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SPOTLIGHT REVIEW

AMPK activation, a preventive therapeutic target in the transition from cardiac injury to heart failure

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Abstract Heart failure is a progressive muscular disorder leading to a deterioration of the heart characterized by a contractile dysfunction and a chronic energy deficit. As a consequence, the failing heart is unable to meet the normal metabolic and energy needs of the body. The transition between compensated left ventricular hypertrophy and the de-compensated heart is multifactorial, although metabolic disturbances are considered to play a significant role. In this respect, the AMP-activated protein kinase (AMPK) could be a potential target in heart failure development. AMPK senses the energy state of the cell and orchestrates a global metabolic response to energy deprivation. We briefly review here the current knowledge about the chronic energy deficit of the failing heart, as well as the role of AMPK in energy homeostasis and in the control of non-metabolic targets in relation to cardiac hypertrophy and heart failure. The relative importance of energetic and non-metabolic effects in the potential cardioprotective action of AMPK is discussed.

Keywords AMPK • Heart failure • Hypertrophy

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1. Introduction

Heart failure (HF) is a multifactorial, progressive, and disabling syndrome, characterized by symptoms resulting from ventricular dysfunction, either diastolic (impaired relaxation) or systolic (impaired contraction). The most common cause of HF is coronary artery disease and myocardial infarction, often linked with co-morbidities (e.g. hypertension, diabetes).

HF is in fact a muscular disorder leading to a progressive deterioration of the heart, characterized by contractile dysfunction linked to chronic energy deficit.[1–5] It is also a systemic disease in which a neurohormonal response of the body activates the renin–angiotenin and adrenergic systems, and leads to left ventricular remodelling and dilatation. Consequently, the increased wall stress enhances local oxygen consumption and worsens the energy deficiency and contractile dysfunction. The heart enters a vicious circle, which precipitates the clinical evolution and aggravates the prognosis. In addition, repeated ischaemic attacks decrease the number of functional cardiomyocytes and lead to maladapted cardiac remodelling.

Restoration of muscle contraction by inotropic support acting on beta-adrenergic signalling fails to improve the short- and long-term prognosis.[6] In contrast, the current HF therapies that inhibit the renin–angiotensin system and the catecholamine response, dramatically improve the prognosis of HF patients.[7] Although they do provide benefits, HF remains a life-threatening condition. Alternative therapies that could improve the energetic state and disrupt the vicious circle of the failing heart are of particular interest.

In this context, the AMP-activated protein kinase (AMPK) appears as a potential therapeutic target. AMPK is a highly conserved eukaryotic protein serine/threonine kinase that senses the energy status of the cell and coordinates a global metabolic response to energy deprivation.[8,9] The question then arises of its potential beneficial metabolic and/or therapeutic effects in the energy-deprived failing heart. In this chapter, we summarize the current knowledge on the energetic state of the failing heart, the biochemical characteristics of AMPK, and its role in energy homeostasis. We also briefly describe non-metabolic, anti-proliferative, anti-fibrotic, and angiogenic effects of AMPK that are independent of energy but related to cardiac hypertrophy and HF. The relative importance of these effects in the potential cardioprotective action of AMPK is discussed.

2. Energetic state of the failing heart

There is a continuum between compensated left ventricular hypertrophy and de-compensated HF. The pathological transition is probably

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multifactorial, although metabolic disturbances are considered to play a significant role.[1,2]

Energy depletion characterizes the failing heart, although the extent of the deficit may vary depending on the stage of HF[1,2,4,10]. In several models of left ventricular hypertrophy as well as in patients suffering from HF, measurements of the changes in cardiac energy charge by NMR techniques revealed significant decreases in phosphorylation potential (decreased phosphocreatine and ATP and increased ADP concentrations) that were proportional to the degree of hypertrophy and could be used as predictors of mortality.[2,11–14] The low energetic potential of the failing heart affects contraction and relaxation, both of which depend on ATP. Clearly, energy deprivation results from the decreased ability of the failing heart to produce ATP from the available substrates—'the failing heart: an engine out of fuel'.[3] Actually the defect affects all steps of energy production and includes disturbances in substrate utilization, mitochondrial oxidative capacity, and ATP transfer.[1,2] Moreover, this does not exclude abnormal ATP utilization by non-contractile biochemical systems.

With regard to substrate utilization, fatty acids are preferred substrates. Their oxidation inhibits glucose uptake, whereas glucose together with insulin inhibits fatty acid oxidation. This reciprocal metabolic control, known as the Randle cycle,[2,15,16] is perturbed in HF. In animal models of HF, the failing heart favours glucose utilization at the expense of fatty acids. The changes are however progressive and depend on the stage of HF.[4] At early stages, fatty acid oxidation is either unchanged or even slightly increased, whereas at more advanced stages, fatty acid oxidation is clearly limited. The metabolic shift corresponds to fundamental changes in the expression of genes controlling fatty acid oxidation and mitochondrial biogenesis, thus leading to metabolic inflexibility and lack of substrate adaptability to the energy needs.[2,4,17,18] It is also interesting to note that overexpression of GLUT1 protects against contractile dysfunction and prevents pressure overload-induced HF.[19] It also rescues the contractile dysfunction in peroxisomal proliferator-activated receptor (PPAR) alpha null hearts submitted to high workload.[20] However, increased glucose uptake and oxidation in transgenic mice overexpressing GLUT1 decreases fatty acid oxidation and remodels metabolism towards glucose utilization, but at the same time increases oxidative stress and results in cardiac dysfunction when these mice are fed a high-fat diet.[21]

Mitochondrial dysfunction has emerged as a characteristic feature of the failing heart and contributes to energy deprivation. The failing heart contains more mitochondria, which are however reduced in size and display ultrastructural abnormalities.[18,22–24] Their electron transfer chain complexes and oxidative phosphorylation capacity are decreased.[1,2,18] Fatty acid oxidation is especially affected in severe HF with a decreased content of enzymes, such as carnitine palmitoyl transferase 1 (CPT1) and acyl-CoA dehydrogenses that control fatty acid oxidation. This deficient fatty acid oxidation is explained by a decreased expression of peroxisome proliferator-activated receptor gamma co-activator (PGC1 alpha),[25] which is probably the most important transcriptional factor involved in heart mitochondrial biogenesis.

On top of a deficient oxidative mitochondrial capacity, the failing heart is also unable to couple energy production to utilization through the compartmentalized creatine kinase system. In the failing heart, decreased content and isoform alteration of this energy transfer system concur to an inefficient adaptation of energy production to utilization.[1]

3. AMPK, a metabolic master switch and more

AMPK senses the energy status of the cell—the fuel gauge of the cell[26]—and orchestrates an integrated metabolic response to energy deprivation in order to conserve ATP via short- and long-term metabolic control. AMPK is therefore regarded as a metabolic master switch in normal and pathological conditions.[8]

3.1 AMPK structure

AMPK is a heterotrimeric complex containing a catalytic (alpha) and two regulatory (beta and gamma) subunits. Each subunit has multiple isoforms (alpha 1 and 2, beta 1 and 2, and gamma 1, 2, and 3) giving 12 possible combinations of holoenzyme, which are present in murine and human hearts. The catalytic alpha subunit contains the protein kinase domain and a threonine residue (Thr172) whose phosphorylation by upstream kinases is responsible for AMPK activation.[8] In mouse hearts, AMPK alpha-2 accounts for 60–80% of total AMPK activity, whereas in human hearts both alpha-1 and alpha-2 catalytic subunits equally contribute to the total AMPK activity.[27] The beta subunit acts as a scaffold for the other two subunits. It also contains a glycogen-binding domain, whose physiological role might be to control glycogen metabolism.[28] The beta-2 isoform is the main isoform expressed in the heart.[29] The gamma subunit contains three AMP-binding domains, one of which binds a non-exchangeable nucleotide, whereas the others can bind AMP or ATP, with however a lower affinity for the latter.[30] Mutations in the gamma-2 subunit cause glycogen accumulation and lead to cardiac arrhythmias, also called Wolff–Parkinson–White syndrome.[31]

3.2 Control of AMPK activity

3.2.1 Biochemical mechanisms of activation

AMPK is activated when AMP concentration increases as a result of insufficient ATP production or unmatched energy demand. AMPK can also be activated independently of adenine nucleotides, by changes in calcium concentrations as well as by increased production of reactive oxygen species (ROS). Whatever the stimulus, AMPK activation requires phosphorylation by upstream kinases of a threonine residue (Thr172) located in the activation loop of the alpha catalytic subunit. At least two pathways lead to AMPK activation by phosphorylation of Thr172.[8,32,33] The first one senses energy depletion and is mediated by AMP and LKB1 (Peutz–Jeghers protein), which seems to be specific for AMPK alpha-2, because in heart from LKB1 KO mice, AMPK alpha-2, but not AMPK alpha-1 activation is abrogated.[34,35] The AMPK alpha-1 kinase acting under these conditions is not known. The second activation pathway is triggered by increased calcium concentration and is mediated by calcium/calmodulin-dependent protein kinase kinase-beta, which phosphorylates Thr172.[36–38] Although this protein kinase is present in heart, the demonstration of its participation in AMPK activation has not been reported.

3.2.2 AMPK activation following ATP depletion

Conditions leading to changes in AMP concentrations and AMPK activation are directly related to changes in ATP concentrations, which adenylate kinase translates into relatively larger changes in AMP. Accordingly, ischaemia and mitochondrial inhibitors activate AMPK within a few minutes[39,40] (*Figure 1*). Increased ATP demand also leads to AMPK activation, especially when combined with decreased

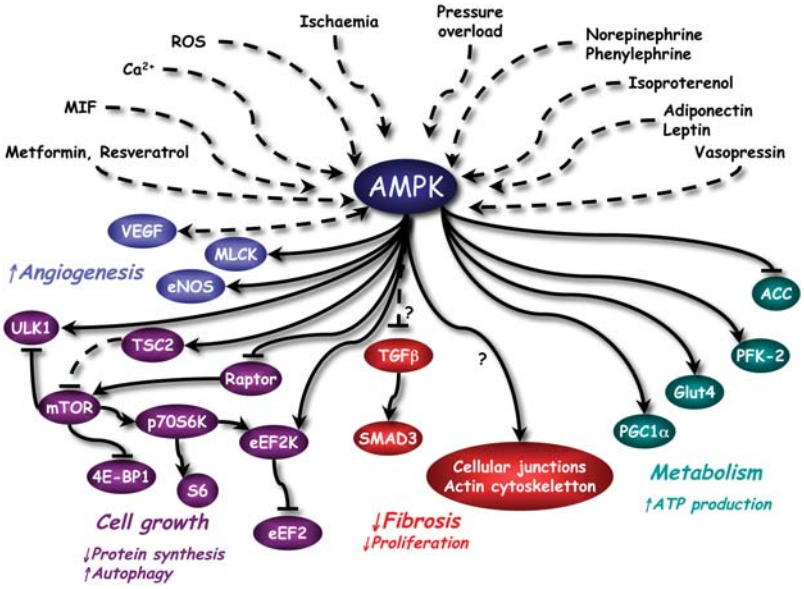

Figure 1 Upstream stimulatory factors and downstream targets of AMPK in the heart. Dashed lines correspond to indirect mechanisms. Question mark signifies that this pathway has not been studied in the heart. Abbreviations: 4E-BP1, 4E binding protein-1;[140] ACC, acetyl-CoA carboxylase;[141] eEF2K, eukaryotic elongation factor 2 kinase;[87,142,143] eNOS, endothelial nitric oxide synthase;[92–94] Glut4, glucose transporter 4;[144] MIF, migration inhibitory factor;[50] MLCK, myosin light chain kinase;[44] mTOR, mammalian target of rapamycin;[145] p70S6K, p70 ribosomal S6 protein kinase;[146] PGC1alpha, peroxisome proliferator-activated receptor gamma co-activator alpha;[77] PFK-2, 6-phosphofructo-2-kinase;[39] ROS, reactive oxygen species;[70-72] S6, ribosomal S6 protein;[147] TGFbeta, transforming growth factor beta;[122] TSC2, tuberous sclerosis factor 2;[85] ULK1, uncoordinated51-like kinase 1;[88-90] VEGF, Vascular endothelial growth factor.[95]

ATP supply, as is the case in contracting skeletal muscle during intense exercise. Remarkably, this is not the case in perfused hearts subjected to high workload, presumably because this organ can adapt its ATP supply to increased energy demand.[41]

3.2.3 Control of AMPK by hormones and agonists

Norepinephrine, phenylephrine, isoproterenol, or vasopressin activate heart AMPK[42–45] (*Figure 1* and *Table 1*). Interestingly, AMPK activation in response to certain cytokines protects the heart against pressure overload or ischaemic injury. The cardioprotective effects of adiponectin[46–48] and leptin,[49] and of the macrophage migration inhibitory factor (MIF), a proinflammatory component released by the ischaemic heart,[50] are mediated, at least in part,[51] by AMPK activation. The precise mechanism of AMPK activation by these agents is however not clear. In addition, AMPK activation also counteracts the angiotensin II-induced hypertrophy.[52] On the other hand, certain hormones inhibit AMPK activation. Insulin antagonizes AMPK activation independently of adenine nucleotide,[53,54] via a hierarchical mechanism whereby phosphorylation by protein kinase B (PKB) of a serine residue (Ser485) in the AMPK alpha subunit prevents subsequent phosphorylation of Thr172 by LKB1.[55] Angiotensin II has also been reported to inactivate AMPK by a still unknown mechanism.[52] But interestingly, if AMPK is activated by a pharmacological agent, it then inhibits cardiac hypertrophy[52] and vascular smooth muscle proliferation induced by angiotensin II.[56]

3.2.4 AMPK activation by pharmacological agents

Among the substances and drugs known to activate AMPK (*Figure 1*), AICA (5-amino-4-imidazole-carboxamide) riboside has been widely used. It is an analogue of adenosine that, in certain cells, is phosphorylated to the corresponding nucleotide, ZMP, which mimics several effects of AMP, including AMPK activation.[57,58] Its use to activate AMPK in cardiomyocytes is not recommended, because of its poor metabolism in these cells,[39,59] and of several unwanted side effects.[60,61] Other more specific tools, such as the Abbott compound A762669, should be preferred.[62,63] Metformin, the most prescribed anti-Type 2 diabetic drug, and its more potent but toxic analogue, phenformin, as well as thiazolidinediones, another class of anti-diabetic drugs, are known to activate AMPK.[64,65] Their initial metabolic effect is to inhibit mitochondrial respiration, thus decreasing ATP production.[66,67] It should however be noted that several effects of metformin are thought to be mediated by p38-MAPK or by an inhibition of mTORC1, independently of AMPK.[68,69] Finally, AMPK could be redox-sensitive: hydrogen peroxide and increased production of ROS activate AMPK,[70,71] possibly by oxidation of two cysteine residues in the alpha subunit of AMPK.[72]

A mechanism connecting caloric restriction and AMPK activation has been described recently. SIRT1, a member of the sirtuin family of NAD-dependent protein deacetylases, is activated by nutrient deprivation and by resveratrol, a cardioprotective polyphenol of red wine. It was initially thought that the anti-ageing effects of resveratrol were mediated by SIRT1.[73] However, resveratrol also activates AMPK, probably by inhibiting mitochondrial respiration,[74] and recent evidence indicates that the protective effect of resveratrol on mitochondrial function is mediated by AMPK, whereas SIRT1 would act downstream of AMPK by de-acetylating PGC1 alpha.[75]

Table 1 Mechanisms of modulation of AMPK activity by hormones and pharmacological agents

Cardiac stimuli	AMPK activity	Mechanisms for modulation of AMPK activity	References
Norepinephrine, Phenylephrine	↑	?	42
Isoproterenol	↑	Phosphorylation of LKB1	43
Vasopressin	↑	?	44,45
Adiponectin	↑	Binding of APPL1 with AMPKα2	46–48
Leptin	↑	?	49
MIF	↑	?	50
H_2O_2 ROS	↑	Inhibition of respiratory chain, AMP↑ ATP↓	70,71
Insulin	↓	Phosphorylation of Ser 485/491	55
Angiotensin	↓	?	52
AICAr	↑	ZMP accumulation	57,58
A762669	↑	Allosteric stimulation	62
Metformin/Phenformin	↑	Inhibition of respiratory chain, AMP↑ ATP↓	64,65
Resveratrol	↑	Inhibition of ATP synthase, AMP↑ ATP↓	74

A762669, Abbott compound A-762669; AICAr, 5-aminoimidazole-4-carboxamide ribonucleoside; APPL1, Adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1; LKB1, also called STK11, serine/threonine kinase 11; MIF, macrophage inhibitory factor; ROS, reactive oxygen species; ZMP, 5-aminoimidazole-4-carboxamide ribonucleotide.

3.3 AMPK targets

3.3.1 Metabolic targets

When activated, AMPK aims at restoring the cellular energy charge by switching off anabolic ATP-consuming pathways, while switching on catabolic ATP-producing pathways. It does so by phosphorylating key metabolic enzymes and transcription factors[8,9] (*Figure 1*). Transcription activation could be mediated through histone H2B phosphorylation.[76] Biosynthetic processes, such as gluconeogenesis, glycogen synthesis, lipogenesis, cholesterol synthesis, and protein synthesis are inhibited, whereas glucose utilization, fatty acid oxidation, and mitochondrial biogenesis are stimulated.[8,9,40] AMPK does not affect the mitochondrial oxidative capacity in the short term. It does however stimulate mitochondrial biogenesis via activation of PGC1 alpha,[77] which is particularly relevant to the energy-deficient failing heart.

3.3.2 Anti-stress effects of AMPK

Recent evidence suggests that AMPK could inhibit (i) endoplasmic stress, although the mechanism remains to be elucidated;[78] (ii) cJUN kinase activation;[79] and (iii) oxidative stress in several cellular models, including cardiomyocytes. The latter could result from the phosphorylation and activation of the forkhead transcription factor 3, which reduces ROS levels by inducing anti-oxidant systems including thioredoxin.[80,81] Finally, AMPK has been reported to inhibit glucose-induced oxidative stress and NADPH oxidase activation in endothelial cells.[82]

3.3.3 Control of protein synthesis, cell growth, and autophagy

AMPK inhibits the mammalian target of rapamycin (mTOR) pathway, which controls protein synthesis and cell growth[9,40,83] (*Figure 1*). This effect is relevant to cardiac hypertrophy. It is mediated by the phosphorylation of upstream controlling elements, such as tuberous sclerosis complex 2 (TSC2) and/or Raptor.[84,85] Downstream of mTOR, p70 ribosomal S6 protein kinase (p70S6K), and 4E-binding protein-1 (4EBP1) are involved in protein translation and cell growth.[83,86] In addition, AMPK directly phosphorylates eukaryotic elongation factor 2 kinase (eEF2K), thereby inhibiting protein elongation through eEF2 phosphorylation.[87] Moreover, AMPK has recently been shown to promote autophagy. It directly phosphorylates and activates ULK1, an initiator of autophagy that is inactivated by mTOR.[88–91] Taken together these data indicate that AMPK inhibits protein synthesis and cell growth and stimulates autophagy.

3.3.4 AMPK and the vascular system

In endothelial cells, AMPK is activated by VEGF and controls eNOS activation.[92,93] eNOS is also known to be a direct target of AMPK in cardiomyocytes,[94] although the role of this phosphorylation is not known. In vascular smooth muscle, direct phosphorylation of myosin light chain kinase by AMPK allows this protein kinase to participate in the control of vascular tone.[44] Interestingly, in skeletal muscle and cardiomyocytes, AMPK activation induces VEGF expression and secretion. The latter plays an important role in muscular adaptation to exercise and coordinates angiogenesis to hypertrophy in response to pressure overload.[95]

4. From hypertrophy to failure

4.1 Cardiac hypertrophy

AMPK is activated in models of chronic pressure overload.[96] This activation is responsible for an increase in glucose metabolism and probably acts as a negative feed-back on hypertrophy by inhibiting the mTOR pathway. Indeed, inhibition of mTOR by its specific inhibitor rapamycin or partial ablation of mTOR blocked p70S6K activation and counteracted the development of cardiac hypertrophy (*Tables 2* and *3*).[97,98] Moreover, pharmacological activation of AMPK inhibits the mTOR pathway and attenuates the development of hypertrophy.[99–101] In addition, in AMPK alpha-2 null mice, cardiac hypertrophy induced by isoproterenol or aortic constriction is significantly larger than in controls and is correlated with p70S6K activation.[102,103] Furthermore, the cardio-specific deletion of LKB1 led to hypertrophy, correlated with a stimulation of mTOR signalling and reduced

Table 2 Changes in cardiac function in AMPK transgenic/KO mice and effects of pharmacological agents

Animal model	Surgery	AMPK activity	Pharmacological treatment	Downstream effects	References
Wild-type (mouse)	TAC	↑	Compound C (inhibits AMPK)	↑ Systolic dysfunction ↑ Hypertrophy, ↓ capillary formation	95
Wild-type (mouse)	CAL	↑	Metformin (activates AMPK)	↓ Systolic dysfunction ↓ Hypertrophy ↑ eNOS phosphorylation ↑ PGC-1α expression, ↑ Mitochondrial respiration	135
AMPKα2-DN(D157A)	CAL	↓	Metformin (no AMPK activation)	No cardioprotective effect	135
AMPKα2-KO	TAC	↓	—	↑ Systolic dysfunction ↑ Hypertrophy, fibrosis	103
AMPKα2-KO	—	↓	Isoproterenol	↑ Hypertrophy ↑ p70S6K activity	102
LKB1-KO (cardiac-specific)	—	↓	—	↑ Systolic dysfunction ↑ Hypertrophy, fibrosis	105
ObR-KO	CAL	↓	AICAr (activates AMPK)	↓ Systolic dysfunction, ↓ hypertrophy ↓ Inflammation, fibrosis, apoptosis	49
APN/KO	TAC	↓	—	↑ Systolic dysfunction ↑ Hypertrophy, ↓ capillary formation	95
Wild-type (dog)	Rapid ventricular pacing	↑	Metformin AICAr (activates AMPK)	↓ Systolic dysfunction ↑ eNOS phosphorylation ↑ Plasma NO levels ↓ Apoptosis	136

AICAr, 5-aminoimidazole-4-carboxamide ribonucleoside; APN, adiponectin; CAL, coronary artery ligation; DN, dominant negative; eNOS, endothelial nitric oxide synthase; KO, knock out; ObR, leptin receptor; p70S6K, p70-ribosomal S6 kinase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; TAC, trans-aortic constriction.

Table 3 AMPK activity in cardiac pathologies

Cardiac pathologies	AMPK activity	Putative role of AMPK	References
Hypertrophy(Left ventricular pressure overload)	↑	Chronically activated, protective	96,101–103,105,132
Ischaemia (*ex vivo, in vivo*)	↑	Acutely activated, protective	34,35,39,48,53,55,79,127–129
Hypertrophic cardiomyopathy (HCM) associated with Wolf–Parkinson–White syndrome	↑ or ↓	Chronically (in)activated, deleterious	See reference[125] for a review

AMPK phosphorylation and could be prevented by overexpressing a constitutively active form of AMPK or by inhibiting mTOR with rapamycin.[104,105] Interestingly, the hypertrophic response to pressure overload is amplified in adiponectin-deficient mice, which exhibit diminished AMPK activity.[106]

4.2 Transition from cardiac hypertrophy to HF

AMPK and its upstream kinase LKB1 not only antagonizes the hypertrophic response, it also delays the transition to HF, as demonstrated by studies resorting to genetic manipulations of these protein kinases. Under normal conditions, the crucial role played by LKB1 in maintaining cardiac function stems from the phenotype following LKB1 deletion. In these LKB1 deficient hearts, the lack of AMPK alpha-2 subunit activation increases mTOR signalling, decreases energy efficiency and VEGF expression, and impairs cardiac function.[104,105,107] Under pathological conditions, as in a model of chronic pressure overload, the lack of AMPK alpha-2 exacerbates hypertrophy and favours the transition to HF.[103] In addition, disruption of the coordination between angiogenesis and hypertrophy is another crucial factor in the pathological transition to HF.[108] And in adiponectin-deficient animals, the lack of AMPK activation by this hormone exacerbates the transition to HF in pressure overloaded hypertrophied hearts due to an angiogenesis deficiency (*Tables 2* and *3*).[109]

As regards energy depletion, AMPK activation, which is expected in energy deficient hearts, is not sufficient to maintain ATP. Several reports indicate that expression of PPAR alpha and PGC1 alpha is decreased and may explain the low-energetic state of the failing heart.[25] However, although PGC1 alpha down-regulation contributes to energy deficiency, it does not suffice by itself to induce HF. In two genetic models of PGC1 alpha deficiency, the overall metabolic disturbances did not lead to HF, except when the hearts were submitted to chronic haemodynamic overloads.[110–112] Thus energy deprivation

alone is not sufficient to cause HF but may contribute to the mal-adaptative response of the heart. It follows that the pathological transition from compensated cardiac hypertrophy to HF implies more than energy depletion.

4.3 Remodelling and fibrosis

Cardiac remodelling occurs following any form of cardiac injury[113] and remodelling of the extracellular matrix (ECM) contributes to contractile dysfunction.[114] It develops in response to increased ventricular walls tension and to different hormones (including angiotensin, catecholamines, and endothelins) and inflammatory cytokines (IL1-b, TGF-b, TNF-a, IL-6 . . .).[115,116] Myocardial fibrosis is a pathological entity of ECM remodelling, which contributes to HF by increasing myocardial stiffness and reducing pumping capacity.[117] The synthesis and turnover regulation of ECM components constitute the primary role of cardiac fibroblasts (CFs), which represent 26–63% of cells within the myocardium of mouse and rat, respectively.[118,119]

Angiotensin II is a critical mediator of cardiomyocyte hypertrophy and cardiac fibrosis.[120] AMPK could interfere with this phenomenon by inhibiting the angiotensin II-induced stimulation of proliferation via a cross-talk with extracellular signal-regulated kinase (ERK), as shown in CFs.[121] Interaction with myodifferentiation has also been studied in mesangial cells in which AMPK inhibits TGF-b-induced smad3-dependent transcription.[122] Finally, AMPK could alter cell–cell or ECM–cell communication in the heart by modulating assembly of cellular junctions, as it does in epithelial kidney cells.[123,124] Together, these *in vitro* results suggest that AMPK activators might have therapeutic potential for HF, in terms of cardiac fibrosis.

5. Cardio-protective effects of AMPK

5.1 Protection against ischaemia/reperfusion injury

The cardioprotective effect of AMPK in ischaemic hearts is well documented and involves a stimulation of glucose uptake and glycolysis.[39,125] However, during reperfusion with fatty acids, AMPK favours fatty acid oxidation, which inhibits glucose oxidation and may decrease cardiac efficiency.[126,127] In mice lacking AMPK alpha-2 or expressing a cardio-specific dominant negative mutant of the same subunit, the ischaemia-induced stimulation of glucose uptake and glycolysis was inhibited leading to ATP depletion and ischaemic contracture, which were obviously not prevented by the residual activity of the AMPK alpha-1.[35,128–130] Similarly, the infarct size following coronary ligation was larger in mice expressing a dominant negative AMPK than in controls (*Tables 3* and *4*).[131]

5.2 Potential protection by hormones and pharmacological activators against transition to HF

Several indirect arguments indicate that AMPK could prevent the deleterious effects of hypertrophy on cardiac metabolism and function. Leptin has been reported to protect against cardiac injury in the failing heart by increasing STAT-3 and AMPK activation. It diminished cardiac hypertrophy, inflammation, and cardiac dysfunction.[49] Similarly, adiponectin could prevent the transition between cardiac hypertrophy to HF by promoting an AMPK-dependent angiogenic regulatory axis[95] and/or by inhibiting NF-kappaB activation.[132] It has also been reported that the metabolic changes and hypertrophy induced by angiotensin II in cultured H9C2 cardiomyocytes are prevented by AMPK activation.[52]

A large number of papers report the beneficial effects of metformin (*Table 2*). For example, clinical studies have shown that metformin is cardioprotective and improves outcomes in patients with HF.[133,134] In addition, metformin exerts beneficial effects on cardiac function and survival in murine models of HF.[135] The effects of MF are mediated, at least in part, by activation of AMPK[40,51,65] and, interestingly, the cardioprotective effects of MF on murine models of HF are mediated by AMPK activation.[135] In dogs, metformin attenuated oxidative stress-induced cardiomyocyte apoptosis and prevented the progression of HF along with AMPK activation.[136] In addition, metformin could also affect the fibrotic response induced by pressure overload.[137] The protection results from an inhibition of the

Table 4 Changes in metabolism and function in hearts of AMPK transgenic/KO mice submitted to ischaemia

Animal model	Surgery/perfusion	AMPK activation	Pharmacological treatment	Downstream effects	References
AMPKα2-DN (D157A)	No flow ischaemia (*ex vivo*)	↓	—	↓ Glucose uptake More rapid ischaemic contracture	130
AMPKα2-DN (D157A)	CAL	↓	Metformin	↑ Myocardial infarct size	131
AMPKα2-DN (K45R)	No flow ischaemia	↓	—	↓ Glucose uptake, glycolysis ↓ Myocardial function recovery	128
AMPKα2-KO	No flow ischaemia	↓	—	↓ Glycogen content, ↓ glycolytic flux More rapid ischaemic contracture No effect on myocardial function recovery	35
AMPKα2-KO	Low-flow ischaemia	↓	–	↓ Glucose uptake More rapid ischaemic contracture Delayed post-ischaemic contractile recovery	129

CAL, coronary artery ligation.

TGF-beta-smad-signalling pathway but is however independent of AMPK activation.[137]

6. Conclusions and perspectives

Despite its known involvement in energy homeostasis, AMPK activation fails to restore energy balance in the failing heart. Once de-compensated, the heart takes little advantage from AMPK, probably because other deficiencies have brought the failing heart to a point of no return. Clearly, the cardio-protective effects of AMPK activators are obtained on the long term by preventing or delaying the pathological transition from hypertrophy to HF. Whether these beneficial effects only result from energetic recovery of the heart remains to be demonstrated. We speculate that they could instead result from non-metabolic effects, which include anti-proliferative, anti-fibrotic, and angiogenic effects of AMPK.

The evidence for cardioprotective effects of AMPK is only circumstantial and indirect. It relies on the use of pharmacological drugs, with off-target effects, and on the phenotype analysis of mice with whole-body deletion of AMPK alpha subunits. To validate AMPK as a potential target in HF progression, new AMPK-specific, and ideally heart-specific, AMPK activators are needed but remain to be discovered. Similarly, our understanding of the importance of AMPK in the transition between hypertrophy and HF would benefit from the study of mice with a heart-specific deletion of AMPK specific isoforms. In addition, a comprehensive and comparative analysis of the various AMPK isoforms expressed in mice and humans could help to improve an AMPK-mediated therapeutic approach.[138] Hopefully, the tools to achieve these goals do not seem out of reach.

Finally and as stated in the introduction, HF is a many-sided muscular disorder in which chronic energy deficit is but one aspect. Dysfunctions of calcium handling and of the contractile machinery are integral parts of HF and go together with energy deficit. And the lack of coordination between contraction, calcium, and energy in HF has been appropriately called 'failing complexity'.[139] As far as we know, the pathological transition to HF is multifactorial and cannot be reduced to a single preponderant disturbed event. To understand this transition and hence to develop a coherent therapeutic approach, the temporal changes of these disturbances should be analysed by systems level integration.[4,139]

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