial interest in AMPK in the heart as a cardioprotective strategy (Table 2).

AMPK regulation

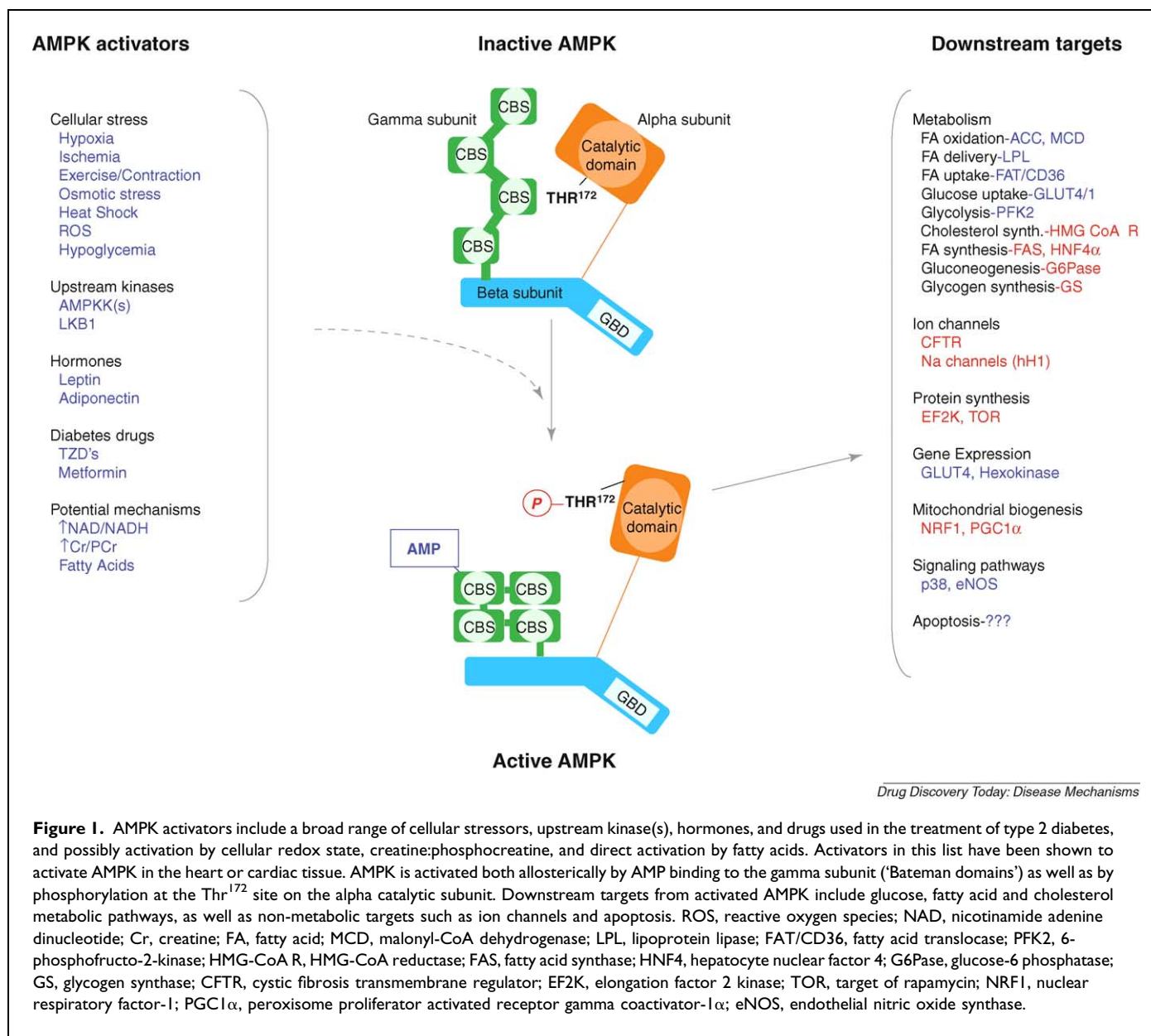
Classically, descriptions of AMPK activation have focused on the role of intracellular AMP; a rise in AMP activates the enzyme either by direct allosteric activation or by promoting phosphorylation of the alpha subunit Thr¹⁷² site (Fig. 1). AMPK activation is inhibited at physiological concentrations of ATP, whereas a fall in ATP further increases the AMP:ATP ratio and activates the enzyme [7]. Anti-diabetic drugs including the THIAZOLIDINEDIONES (TZDs, see Glossary) and metformin can also activate AMPK [10]. AMPK is inactivated by protein phosphatase 2C (PP2C) [11]. Interestingly, PP2C is upregulated in the hearts of insulin-resistant obese Zucker rats and *ob/ob* mice, and TZD treatment prevents this upregulation, promoting the activation of AMPK [12]. Insulin can also inhibit AMPK in the heart, which correlates with the antagonism between the anabolic effects of insulin and the catabolic effects of AMPK [13].

Alpha subunit Thr¹⁷² phosphorylation by upstream kinase(s), termed AMPK kinases or AMPKKs, increases AMPK activation. Although AMPKK(s) in the heart have not been well characterized, we have recently shown that biochemically enriched AMPKK is activated by ischemia in rat hearts both *in vitro* and *in vivo* [14]. AMP increased and ATP decreased the ability of AMPK to be activated by heart AMPKK, and these interactions required the presence of functional gamma subunits in the heterotrimeric AMPK complex (Fig. 2). Use of either a truncated alpha 1 subunit or an alpha1/beta1/gamma1 heterotrimer with an R70Q mutation in the gamma 1 CBS

Table 1. Myocardial metabolism during several physiologic and pathologic states^a

	Normal	Exercise	Ischemia	Reperfusion	Diabetes	Hypertrophy
Glucose uptake	+	↑↑	↑	↑	↓	↑
Glycolysis	+	↑↑	↑↑	↑	↓	↑
Glucose oxidation	+	↑↑	↓↓	↓	↓	↑
Fatty acid oxidation	+++	↑↑	↓↓	↑↑	↑↑	↓
Lactate oxidation	+	↑↑	↓↓↓	↓	↓	↑

^a These represent semi-quantitative assessments, dependent upon models and conditions. For reviews, see Refs. [1,51–53].



domain/AMP binding site eliminated the ability of AMP and ATP to modulate AMPK phosphorylation. However, AMPKK activity itself was not affected by changes in AMP or by the AMPK activator AICAR (see Glossary), that would appear to exert their action on AMPK itself. Interestingly, recent research has also demonstrated increased AMPKK activity from hearts subjected to mild ischemia where the AMP:ATP ratio did not appear to be altered [15].

The tumor suppressor kinase, LKB1 (see Glossary), is an important and AMP-independent AMPKK in the liver [16,17]. LKB1 depends on STRAD/MO25 modifier proteins to exert its effects, and its action can also involve phosphorylation of one or more AMPK-related kinases [18]. The expression and function of these modifier proteins and AMPK-related kinases in the heart are not known. LKB1 does not appear to be activated by ischemia in the heart, but additional

as yet unidentified AMPKK proteins probably account for increased AMPK phosphorylation during ischemia [15]. Further identification and characterization of these AMPKKs in the heart is an important area of research.

Additional aspects of AMPK regulation remain controversial, including its regulation by creatine:phosphocreatine [19] and NAD:NADH concentrations ratios [20]. Furthermore, AMPK is activated by supra-therapeutic (0.5–2 mM) doses of metformin as well as by osmotic stress in the absence of detectable increases in [AMP] [10]. There is also some suggestion that fatty acids can activate AMPK directly in an AMP-independent fashion in the heart, indicating a potential mechanism by which AMPK could sense substrate availability and coordinate cellular substrate utilization [21].

Intriguingly, the adipocyte hormones adiponectin and leptin also activate AMPK. Leptin does so in an AMP-independent

Table 2. Targets and related therapies: AMPK as a target for cardiovascular disease therapies

Strategic approach to target	Expected outcome of intervention at target	Advantages or disadvantages	Who is working on the this	Clinical trials	Refs
AMPK					
Adiponectin	↓ Hypertrophy ? Beneficial metabolism	? Increase insulin sensitivity, treatment of type 2 diabetes, anti-atherogenic effects; unavailability	Walsh, K Ruderman, NB	n.a.	[24–26]
AMP mimetic-AICAR	↑ Response of AMPK to upstream kinase	Lack of specificity, difficulty with delivery	Mangano, DT (meta-analysis)	Yes (see Ref. [55])	[55]
Direct AMPK activators	↑ AMPK activity	Currently under development, potential side effects (? glycogen overload)	n.a.	n.a.	n.a.
Inhibition of PP2C-TZDs	↓ AMPK deactivation	Used in treatment of type 2 diabetes, ↑ insulin sensitivity, possible anti-athero	Unger, RH; PROactive study group	[54]	[12]
Overexpression of AMPK	Constitutive AMPK activity	Not clinically available, viral toxicity	Dyck, JR	n.a.	[56]
Preconditioning (PC)	↑ AMPK, ↑ GLUT4	Ischemic PC-lack of feasibility Pharmacologic PC-agents not available	Shimamoto, K	n.a.	[57]
Genetics	Identification of cardiomyopathy, screening	Family counseling, identification of high risk individuals	Seidman, CE; Seidman, JG and Arad, M Roberts, R and Gollob, MH		[46] [47]

fashion in skeletal muscle [22], although it does not appear to stimulate AMPK in isolated rat hearts [23]. Activation of AMPK by adiponectin increases skeletal muscle glucose uptake and fat oxidation [24] and is pro-angiogenic [25] and anti-hypertrophic in the heart [26]. Taken together, these data suggest that the regulation of AMPK in the heart is multifaceted.

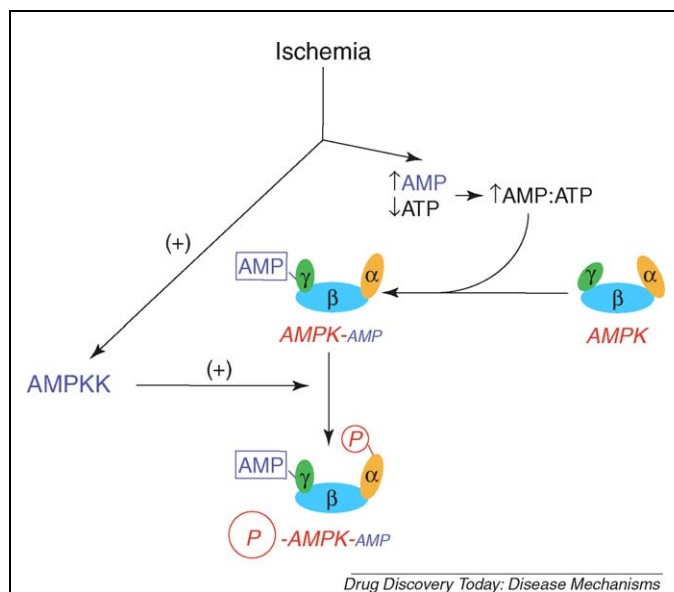


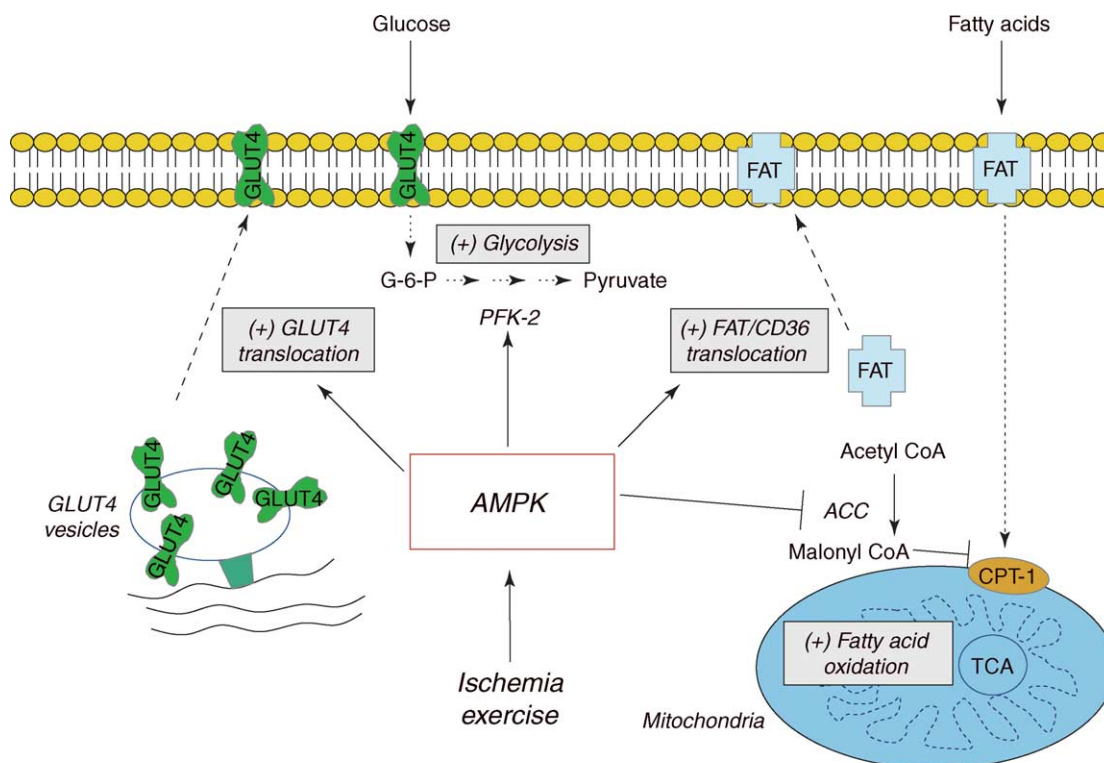
Figure 2. Proposed schema for AMPK regulation in the heart based on recent data from Baron *et al.* [14] and Altarejos *et al.* [15]. Ischemic stress leads to increased intracellular AMP and a fall in ATP, increasing the AMP:ATP ratio. AMP binds to the AMPK gamma subunit via the CBS domains, increasing its ability to be activated by phosphorylation by an upstream kinase. In addition, ischemia increases the activity of the upstream kinase directly, through a mechanism that is independent of AMP.

AMPK's metabolic effects in the heart

Glucose metabolism

Glucose uptake mediated by specific membrane transporters is a rate-limiting step in glucose metabolism. Ischemia-induced glucose uptake in the heart is dependent upon translocation of GLUT4 transporters (see Glossary) from intracellular membrane vesicles to the sarcolemma [27] and is not mediated by the PI3-kinase pathway that is responsible for insulin-stimulated glucose uptake. AMPK was first shown to stimulate GLUT4 translocation in the heart in isolated rat heart papillary muscles exposed to AICAR [3]. Pressure-overload hypertrophy also increases AMPK activation and is associated with increased GLUT4 translocation to the plasma membrane [28]. In the heart, AMPK-deficient transgenic mice expressing a dominant negative alpha 2 isoform have impaired glucose uptake during ischemia [5] and post-ischemic reperfusion [5,29]. In addition to mediating ischemic glucose uptake, AMPK activation is also associated the GLUT4 translocation during exercise [9]. However, it is still unclear whether AMPK has a crucial role in this response to exercise. AMPK appears to regulate glucose transporter translocation in part by interacting with a complex network of signaling pathways including p38/MAPK [30] and eNOS [31] (see Glossary). Although the direct downstream mechanisms by which AMPK mediates GLUT4 translocation are unclear, additional possible targets include proteins involved in the cytoplasmic retention of GLUT4 vesicles and/or pathways involved in the docking or fusion of these vesicles at the sarcolemmal membranes (Fig. 3).

After glucose is transported into the cell it can enter the glycolytic pathway to eventually form pyruvate, which is



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Figure 3. AMPK controls both glucose and fatty acid metabolism in the heart. Ischemic AMPK activation leads to translocation of GLUT4-containing vesicles to the sarcolemma. Possible mechanisms include the release of GLUT4 vesicles from their tethered location and/or an increase in the ability of these vesicles to dock and fuse with specific components of the plasma membrane. Following GLUT4 insertion, glucose transport across the sarcolemma is increased, and AMPK accelerates glycolysis by stimulating phosphofructokinase-2 (PFK-2). AMPK's control of fatty acid metabolism includes a recently described increase in the translocation of the FAT/CD36 fatty acid transporter. After fatty acids enter the cell, their oxidation in the mitochondria is controlled at the level of transport into the mitochondrial matrix as acyl CoA units. This is regulated by the mitochondrial transport protein, carnitine palmitoyl transferase-1 (CPT-1), which is inhibited by malonyl-CoA. AMPK phosphorylates and inhibits acetyl-CoA carboxylase (ACC) which leads to a decrease in malonyl-CoA production and a removal of inhibition of CPT-1, increasing fatty acid mitochondrial transport and oxidation. G-6-P, glucose-6-phosphate; TCA, tricarboxylic acid cycle.

either oxidized or converted to lactate. The enzyme, 6-phosphofructo-2-kinase (PFK-2), is activated by insulin and AMPK [32], leading to the synthesis of fructose-2,6-bisphosphate (F26BP), a positive regulator of the important glycolytic enzyme 6-phosphofructo-1-kinase (PFK-1). Therefore, AMPK mediates both the uptake of glucose in the ischemic heart and stimulates its flux through glycolysis.

Fatty acid metabolism

One of the earliest identified targets of AMPK was acetyl-CoA carboxylase (ACC) [7] which catalyzes malonyl-CoA production. AMPK phosphorylates and inactivates ACC, decreasing the production of malonyl-CoA, which relieves inhibition of the mitochondrial fatty acid transporter, carnitine palmitoyl transferase-1 (CPT1) (Fig. 3). Via this mechanism, AMPK activates fatty acid oxidation during post-ischemic reperfusion [4,5] and possibly during exercise [9]. Mouse hearts deficient in AMPK activity show decreased reperfusion fatty acid oxidation [5]. AMPK can also increase fatty acid uptake and delivery to the heart. First, AMPK increases the translo-

cation of the fatty acid transporter, fatty acid translocase (FAT/CD36) [33] to its active site in the sarcolemma. Secondly, AMPK increases the activity of lipoprotein lipase (LPL) at the capillary lumen, thereby increasing the ability of the heart to metabolize plasma triglycerides [34].

Glycogen

Glucose transported across the sarcolemmal membrane can also be stored as glycogen. The production of glycogen is controlled by glycogen synthase (GS). GS is regulated by glycogen concentration, allosterically by glucose-6-phosphate (G6P), and covalently by phosphorylation. Phosphorylation of GS by kinases at specific serine residues ('site 2' or 'site 3') inhibits the enzyme. AMPK was shown to co-immunoprecipitate with skeletal muscle GS [35] and to be a GS kinase *in vitro* [36]. Indeed, studies from AMPK alpha 2 knockout animals show that AMPK is required for the deactivation of AICAR of GS in skeletal muscle [37]. The AMPK beta subunit contains a glycogen-binding domain, perhaps compartmentalizing AMPK in an intracellular location

where it senses glycogen stores and modulates glycogen synthesis [38]. Although AICAR also activates glycogen phosphorylase [39,40] by increasing glycogenolysis, this reflects the intracellular conversion of AICAR to ZMP (an AMP analogue) and allosteric activation of the enzyme, rather than an AMPK effect *per se*.

The interesting phenotype of glycogen overload was first described in skeletal muscle from Hampshire pigs (RN⁻) that have a mutation in the AMPK gamma 3 subunit gene (PRKAG3, GenBank accession no. NM_017431) in the CBS domain responsible for AMP binding [41]. A recent proteomic analysis suggest this glycogen overload is secondary to constitutively active AMPK [42], with increased expression and enzymatic activity of UDP-glucose pyrophosphorylase and PFK-2. Although the heart does not express the AMPK gamma 3 subunit, analogous mutations in the human and mouse cardiac AMPK gamma 2 subunit gene (PRKAG2, GenBank accession no. NM_016203) cause a hypertrophic cardiomyopathy characterized by glycogen-laden cardiomyocytes and a Wolff–Parkinson–White ventricular pre-excitation phenotype [43].

Further support for the concept that AMPK regulates muscle glycogen comes from AMPK-deficient models. The AMPK alpha 2 dominant negative mouse (K45R mutation) has decreased glycogen stores in both heart [5] and skeletal muscle [44]. The alpha 2 knockout mice also show diminished skeletal muscle glycogen content and decreased glycogen synthesis rates during hyperinsulinemia [37]. Further, increased skeletal muscle glycogen accumulation occurs with chronic, *in vivo* AMPK activation [45].

Whether the PRKAG2 mutations are associated with increased or decreased AMPK activity is controversial. AMPK activity was increased in transgenic mice expressing an N488I PRKAG2 missense mutation [46]. However, mice expressing the R302Q PRKAG2 mutation showed diminished gamma 2 subunit-related and total AMPK activity [47]. How increased AMPK activity might increase glycogen is uncertain but glycogen accumulation could reflect a combination of increased glucose uptake and inhibition of glycolysis by the Randle cycle mechanism in the absence of increased metabolic demand. The stimulatory effect of AMPK activity on glucose transport might overcome its inhibition of GS, leading to increased intracellular glucose and glycogen storage. Studies describing the effects of AMPK activators on heart glycogen will be important to understand the physiologic effects as well as the safety of chronic AMPK activation in the treatment of type 2 diabetes.

AMPK and cardioprotection

AMPK exerts diverse metabolic effects described above, preserving the cellular energy state. These effects alone can mediate AMPK action as a cardioprotective pathway. In addition, it is possible that AMPK plays a role in myocardial

preconditioning, one of the most powerful known cardioprotective mechanisms. AICAR potentiates preconditioning in rabbits undergoing coronary ligation [48], lengthening the preconditioning time window [49], and preconditions rat livers from ischemia *in vivo* [50]. AMPK also exerts anti-apoptotic effects. Mouse hearts expressing a dominant negative AMPK alpha 2 subunit showed a marked decrease in myocardial function and energetics after global, low-flow ischemia and reperfusion [5]. These AMPK-deficient hearts also had increased creatine kinase release, as well as greater TUNEL staining and caspases-3 activity indicating increased apoptosis [5]. The mechanisms by which AMPK protects against apoptosis are currently under investigation. Although AMPK deficiency appears detrimental during ischemia-reperfusion, whether increased AMPK activity in the heart would provide a cardioprotective benefit is an important clinical issue and requires further study.

Conclusions

AMPK plays a fundamental role in the stress response of the heart. It exerts pleiotropic metabolic and non-metabolic effects, with new target pathways continuing to be described. Its activation includes AMP-dependent and AMP-independent mechanisms, as well as modulation by upstream kinases whose identities in the heart are still unclear. In the heart, AMPK increases glucose uptake, glycolysis and fatty acid oxidation, and affects glycogen metabolism. Overall, AMPK appears to be cardioprotective, either through energy-conserving metabolic effects or potentially via additional actions. Further definition of the effects of long-term AMPK activation and effects of the AMPK in various ischemic models is required to further understand the clinical ramifications of this potentially powerful signaling pathway in the heart.

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