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AMPK: Lessons from transgenic and knockout animals

Benoit Viollet^{1,2}, Yoni Athea^{3,4}, Rémi Mounier^{1,2}, Bruno Guigas^{5,6}, Elham Zarrinpashneh⁷, Sandrine Horman⁵, Louise Lantier^{1,2}, Sophie Hébrard^{1,2}, Jocelyne Devin-Leclerc^{1,2}, Christophe Beauloye⁷, Marc Foretz^{1,2}, Fabrizio Andreelli^{1,2,8}, Renée Ventura-Clapier^{3,4} and Luc Bertrand⁷

¹Institut Cochin, Université Paris Descartes, CNRS (UMR 8104), Paris, France.

²Inserm, U567, Paris, France.

³Inserm, U769, Châtenay-Malabry, France.

⁴Univ Paris-Sud, Châtenay-Malabry, France.

⁵Université catholique de Louvain and Institute of Cellular Pathology, Hormone and Metabolic Research Unit, Brussels, Belgium.

⁶Leiden University Medical Center, Department of Molecular Cell Biology, Leiden, Netherlands

⁷Université catholique de Louvain, Division of cardiology, Brussels, Belgium.

⁸Inserm U695, IFR Xavier Bichat, Paris, France.

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Abstract

AMP-activated protein kinase (AMPK), a phylogenetically conserved serine/threonine protein kinase, has been proposed to function as a 'fuel gauge' to monitor cellular energy status in response to nutritional environmental variations. AMPK system is a regulator of energy balance that, once activated by low energy status, switches on ATP-producing catabolic pathways (such as fatty acid oxidation and glycolysis), and switches off ATP-consuming anabolic pathways (such as lipogenesis), both by short-term effect on phosphorylation of regulatory proteins and by long-term effect on gene expression. Numerous observations obtained with pharmacological activators and agents that deplete intracellular ATP have been supportive of AMPK playing a role in the control of energy metabolism but none of these studies have provided conclusive evidence. Relatively recent developments in our understanding of precisely how AMPK complexes might operate to control energy metabolism is due in part to the development of transgenic and knockout mouse models. Although there are inevitable caveats with genetic models, some important findings have emerged. In the present review, we discuss recent findings obtained from animal models with inhibition or activation of AMPK signaling pathway.

Introduction

To sustain metabolism, intracellular ATP concentration must be maintained within a narrow range. This is achieved both at the cellular level as well as the systemic level, encompassing intracellular regulation of anabolic and catabolic pathways in addition to substrate storage and release. It has been recently described how this co-ordination may be achieved through the functions of AMP-activated protein kinase (AMPK), a phylogenetically conserved serine/threonine protein kinase. AMPK has been viewed as a signal integrator monitoring systemic and cellular energy status (1). This kinase is typically, but not exclusively, activated by an increase AMP/ATP ratio. Once activated, AMPK regulates a large number of downstream targets, shutting down anabolic pathways and stimulating catabolic pathways, thus simultaneously sparing limited energy resources and acquiring extra energy. A synergistic response entails inhibition of anabolic pathways that mediate the synthesis of macromolecules such as protein, fatty acids, lipids, cholesterol and glycogen. Concomitantly AMPK stimulates energy providing pathways like β -oxidation, glucose uptake and cardiac glycolysis, the net result of AMPK activation being stabilisation of ATP levels and restoration of energy balance. AMPK acts first by directly affecting key enzymes activities, *e.g.* in glucose and fat metabolism, and second by longer-term transcriptional control of key players of these metabolic pathways. Important progress has recently been made in the understanding of the pathophysiological role of AMPK at both the cellular and whole organism level. This is in part due to the development of transgenic and knockout (KO) mouse models, which have made it possible to study distinct physiological functions for AMPK isoforms.

Structure and function of AMPK complexes

AMPK exists as a heterotrimeric complex consisting of a catalytic subunit α and two regulatory β and γ subunits (1). The conventional serine/threonine kinase activity of AMPK is supported by α subunit which is characterized by the presence in the activation loop of a threonine residue (Thr172) whose phosphorylation is required for activation. The C-terminal region of α subunit is required for the association with the other two β and γ subunits. The β subunit contains a C-terminal region required for the association with α and γ subunits and a central region that allowed AMPK complex to bind glycogen. The γ subunit contains four tandem repeats known as cystathionine β -synthase (CBS) motifs which bind, together, two molecules of AMP or ATP in a mutually exclusive manner. Binding of AMP (on γ subunit)

activates AMPK via a complex mechanism involving direct allosteric activation and phosphorylation of α subunit on Thr172 by upstream kinases as the protein kinase LKB1 (a tumour suppressor whose germline mutations in humans are the cause of Peutz-Jeghers syndrome), the CaMKKII β (calmodulin-dependent protein kinase kinase II β) and perhaps also TAK1 (mammalian transforming growth factor β -activated kinase) (1). Although it was originally proposed that AMP binding promoted AMPK phosphorylation by upstream kinases, recent works suggested that it occurs entirely by inhibiting dephosphorylation of Thr172, probably catalysed by protein phosphatase 2C α (2, 3).

Homologues of all three subunits have been identified in mammals, fruitfly (*Drosophila melanogaster*), worm (*Caenorhabditis elegans*), yeast (*Saccharomyces cerevisiae*), plants (*Arabidopsis thaliana*) and the primitive protozoon *Giardia lamblia*, with a high degree of conservation that suggests that this ancient signaling circuit evolved at least a billion years ago to regulate a wide spectrum of actions on metabolic homeostasis. In mammals, two to three isoforms of each subunit (α 1, α 2, β 1, β 2, γ 1, γ 2, γ 3) encoded by different genes are known giving rise to a large variety of heterotrimeric combinations, with splice variants (for the γ 2 and γ 3 genes) adding to the diversity. Furthermore, differences in the tissue distribution of catalytic and regulatory isoforms expression patterns have been reported (1). Thus, recent investigation of isoform composition of AMPK complexes in human skeletal muscle found that only 3 of the 12 theoretically possible AMPK complexes were present (α 2 β 2 γ 1 >> α 2 β 2 γ 3 = α 1 β 2 γ 1) and were activated differently depending on exercise intensity and duration (4-6). Moreover, a specificity of each catalytic isoforms for their preferentially upstream kinase has been clearly shown both in skeletal muscle (7) and heart (8). Indeed, in LKB1 $^{-/-}$ mice, ischemia in the heart and contraction in skeletal muscle were no more able to activate AMPK α 2 subunit whereas AMPK α 1 activation is only slightly affected. Interestingly, expression of the γ 3 subunit appeared highly specific to glycolytic skeletal muscle whereas γ 1 and γ 2 showed broad tissue distributions. In skeletal muscle, the β 2 subunit is also highly expressed but the β 1 subunit predominates in the liver. AMPK α 1 and α 2-containing complexes account each for about half of total AMPK activity in liver. In adipose tissue, AMPK complexes containing the α 1 catalytic subunit are mainly expressed whereas, in skeletal and cardiac muscles, AMPK complexes containing the α 2 catalytic subunit are predominant. In addition of differences in tissue distribution, it is now accepted that AMPK complexes distribution is also regulated at the intracellular level. Indeed, AMPK α 2-containing complexes were found in both the nucleus and the cytoplasm raising the

possibility of the direct phosphorylation of co-activators and transcription factors (9, 10). In contrast, AMPK α 1-containing complexes are predominantly localized in the cytoplasm but have been also observed at the plasma membrane in airway epithelial cells (11) and carotid body cells (12). Although the functional significance of different AMPK isoform combination remains unclear, it raised important questions about the function of each heterotrimeric AMPK complex in relation with their particular sensitivity to AMP and ATP, subcellular localization and/or specific targets (13). As a matter of fact, it has been hypothesized that regulation of exercise-induced glucose transport in human skeletal muscle could be rather associated with α 2 β 2 γ 1 than α 2 β 2 γ 3 heterotrimeric complex activation (6). Recently, it has been suggested that isoform combination may also determine subcellular targeting of AMPK and hence targeting substrates. Very fascinatingly, it has been demonstrated that post-translational modification of the β 1 subunit may target AMPK complexes to the plasma membrane (14). In addition, it was found that plectin, a cytoskeleton linker protein which has been shown to bind the γ 1 subunit, affects the subunit composition of AMPK complexes in differentiated myotubes (15). Thus, the selective expression of a particular heterotrimeric AMPK complex could determine a specialized cellular and systemic response to different metabolic stresses.

Development of animal models for the study of AMPK functions

As most of the action of AMPK has been described on the basis of incubation of eukaryotic cells with pharmacological activators of AMPK such as 5-aminoimidazole-4-carboxamide riboside (AICAR) and the antidiabetic drug metformin (16), it has been difficult to assign specific functions for AMPK heterotrimeric complexes since pharmacological activation of AMPK would supposedly concern all AMPK complexes. To answer this question, the use of mouse transgenic and KO models (Table 1A and 1B) has proved useful in this respect. The generation of catalytic subunit (α 1 $^{-/-}$ and α 2 $^{-/-}$) knockout animals has emphasized the distinct and critical role for AMPK α 1 and AMPK α 2 catalytic subunit in the control of energy metabolism. Unfortunately, a double knockout is embryonic lethal at ~10.5 days post-conception (BV, unpublished results), but the development of tissue-specific and conditional knockout for AMPK α 2 isoform has allowed the generation of animal models completely lacking hepatic AMPK activity (AMPK α 1 α 2 $_{LS}^{-/-}$ mice) by crossing liver-specific AMPK α 2 $^{-/-}$ mice with AMPK α 1 $^{-/-}$ mice. In addition, conditional knockout for AMPK α 1 isoform are

also now being generated (BV and MF, unpublished results) and will allow the establishment of tissue-specific deletion of both AMPK α 1 and α 2 catalytic subunits in the near future. Another approach to suppress AMPK activity *in vivo* is the generation of transgenic mice over-expressing a kinase-inactive mutant α subunit acting as a dominant-negative form. This strategy has been used to inhibit AMPK in heart and skeletal muscle. Genetic models with deletion of the β subunit isoforms of AMPK have been recently generated and have revealed that AMPK β subunits are unable to compensate for the loss of each other at least in liver and skeletal muscle (B. Kemp, personal communication). Ablation of AMPK γ 3 subunit expression has also been realized making available another model with an altered AMPK signaling pathway in skeletal muscle. Furthermore, the introduction of transgenic mouse models involving the γ isoforms harbouring naturally occurring mutations in human γ 2 and pig γ 3, leading to metabolic abnormalities in heart and skeletal muscle, respectively, has enhanced our understanding of AMPK signaling in these tissues. Animal models over-expressing constitutively active forms of AMPK (mutated form of the α 2 or γ 1 subunit) in liver and skeletal muscle have been also generated and provide useful models for the study of acute and chronic activation of AMPK. In addition to all these mammalian genetic models, the consequence of AMPK deletion or over-expression in the *C. elegans* and *Drosophila* model systems has been described very recently and gain insight into the multiple roles of AMPK in the regulation of energy metabolism and other physiological processes.

Distinct physiological roles for α 1/ α 2 catalytic subunits of AMPK in the control of whole-body energy metabolism and insulin sensitivity

Important progress has recently been made in the understanding of distinct physiological functions for AMPK α subunits thanks to the metabolic exploration of AMPK α 1 $^{-/-}$ and AMPK α 2 $^{-/-}$ mice (17, 18). An important issue was to investigate the specific targets and roles for α 1 and α 2 catalytic subunits. To firstly examine this point, AICAR tolerance tests were performed in both AMPK α 1 $^{-/-}$ and AMPK α 2 $^{-/-}$ mice. Interestingly, AMPK α 2 $^{-/-}$ but not AMPK α 1 $^{-/-}$ mice are resistant to hypoglycemic AICAR effects (17, 18) suggesting that AMPK α 2-containing complexes are the main contributors of AMPK activation during AICAR stimulation at the whole-body level. This is remarkable if we consider that both catalytic subunits are present in the most important tissues (as skeletal muscle and liver) for the regulation of glucose homeostasis. Thus, if AMPK α 2 $^{-/-}$ mice are resistant to AICAR hypoglycemic action, this suggested that remaining complexes containing AMPK α 1 could

not fully compensate for the lack of AMPK α 2. Furthermore, no experimental data (and especially from *in vitro* studies) suggested that sensitivity to AMP (or ZMP) would be modulated by isoform composition of AMPK complexes. Discrepancy between α 1 and α 2 catalytic subunits not only concerns AICAR hypoglycemic action. Indeed, whole-body deletion of AMPK α 1 has no detectable metabolic phenotype whereas whole-body AMPK α 2 deletion results in mild insulin-resistance and in impaired glucose tolerance associated with insulin secretory defect (17, 18). These metabolic alterations are both features of type 2 diabetes and emphasize AMPK α 2 $^{-/-}$ mice as a new animal model of this disease. But, in contrast to the multiple defects in intracellular insulin pathway observed in type 2 diabetes, AMPK α 2 $^{-/-}$ mice do not display typical features of this disease at a molecular level. In particular, insulin release by isolated pancreatic islets and insulin-stimulated glucose uptake by isolated muscles were all normal (18). Normal glucose tolerance in transgenic mice overexpressing a kinase-dead AMPK α 2 mutant (K45R mutation) in cardiac skeletal muscle (AMPK α 2-KD) (19) argues against the hypothesis that muscular AMPK is sufficient by itself to deteriorate the glucose homeostasis (18). Another known important mechanism of insulin resistance in skeletal muscle is increased lipid availability or increased muscular uptake of free fatty acids (FFA). Surprisingly, intramuscular lipid content in AMPK α 2 $^{-/-}$ and control mice were comparable indicating no alteration in lipid oxidation ability in AMPK α 2 $^{-/-}$ mice. This conclusion is also supported by similar 24-hour respiratory quotient and energy expenditure in AMPK α 2 $^{-/-}$, AMPK α 1 $^{-/-}$ and control mice (Table 2). Thus, lipid oxidation cannot be altered at the whole-body level by the deletion of either AMPK α 2 or AMPK α 1 subunits. At this time, how can one explain that lack of AMPK α 2 subunit can alter both insulin sensitivity and insulin secretion *in vivo*? It is now established that *in vivo* impaired insulin action and reduced insulin secretion observed in AMPK α 2 $^{-/-}$ mice are both linked to altered function of the autonomic nervous system in integrating peripheral metabolic signals. Indeed, increase in catecholamine release in AMPK α 2 $^{-/-}$ mice could explain development of insulin resistance and glucose intolerance *in vivo* (18). This suggested that the hypothalamic AMPK α 2 contributes to the balance between the whole-body metabolic changes and the sympathetic tone which in turn regulates the peripheral metabolism.

Role of AMPK in hepatic metabolism

Hepatic metabolism plays a key role in the control of whole-body energy status since liver is the major site for storage and release of carbohydrates and for the synthesis of fatty acid. In the liver, AMPK coordinates the changes in the activity of enzymes of the lipid metabolism and, so, regulates the partitioning of fatty acids between oxidative and biosynthetic pathways. In the cholesterol synthesis pathway, AMPK phosphorylates and inhibits hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase blocking the conversion of HMG-CoA to mevalonate. In total and liver-specific AMPK α 2 $^{-/-}$ mice, plasma levels for total and HDL cholesterol are not statistically different compared to controls but have a tendency to be higher. This suggested that remaining α 1 subunit activity in AMPK α 2 $^{-/-}$ mice is sufficient to control hepatic cholesterol synthesis and that HMG-CoA reductase is probably a target for both AMPK catalytic subunits. Acetyl-CoA carboxylase (ACC) is an important rate-controlling enzyme for the synthesis of malonyl-CoA, which is both a critical precursor for biosynthesis of fatty acids and a potent inhibitor of mitochondrial fatty acid oxidation. Phosphorylation and inhibition of ACC by AMPK leads to a fall in malonyl-CoA content and a subsequent decrease in triglyceride synthesis concomitantly with an increase in β -oxidation. This was first evidenced by the decrease in plasma triglyceride levels during AICAR infusion in lean and obese rodents. These results are consistent with *ex vivo* findings demonstrating the AICAR-induced inhibition of mitochondrial glycerol-3-phosphate acyltransferase (GPAT) activity and subsequent inhibition of triacylglycerol synthesis (20). In addition, overexpression of AMPK α 2 in the liver decreases plasma triglyceride levels and increases plasma ketone bodies levels, a surrogate marker for hepatic β -oxidation (21). Conversely, liver-specific AMPK α 2 deletion leads to increased plasma triglyceride levels and reduction in plasma ketone bodies levels. This emphasizes the critical role for AMPK α 2 subunit in the control of the balance between hepatic lipogenesis and β -oxidation.

As reported recently, AMPK has been also involved in the control of mitochondrial biogenesis in the liver. First evidences were brought by treatment with resveratrol, a polyphenol constituent of red wine, which increases mitochondrial number in liver in association with AMPK activation (22). Second, AMPK α 1 α 2 $_{LS}$ $^{-/-}$ mice have reduced mitochondrial biogenesis as suggested by decreased transcript and protein expression of key mitochondrial constituents such as peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α), cytochrome c oxidase I (COX I), COX IV and cytochrome c genes (23). Interestingly, both mitochondrial respiration and basal level of ATP were significantly lower in hepatocytes isolated from AMPK α 1 α 2 $_{LS}$ $^{-/-}$ mice compared with control mice (24). This

result emphasizes the importance of AMPK in the regulation of cellular energy homeostasis via the control of adaptive mitochondrial function. Thus, diminished AMPK activity may be an important contributing factor in the reduced mitochondrial function and dysregulated intracellular lipid metabolism associated with hepatic insulin resistance.

Recent results from various animal models confirm the physiological importance of hepatic AMPK for whole-body glucose homeostasis. It has been first shown that systemic infusion of AICAR in normal and insulin-resistant obese rats leads to the inhibition of hepatic glucose production (HGP) (25). Short-term activation of AMPK specifically in the liver by adenovirus-mediated expression of a constitutively active form of AMPK α 2 (AMPK α 2-CA) is sufficient for controlling hyperglycemia in murine models of diabetes (21). Liver-specific deletion of AMPK α 2 results in mild hyperglycemia and glucose intolerance due to increased fasted HGP (26). These data indicate that remaining hepatic AMPK α 1 is not sufficient to control HGP in the post-absorptive state. Thus, these results demonstrate that hepatic AMPK α 2 is essential to control HGP and maintain fasting blood glucose levels in the physiological range. In addition, it has been also recently demonstrated that in the absence of hepatic LKB1, AMPK was almost completely inactive and fasting blood glucose levels were highly increased (27). In liver-specific LKB1 $^{-/-}$ mice, the antidiabetic drug metformin no longer reduced blood glucose levels providing the apparent possibility that LKB1-mediated activation of AMPK in the liver might be required to lower blood glucose levels (27). Knowing the fact that LKB1 regulates thirteen different downstream protein kinases including AMPK, these data raised the question whether glucose-lowering function of LKB1 is mediated by one or several of these AMPK-related kinases rather than AMPK itself. In order to evaluate the respective contribution of AMPK in liver and skeletal muscle for the control of blood glucose levels, mice lacking both α 1 and α 2 catalytic subunits in the liver (AMPK α 1 α 2 $_{LS}$ $^{-/-}$) were submitted to hypoglycemic effect of AICAR. AMPK α 1 α 2 $_{LS}$ $^{-/-}$ mice showed severe AICAR resistance (Figure 1). Since AICAR effect in skeletal muscle is similar in both AMPK α 1 α 2 $_{LS}$ $^{-/-}$ and control mice, the resistance to the hypoglycemic effect of AICAR observed in AMPK α 1 α 2 $_{LS}$ $^{-/-}$ mice is due to the lack of hepatic AMPK. The difference between the glycemic excursion in AMPK α 1 α 2 $_{LS}$ $^{-/-}$ and control mice corresponds to the contribution of hepatic AMPK for the inhibition of HGP. Thus, the participation of the liver in AICAR hypoglycemic action represents about 40% (Figure 1), indicating that hepatic AMPK has a crucial role in the control of blood glucose levels. Furthermore, the potent effects of circulating adipocyte-derived hormones on whole-body glucose metabolism

recently highlighted the involvement of AMPK in the control of glucose output by the liver. Indeed, a physiological link has been established between increased resistin plasma levels (as observed in insulin resistant rodent models) and increased liver glucose output through the inhibition of AMPK activity (28). Moreover, it has been recently demonstrated that hypoglycemic effect of adiponectin appears to be mediated by hepatic AMPK activation (29). This was corroborated with the incapacity of adiponectin to regulate HGP in the absence of the AMPK α 2 subunit in the liver (26).

Although the action of AMPK in systemic energy balanced is achieved by rapid and direct phosphorylation of metabolic enzymes, long-term effects has also been clearly demonstrated on glycolytic and lipogenic gene expression in the liver by adenovirus-mediated gene transfer of AMPK α 2-CA (21). Obese *ob/ob* mice provide an animal model of non-insulin dependent diabetes mellitus, exhibiting hyperglycemia, hyperinsulinemia and obesity. *ob/ob* mice maintains a high level of hepatic lipogenesis linked with increased expression of lipogenic and glycolytic genes. Overexpression of AMPK α 2-CA in the liver of *ob/ob* mice allows the normalization of expression pattern of these genes (Figure 2). Of note, AMPK activation reduces expression of sterol regulatory element-binding protein-1c (SREBP1c) and carbohydrate response element-binding protein (ChREBP), transcription factors playing a key role in the transcriptional regulation of lipogenic and glycolytic genes by insulin and glucose, respectively. Polyunsaturated fatty acids (PUFAs) are also known to exert repression on glycolytic and lipogenic gene and raised the question about a role of AMPK in mediating the effect of PUFAs on gene transcription. The ability of PUFAs to directly modulate AMPK activity remains controversial and to address the role of AMPK in the inhibitory effect of PUFAs in liver, a series of experiments have been performed in AMPK α 1 $^{-/-}$, AMPK α 2 $^{-/-}$ and AMPK α 1 α 2 $_{LS}^{-/-}$ mice. Under both *in vivo* and *in vitro* experimental conditions, no change in AMPK activation was observed in the presence of PUFAs (30). Indeed, results obtained in AMPK KO mice clearly show that AMPK is not involved in the control of ChREBP nuclear translocation and in mediating the negative effect of PUFAs on both glycolytic and lipogenic gene expression (9, 30). Together, these data demonstrate that PUFAs inhibit ChREBP nuclear translocation and repress glycolytic/lipogenic gene expression by an AMPK-independent mechanism. AMPK has been also implicated in the transcriptional regulation of drug-metabolizing enzyme cytochrome P450 (CYP) gene family. The CYP genes plays a crucial role in the transformation of xenobiotics by the liver and their expression is strongly induced in response to phenobarbital (PB) administration. In

AMPK α 1 α 2^{LS}^{-/-} mice, PB failed to induce the expression of CYP genes indicating that AMPK play a crucial role in the regulation of drug metabolism (31).

Role of AMPK in adipose tissue

Adipocytes have both metabolic and endocrine functions. Energy storage under food availability is a determinant of survival in period of increased energy expenditure or fasting. Triacylglycerol (TAG) exists as the most efficient macromolecule for this storage. When needed, triglycerides are hydrolyzed (lipolysis) into fatty acids and glycerol which are exported back into the blood. It has been reported that activation of AMPK in adipocytes results in the inhibition of both lipolysis and lipogenesis by regulating directly the enzymes engaged in lipid metabolism (32), as well as by downregulating PPAR γ expression (33). Another consequence of AMPK activation in adipose tissue is an increase in fatty acid oxidation. This has been demonstrated when the uncoupling mitochondrial protein UCP-1 is overexpressed in adipocytes. In this case, the resulting activation of AMPK and inactivation of ACC (34) leads to a fall in malonyl-CoA content and a subsequent increase in mitochondrial fatty oxidation via the allosteric regulation of carnitine palmitoyltransferase-1 (CPT-1) which catalyzes the entry of long chain fatty acyl-CoA into mitochondria. In addition, UCP-1 overexpression is concomitant to mitochondrial biogenesis and, so, promotes fatty acid oxidation process (35). A crucial function of adipocytes is to release stored triglycerides when needed. AMPK activation in adipocytes, using AICAR or overexpression of a constitutively active form of AMPK has been shown to inhibit β -adrenergic-induced lipolysis (32, 36, 37). This correlates with decreased plasma triglycerides, fatty acid concentration and glycerol turnover in both lean and obese rats infused with AICAR (25). This is explained by both HSL phosphorylation on Ser565 in adipocytes and reduction of isoproterenol-induced HSL translocation to the lipid droplet (37). Whether perilipin is a target of AMPK is presently unknown. Interestingly, in mice lacking the predominant AMPK α 1 isoform, the size of adipocytes is reduced and both basal and isoproterenol-induced lipolysis is higher than that of control adipocytes (37). Even if AMPK α 1 subunit is predominant in adipocytes, deletion of AMPK α 2 subunit is also followed by modifications in adipose tissue. Indeed, AMPK α 2^{-/-} mice submitted to high fat diet exhibited increased body weight and fat mass (38). The increase in adipose tissue mass was due to the enlargement of the pre-existing

adipocytes with increased lipid accumulation. This study demonstrates that lack of AMPK α 2 subunit may be a factor contributing to the development of obesity.

Role of AMPK in skeletal muscle

In skeletal muscle, AMPK is activated in response to both endurance exercise (*e.g.* in rat muscle during treadmill running and in human muscle during cycle exercise) (39, 40) and *ex vivo* contraction (electrical stimulation) (41). AMPK is therefore believed to be an important signaling molecule in regulating muscle metabolism during exercise (42). Indeed, a decrease of the voluntary activity of AMPK α 2-KD mice, named “lazy mice” for this reason, has been reported (19). Moreover, muscle-specific transgenic mice expressing an inactive AMPK α 2D157A mutant (AMPK α 2i) have significantly lower exercise tolerance (43) suggesting a direct role of AMPK in the adaptation to exercise. Surprisingly, AMPK α 2-/- mice ran the same distance per day at the same maximal speed as their littermate controls over 8 weeks of running wheels training (Figure 3A and B). The sole effect of AMPK α 2 deletion, if any, was a decrease in the stride length for AMPK α 2-/- mice, which was compensated by an increase in number of runs compared to the controls (Figure 3C and D). Thus, the lack of AMPK α 2 would not change the mice ability to perform physical exercise and training. Accordingly, no alteration in exercise-induced activation of metabolic genes in skeletal muscle has been detected in control and AMPK α 2-/- mice during voluntary wheel activity (44) or forced activity on treadmill (45). In AMPK α 2-KD and AMPK α 2i mice, it has also been reported similar results with a normal increase of glucose transporter GLUT4 mRNA after treadmill running (46). However, in AMPK α 2i mice, exercise training did not upregulate hexokinase II gene expression (47) suggesting increased capacities for glucose phosphorylation as an adaptation to endurance training. This discrepancy could be due to differences in the expression of the remaining AMPK α 1 subunit in these various genetic animal models. AMPK α 1 subunit is still present in AMPK α 2-/- and AMPK α 1 activity can be detected in AMPK α 2-KD mice and may sustain alone the coordination of muscle metabolism and adaptation to exercise. It has been reported that AMPK α 1 activity is higher in AMPK α 2-/- muscle as compared to control muscles, suggesting a compensatory effect (44). Nevertheless, recent studies using transgenic mice over-expressing an active γ 1R70Q mutant in skeletal muscle supported the critical role of AMPK in training adaptation. Indeed, chronically increased muscle AMPK activity significantly rised the relative proportion of type

Ila/x fibers and the activity of mitochondrial markers in sedentary transgenic animals compared to their sedentary controls, without any further increase with exercise training (47). Moreover, the proportion of type Ila/x fibers in trained AMPK α 2i mice was significantly lower than in their trained controls but no difference for mitochondrial markers was observed in response to exercise training (47).

While it has been known for more than 75 years that physical activity is associated with increased mitochondrial content in muscle, the molecular mechanisms for this adaptive process has only recently been partly elucidated. The first major regulator of mitochondrial biogenesis discovered was PGC-1 α which induces mitochondrial biogenesis by activating the transcription of nuclear respiratory factors 1 and 2 and of mitochondrial transcription factor A whose responsive elements have been found in the gene promoters of a number of mitochondrial proteins. Interestingly, activation of AMPK with AICAR (48) or with β -guanidinopropionic acid (β -GPA) (49), a creatine analogue that is known to induce muscle adaptations similar to those induced by exercise training, led to activation of the mitochondrial transcription cascade. Furthermore, activation of AMPK by physical exercise or by over-expressing an active γ 1R70Q mutant in muscle has been associated with an increase in PGC-1 α and mitochondrial markers gene expression, making a direct link between AMPK and mitochondrial biogenesis (50, 51). A recent report revealed that PGC-1 α is required for AMPK-dependent activation of gene expression, including PGC-1 α itself, GLUT4 and mitochondrial genes (10). In addition, AMPK directly phosphorylates PGC-1 α suggesting that posttranslational modifications of PGC-1 α may participate in the regulatory functions of AMPK in skeletal muscle, integrating environmental changes into the corresponding metabolic adjustments (10). Accordingly, a decrease in the expression of PGC-1 α and several mitochondrial markers was observed in resting muscles from AMPK α 2-/- mice (44, 45) and AMPK α 2i transgenic mice (51). Finally, chronic muscle energy deprivation induced by β -GPA treatment had no effect on mitochondrial content and PGC-1 α expression in AMPK α 2-KD (52) suggesting that AMPK might be a regulator involved in the initiation of mitochondrial biogenesis.

Reductions in AMPK-stimulated activity have recently been implicated in the reduced mitochondrial function and dysregulated intracellular lipid metabolism associated with aging-induced insulin resistance and type 2 diabetes (53). Oxidative capacity of fast/glycolytic (plantaris) and slow/oxidative (soleus) skeletal muscles from AMPK α 2-/- mice were directly measured in permeabilized fibers from sedentary animals to evaluate the role of AMPK in

mitochondrial function. No modification in muscle oxidative capacity of soleus or plantaris muscles was observed in AMPK α 2^{-/-} mice (Table 3). Both basal and maximal respiration were similar in control and AMPK α 2^{-/-} soleus and plantaris muscles. Coupling between respiratory chain and phosphorylation was also intact in the absence of AMPK α 2 in skeletal muscle (Table 3). Following endurance training for 8 weeks, an increase of mitochondrial respiration with the addition of different substrates (which became significant with the last addition of substrates, namely pyruvate and glutamate), was observed in the soleus muscle of trained control mice (Figure 4A, left panel) but not in trained AMPK α 2^{-/-} soleus muscle (Figure 4A, right panel). In the case of plantaris muscle, no effect of training was observed on respiration rates either for control (Figure 4B, left panel) or AMPK α 2^{-/-} (Figure 4B, right panel) mice. These results indicate that AMPK α 2 could participate in the adaptation to endurance training in oxidative muscle by improving the mitochondrial respiration under different substrates.

Endurance training is known to induce a partial fast-to-slow muscle phenotype transformation and mitochondrial biogenesis but no growth. In contrast, resistance training mainly stimulates muscle protein synthesis resulting in hypertrophy. For muscle growth to occur, the rate of protein synthesis must exceed that of protein breakdown. Since protein synthesis can account for up to 30-50% of the cellular energy expenditure, a reduction in protein synthesis seems an efficient mechanism to save energy. One critical signaling pathway controlling protein synthesis during skeletal muscle growth involves the mammalian target of rapamycin (mTOR) kinase. mTOR has emerged as an essential factor for growth and development activated in skeletal muscle by a variety of anabolic signals like resistance exercise. The use of mTOR inhibitor rapamycin has demonstrated that mTOR is required for hypertrophy. On the contrary, AMPK activity is thought to inhibit protein synthesis, which is important for skeletal muscle hypertrophy, and may therefore modulate skeletal muscle mass and hypertrophy (54). Increased AMPK phosphorylation in fast-twitch plantaris muscle has been associated with an impaired hypertrophic capacity (55, 56). In parallel, with the mTOR pathway, studies suggest that AMPK reduces both the initiation and the elongation of ribosomal peptide synthesis (57). In rat skeletal muscle, activation of AMPK by AICAR is shown to associate with a reduced protein synthesis (54). The effect of AMPK on protein synthesis is likely to be due, in part, to AMPK-induced inhibition of the mTOR/ ribosomal protein S6 kinase (S6K) signaling pathway (54, 58-60). Indeed, this pathway contains multiple potential sites for regulatory integration with AMPK (54, 61). For example, AMPK

phosphorylates and activates the GAP protein named tuberous sclerosis complex 2 (TSC2). Once activated, TSC2 is able to inactivate its G-protein Rheb thereby blocking activation of mTOR (9, 61). It has been also shown that AMPK could directly phosphorylate and inactivate mTOR (62). In parallel, with the mTOR pathway, AMPK reduces protein synthesis by a specific phosphorylation (Ser398) on the eEF-2 kinase (59), which is a key component in protein synthesis as it promotes peptide elongation. In addition to the AMPK-induced mTOR regulation, it has been recently shown that, on the other way, the suppression of S6K1 activity simultaneously promotes AMPK signaling (63).

Numbers of studies have investigated whether AMPK is an important signaling molecule in the control of skeletal muscle glucose transport. Muscles from AMPK α 2^{-/-}, AMPK γ 3^{-/-}, AMPK α 2-KD and AMPK α 2i mice completely abolished *ex vivo* AICAR- or hypoxia-stimulated glucose uptake (17, 19, 64, 65). Based on these observations, AMPK has been proposed to be a key player in initiating signaling to contraction-stimulated glucose uptake. Nevertheless, it has been difficult to verify this hypothesis with genetic approaches. In AMPK α 2^{-/-}, AMPK γ 3^{-/-}, AMPK α 2-KD and AMPK α 2i mice *ex vivo* contraction-stimulated glucose uptake is normal (17, 65) or only moderately reduced (64, 66). Recent observations using aforementioned genetic animal models suggested that Akt substrate of 160 kDa (AS160), a key protein in the regulation of insulin-dependent GLUT4 trafficking and glucose uptake, may represent a point of convergence between AMPK and insulin-signaling pathways for the control of glucose uptake. AICAR-induced AS160 phosphorylation was completely prevented in AMPK α 2^{-/-}, AMPK γ 3^{-/-} and AMPK α 2-KD mice (67, 68). However, the situation was different in response to contraction as AS160 phosphorylation was impaired in AMPK α 2^{-/-} and AMPK α 2-KD mice but not in AMPK γ 3^{-/-} mice (68). These results highlighted the importance of isoform composition of AMPK complexes in the regulation of exercise-induced phosphorylation of AS160 with α 2 β 2 γ 1 playing probably a central role (6). Interestingly, muscle-specific LKB1 KO mice which lack the ability of AMPK α 2 to be phosphorylated and activated displayed an impaired capacity to stimulate both contraction and AICAR-stimulated glucose uptake (7). However, these results do not directly link AMPK activity and glucose transport, as LKB1 phosphorylates and activates several other kinases which could contribute to glucose transport during contraction. Thus, taken together, these data indicate that AMPK can regulate glucose uptake in resting muscle and that AMPK plays, at least partially, a role in the contraction-stimulated glucose uptake.

AMPK is also pivotal in the regulation of skeletal muscle fatty acid metabolism. AMPK activation with AICAR, leptin or adiponectin induces increasing rates of fatty acid oxidation via phosphorylation and inactivation of ACC (29, 69, 70). Interestingly, leptin was found to selectively activate and phosphorylates AMPK α 2 in skeletal muscle via direct and indirect mechanisms; the latter involving the hypothalamic-sympathetic nervous system and α adrenergic receptors in muscle (70). During muscle contraction, the activation of AMPK inhibits ACC resulting in long-chain fatty acids β -oxidation. First evidence for the role of AMPK in the regulation of fatty acid oxidation in skeletal muscle has been obtained from mice overexpressing the activating γ 3 R225Q mutation in muscle. These transgenic mice showed increased fatty acid oxidation when challenged with a fat-rich diet or after swimming exercise leading to reduced intramuscular accumulation of triglycerides (65, 71). Thus, the AMPK γ 3 isoform plays a major role in modulating intramuscular fuel utilization toward fat oxidation during exercise. Nonetheless, recent studies demonstrated the importance of alternative pathways on upregulation of fatty acid oxidation in the absence of AMPK activity during muscle contraction (72, 73) and the additive effects of contraction and AICAR on fatty acid oxidation (74) suggesting parallel pathways for regulating fatty acid oxidation during muscle contraction. However, because oxidative metabolism is important during endurance exercise, changes in lipid metabolism in response to AMPK activation may also affect glycogen metabolism in skeletal muscle. The level of muscle glycogen is essential for muscle performance. Indeed, glycogen represents the main source of glucose for muscle ATP synthesis early during a bout of exercise. Glycogen synthase (GS) is believed to be the rate limiting enzyme in the glycogen synthesis. In mouse white skeletal muscle, AMPK α 2 but not AMPK α 1 is able to phosphorylate and inactivate GS *in vivo* in response to AICAR treatment (75), which is in agreement with the role of AMPK in the control cellular of energy balance. Recently, the role of AMPK in the regulation of muscle glycogen has been further investigated with AMPK γ 3 $^{-/-}$ and AMPK α 2-KD mice and transgenic mice over-expressing AMPK γ mutants specifically in skeletal muscle (65, 76, 77). Over-expression of γ 3R225Q and γ 1R70Q mutant activates AMPK and increases muscle glycogen content accompanied by an higher exercise capacity (71, 77) but glycogen resynthesis after exercise is blunted in AMPK γ 3 $^{-/-}$ and AMPK α 2-KD mice (19, 65).

Although, pharmacological activation of AMPK in resting muscle has been demonstrated to act on muscle metabolism, the available data from current animal models do not allow to drive firm conclusions about the physiological role of AMPK during exercise and muscle

contraction. All the studies using AMPK animal models have brought a quantity of results which are, to some extent, difficult to connect, and even more are contradictory. This conflict could come from the difficulties to compare different genetic animal models. Indeed, expression of a dominant-negative form of AMPK is clearly not equal to the deletion of a particular AMPK subunit. Another reason may be the many additional signaling pathways that are activated during exercise which may have overlapping actions to AMPK. Clearly, more *in vivo* approaches using new animal models or specific inhibitor strategies are needed to comprehensively understand the role of AMPK in muscle metabolic adaptation during exercise training.

Role of AMPK in the heart

In comparison to hepatic and adipose tissue, the heart could be considered as a simplistic organ, which its function is to be a modest circulating pump. However, this cardiac pump has to continuously adapt its function and, so, its energy consumption to the oxygen demand and circulating substrates and hormones in the body. In normal conditions, the human heart produces and directly consumes 35kg of ATP each day. This energy comes from the oxidation of different substrates, including fatty acids and glucose (Figure 5A). This oxidation requiring a continuous flux of oxygen, any reduction of this flux, for example during an ischemic episode, will inevitably induce an energetic imbalance, an increase in AMP/ATP ratio and, so, AMPK activation (Figure 5B). It has been postulated that this resulting AMPK activation acts as an emergency signal to restore cell energy homeostasis (78-81). Indeed, it is assumed that AMPK promotes glycolysis, the sole energy providing pathway under anaerobic conditions, by a double mechanism (Figure 5B). First, it increases glucose uptake by stimulating the translocation of glucose transporter GLUT4 to the sarcolemmal membrane. Second, AMPK indirectly stimulates 6-phosphofructo-1-kinase (PFK-1) activity by phosphorylating and activating 6-phosphofructo-2-kinase, the enzyme that synthesizes fructose 2,6-bisphosphate, a potent PFK-1 stimulator. By participating in the stimulation of glycolysis and, so, in the ATP production, AMPK is considered to play a protective role during an ischemic episode. By contrast, it has been postulated that AMPK could play a deleterious role during early reperfusion. Indeed, during reperfusion, the still existing AMPK activation helps fatty acid oxidation to predominate over glucose oxidation by phosphorylating and inactivating ACC. This ACC inactivation decreases the concentration of malonyl-CoA and so increases fatty acid oxidation (Figure 5C). During the early phase of

reperfusion, this resulting stimulation of fatty acid oxidation occurs in parallel to the still present glycolytic stimulation, inducing a deleterious uncoupling of glucose oxidation and glycolysis (78). Transgenic animal models have been extensively used to answer to the question if AMPK is a friend or a foe for the heart during an ischemia/reperfusion episode. The study of AMPK α 2^{-/-} mouse heart (82), the AMPK α 2 isoform being predominant in the heart, and of AMPK α 2-KD mice where a kinase-dead AMPK α 2 isoform (K45R mutation) is expressed in the heart (19, 83), confirmed the major role of AMPK in the regulation of cardiac metabolism. Indeed, in the absence of the AMPK α 2 isoform, ACC phosphorylation in both normoxic and ischemic conditions was clearly decreased (82). This correlates to the absence of the stimulation of fatty acid oxidation during reperfusion in AMPK α 2-KD mouse hearts (83). Similarly, in both models (83, 84) as well as in heart AMPK α 2i model (D157A mutation) (85), basal and/or ischemia-stimulated glucose uptake were downregulated, whereas the stimulation by ischemia of glycolysis, measured by lactate production, was decreased (82, 83). The decrease in glycolytic stimulation during ischemia induced a major energetic imbalance. Indeed, at the end of the ischemic episode, AMP/ATP ratio reached values 10-times higher in AMPK α 2^{-/-} mouse hearts in comparison to that of wild-type animal (82). In term of cardiac function, this robust energetic imbalance is accompanied by a more rapid and severe ischemic contracture of the AMPK α 2^{-/-} mouse hearts (82, 84). Similar results were found in AMPK α 2-KD model where the left ventricular developed pressure is clearly lower than in wild-type hearts during ischemia (83). Even if the link between modifications of metabolic regulation and heart function is difficult to establish, these results, taken together, showed that the decrease of AMPK activation is detrimental for the heart during ischemia.

However, the effect of AMPK activity deletion on the heart function during reperfusion is less clear. Indeed, AMPK α 2-KD hearts demonstrated impaired recovery of left ventricular function accompanied by an increase in myocardial injury and apoptosis (83). By contrast, in AMPK α 2^{-/-} hearts, despite the apparent worse metabolic adaptation during ischemia, the absence of AMPK α 2 isoform does not exacerbate impairment of the recovery of post-ischemic contractile function in the absence of fatty acids (82, 84) and has transient and limited effect in the presence of fatty acids (84).

Another important role of AMPK in the heart is its contribution in the regulation of glycogen metabolism, even if the pathways controlled by AMPK and involved in the regulation of glycogen synthesis and breakdown remain to be fully elucidated (78, 86). Different mutations

of the AMPK γ subunit have been shown to induce an increase in glycogen storage and cardiomyopathy characterized by a ventricular pre-excitation, named Wolff-Parkinson-white (WPW) syndrome. Different transgenic models overexpressing these mutations in the heart have been extensively studied (78, 86). These models are all characterized, amongst others, by an increase in glycogen content. By contrast, the action of these mutations on AMPK activity and AMPK sensitivity to AMP seemed to be partially different, if not opposite. If it is rather difficult to conciliate, at first sight, a same increase in glycogen content but different AMPK activity, a model of AMPK/glycogen relationship has been proposed (86). Firstly, these mutations induce inappropriate activation of AMPK under basal conditions. This abnormal AMPK activation leads to a concomitant increase in both glucose uptake and fatty-acid oxidation, inducing, via the Randle effect, an inhibition of glucose oxidation and, so, the storage of the exceeding glucose into glycogen. This increase in glycogen content, subsequently, inhibits AMPK, reaching a new steady state. This putative model is in agreement with the results obtained in different genetic models, with altered AMPK activity. In AMPK α 2-KD (83) and AMPK α 2 $^{-/-}$ hearts (82, 84), loss of AMPK activity is correlated to the reduction of glycogen content. However, it should be mentioned that the relationship between glycogen storage and AMPK is not simply related to its activity/activation. Indeed, LKB1 $^{-/-}$ hearts (8) possess an AMPK activity profile similar to that of AMPK α 2 $^{-/-}$ hearts (82). That is a complete inactivation of the AMPK α 2 isoform without (for AMPK α 2 $^{-/-}$) or with only a little decrease (for LKB1 $^{-/-}$) in AMPK α 1 activity or ischemia-induced activation. Under the same ischemic condition, LKB1 $^{-/-}$ heart was characterized by a 10-fold higher energetic resistance measured by the increase in AMP/ATP ratio (AMP/ATP ratio reached after 10 min of ischemia: 0.33 and 3.5 for LKB1 $^{-/-}$ and AMPK α 2 $^{-/-}$, respectively) (8, 82). This is linked to superior glycolytic stimulation measured by lactate accumulation (Figure 6) and could be associated to the higher glycogen content under normoxic condition (Figure 6). So, similar AMPK activity coexists with different glycogen storage. One of the main differences between LKB1 $^{-/-}$ and AMPK α 2 $^{-/-}$ animals does not reside in the activity but in the physical presence of the protein. Indeed, the expression of the different AMPK subunits, including AMPK α 2 is not modified in LKB1 $^{-/-}$ mouse hearts, whereas AMPK α 2 is, obviously, not expressed in AMPK α 2 $^{-/-}$ mice. Interestingly, the modification of the amount of the AMPK α 2 subunit is accompanied by the decrease in AMPK β subunit (82). Knowing the fact that the AMPK β subunit possesses a glycogen binding domain, we can postulate that

glycogen storage is also, at least partially, regulated via its direct interaction with the AMPK heterotrimer, independently of its intrinsic activity.

As already explained, mutations of the AMPK γ subunit have been shown to induce cardiomyopathy characterized by a ventricular pre-excitation WPW syndrome. The study of different mouse genetic models of AMPK γ 2 missense mutation, including transgenic R302Q (87), N488I (88, 89), R531G (90) and T400N (91), concluded that the WPW cardiomyopathy resulted, at least partially, from physical disruption of conduction cells by glycogen engorgement (86). In addition, other mechanisms have been proposed to act in parallel, including AMPK-induced phosphorylation of ion channels. Similarly to these AMPK γ mutations, the cardiac-specific dominant-negative TAK1 mice were characterized by electrophysiological and biochemical properties similar to WPW syndrome and impaired AMPK activation (92). The author of this study concluded that TAK1 plays a pivotal role in the LKB1/AMPK signaling.

Finally, the study of genetic models of AMPK revealed new roles for this enzyme. Indeed, using AMPK α 2-KD, it has been recently reported that AMPK plays an important role in p38 MAPK activation during ischemia by promoting p38 MAPK autophosphorylation through interaction with the protein TAB1 (93). On the same way, in the heart AMPK α 2i model (85), it has been shown that AMPK mediates cardiac preconditioning by regulating the activity and recruitment of sarcolemmal K(ATP) channels (94). On the other hand, the study of AMPK α 2-/- mouse heart revealed that AMPK α 2 participates in the regulation of cardiac muscle oxidative capacity (95). The absence of AMPK α 2 induced the decrease of the maximal oxidative capacity and an impairment of the complex I of the respiratory chain. This was associated to a decrease in mitochondrial cardiolipin content that could be explained by the downregulation of mRNA of enzymes involved in cardiolipin synthesis and remodeling.

Role of AMPK in vascular reactivity

AMPK is expressed both in endothelial and in smooth muscle cells. Previous studies have established that the predominant isoform expressed in vascular endothelial cells is α 1 (96, 97). Both α 1 and α 2 catalytic subunits are expressed in arterial smooth muscle cells, although their relative proportion differs between different arteries (12, 98). Vasodilatation is a vital mechanism of systemic blood flow regulation that occurs during periods of increased energy demand. As a metabolic sensor, vascular AMPK could be involved in the metabolic

regulation of blood flow. Hypoxia increases the AMP/ATP ratio in pulmonary artery smooth muscle cells and induces a two-fold increase in AMPK activity (12). Metabolic challenge also rapidly and reversibly activates AMPK α 1 in porcine carotid arteries (98), suggesting that AMPK activation may participate in the regulation of blood pressure. Indeed, AICAR treatment decreased blood pressure in rats displaying features of the insulin resistance syndrome (99). A target of AMPK is endothelial nitric oxide synthase (eNOS), an important modulator of angiogenesis and vascular tone. It has been clearly established that AMPK may associate with, and phosphorylate eNOS in cardiomyocytes and endothelial cells, thus increasing eNOS activity and NO production (96, 100). Activation of AMPK with AICAR stimulates NO synthesis in human aortic endothelial cells (101).

Thus, AMPK could trigger vasodilatation and participate in blood flow regulation. Indeed, pharmacological activation of AMPK by AICAR induces relaxation of mouse aorta (102). This AICAR-induced vasorelaxation is not inhibited by the addition of adenosine receptor antagonists. Moreover, when aortic rings are freed of endothelium or pretreated with L-NMMA to inhibit nitric oxide synthase activity, AICAR still induces aortic ring relaxation, suggesting a direct effect of AICAR on smooth muscle cells. Finally, AICAR-induced relaxation of aortic rings is completely abolished in AMPK α 1 $^{-/-}$ but not AMPK α 2 $^{-/-}$ mice (102). Therefore, activation of AMPK α 1 but not AMPK α 2 is able to induce aortic relaxation in mice, in an endothelium and eNOS-independent manner. AMPK activation may be an additional mechanism by which hypoxia or metabolic challenge can induce vasorelaxation of large vessels, thereby increasing oxygen availability in peripheral tissues. AMPK thus appears as a new player in the complex signaling pathways that regulate vascular tone.

Role of AMPK in the hypothalamus

The central nervous system, in particular the hypothalamus, plays an essential role in the regulation of feeding behavior and energy expenditure by integrating information from the periphery through nutrients, hormones and afferent neural inputs (103). The emerging role of hypothalamic AMPK in these pathways involved in whole body energy homeostasis has received considerable attention during the last years (104-106), highlighting a more central and integrated function for AMPK than the one primarily recognized as cellular energy sensor. The most striking proof of concept for AMPK involvement in appetite regulation came from experiments showing that expression of a constitutively active or a dominant negative form of AMPK in the hypothalamus induced reciprocal modifications of neuropeptides expression, food intake and body weight in mice (107). Thus, elevated hypothalamic

AMPK activity led to stimulation of food intake whereas its reduction was associated with hypophagia. In agreement with this genetic approach, it has been shown that acute intracerebroventricular (icv) administration of pharmacological AMPK activator (AICAR) or inhibitor (compound C) increased or decreased food intake, respectively (108, 109). Furthermore, antagonistic effects on food intake were also observed after modulation of intra-hypothalamic ATP levels by icv injection of 2-deoxyglucose (orexigen, (110)) or the fatty acid synthase inhibitor C75 (anorexigen, (111)). Interestingly, a number of studies demonstrated that hypothalamic AMPK is also regulated under physiological conditions, its activation during starvation and inactivation upon refeeding being associated with nutritional changes in circulating hormones and nutrients (107, 111). Thus, it has been shown that hormones known to affect food intake could exert part of their effects through modulation of hypothalamic AMPK activity. The adipocytokine leptin and insulin, which exert anorexigenic effects, were reported to inhibit AMPK in both arcuate (ARC) and paraventricular hypothalamic nucleus by a mechanism that presumably involved the melanocortin 4 receptors (107). In other hand, the gut hormone ghrelin induced hypothalamic AMPK activation and stimulation of food intake (108, 112). Furthermore, it has been very recently demonstrated that trimeric and hexameric forms of adiponectin were able to cross the blood brain barrier and to concomitantly increase AMPK activity in ARC nucleus and food intake in mice (113). In addition, adiponectin also reduced energy expenditure and remarkably reversed the leptin-induced suppression of hypothalamic AMPK activity and its subsequent inhibitory effect on food intake (113). All these effects required the presence of the adiponectin receptor adipoR1 (113). Finally, icv administration of resistin promoted short-term satiety in rats (114), although its inhibitory effect on AMPK reported in various tissues (115, 116) remains to be specifically addressed in the hypothalamus. In addition to these hormonal signals, nutrients may also be involved in the AMPK-mediated regulation of food intake. While opposite effects of glucose modulation on feeding behavior are known for decade, it has been recently shown that the hypophagia following icv injection of glucose could be mediated by suppression of AMPK activity in multiple hypothalamic areas (107, 109). Thus, AMPK is physiologically inhibited by elevated glucose level associated with refeeding and activated by a reduction of glucose availability during prolonged fasting.

The mammalian target of rapamycin (mTOR) has been recently implicated in the hypothalamic control of food intake. However, conversely to AMPK, mTOR is activated by leptin, glucose and amino acids, leading to inhibition of food intake (117). Interestingly, since activation of AMPK suppresses mTOR activity, this strongly suggests that both kinases may have overlapping and reciprocal functions in the control of feeding behavior (117). The AMPK-mediated regulation of food intake involves complex modifications of orexigenic and anorexigenic neuropeptides expression,

notably in the ARC nucleus of the hypothalamus, a region containing both neuropeptides Y (NPY)/agouti-related protein (AgRP) and pro-opiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART) neurons. Indeed, inhibition of hypothalamic AMPK was reported to suppress neuronal NPY/AgRP signaling (107), this being eventually mediated by a cAMP response element-binding protein (CREB)-dependent mechanism (111), whereas elevated AMPK activity was associated with increased fasting-induced NPY and AgRP expression (107, 110). However, the underlying mechanism(s) connecting modulation of hypothalamic AMPK activity to expression of these neuropeptides remain unknown to date. It has been suggested that modulation of malonyl-CoA and/or long chain fatty acyl-CoA (LCFA-CoA) concentrations in the hypothalamus could act as potent regulators of food intake (118, 119). Accordingly, AMPK activation during starvation, which leads to phosphorylation and inactivation of ACC, would decrease hypothalamic malonyl-CoA and/or LCFA-CoA content, favor fatty acid oxidation and stimulate feeding (Figure 7). Since AMPK has been shown to phosphorylate and activate malonyl-CoA decarboxylase (MCD) (120), this could also participate to the reduction of malonyl-CoA level by increasing its degradation. In support to this mechanism, CPT1 inhibition (121) and adenovirus-mediated expression of MCD in the hypothalamus (122) have been associated with opposite changes in malonyl-CoA levels and neuropeptides expression, resulting in suppression or stimulation of food intake, respectively. Although recent evidence suggests that the brain-specific CPT1 isoform (CPT1c) may be one of the hypothalamic malonyl-CoA target that relays AMPK signaling to orexigenic and anorexigenic neurons (123), further studies will be needed to address this specific issue. In addition, the signal resulting from hypothalamic lipid sensing could come from LCFA itself since it has been reported that icv administration of fatty acids or manipulation of hypothalamic lipid metabolism also reduced food intake (121).

Taken together, there is a growing body of evidence suggesting that hypothalamic AMPK plays a key role in appetite regulation by integrating nutrient- and hormonal-derived signals. However, it is striking that neither the AMPK α 1 nor the AMPK α 2 knockout mice exhibited apparent changes in body weight and mean daily food intake ((17, 18) and Table 2). While upregulation of the remaining AMPK isoforms should not be excluded, as previously reported in other tissues (18), it should be noted that these phenotype were observed on animals fed a normal chow diet *ad libitum* and may eventually differ in response to other diets (e.g. high fat). In addition, it would also be interesting to measure food intake during the first hours following refeeding in mice submitted to a prolonged fasting period to see if the starvation-induced activation of AMPK in the hypothalamus is involved in the initiation of food intake.

Finally, the forthcoming generation of mice expressing neuron-specific deletion of $\alpha 1$ and $\alpha 2$ isoforms of the AMPK catalytic subunits in specific regions of the hypothalamus would probably constitute a very helpful model to further study the involvement of AMPK in feeding behavior and energy expenditure. Thus, very recent results showed that newly engineered mice lacking AMPK $\alpha 2$ in POMC neurons unexpectedly developed obesity due to reduction of energy expenditure and dysregulation of food intake (124). In other hand, deletion of AMPK $\alpha 2$ in AgRP neurons led to divergent physiological effect, *i.e.* the development of an age-dependent lean phenotype (124). These new opposite findings would certainly lead to a higher degree of complexity with regard to the role of AMPK in hypothalamic functions.

New insights in AMPK functions from non-mammalian animal model systems

****Drosophila melanogaster***

Several groups have recently generated AMPK mutants in *Drosophila* allowing the *in vivo* genetic analysis of AMPK function and identifying new physiological roles. A mutation in the AMPK γ -subunit has been described to cause progressive neurodegeneration in *Drosophila* (125). This result further supports emerging role of AMPK in preserving neuronal integrity in mammals (126). Deletion of the unique AMPK α -subunit in *Drosophila* has been also generated and showed a lethal phenotype (127, 128), demonstrating the necessity of AMPK during embryogenesis as previously shown in mammals. AMPK-null fly embryos showed severe abnormalities in epithelial cell polarity with disruption of the apical-basal polarity of the actin cytoskeleton. AMPK-null embryos contained defective mitotic divisions leading to the frequent formation of polyploid cells. Moreover, AMPK deficient epithelial cells also overproliferate under energetic stress forming outgrowths of unpolarized tumor-like cells. Recent results in mammalian cells have demonstrated AMPK-induced repression of cell proliferation and cell cycle progression mediated by mTOR and p53 (129, 130). Furthermore, the discovery of AMPK activation by the tumor suppressor LKB1 has uncovered a strong connection between metabolic signaling, cell proliferation and cancer. Interestingly, LKB1-null fly mutants showed very similar polarity and mitosis defects to those observed in AMPK-null mutants (127, 128). Remarkably, expression of a constitutive active form of AMPK rescued LKB1-null mutants, suggesting that AMPK is a critical downstream mediator of LKB1, controlling mitosis and cell polarity (127, 128). Intriguingly, it has been established that myosin II regulatory light chain (MRLC) is a critical downstream target of AMPK for the

execution of mitosis and cell polarity establishment both in mammals and *Drosophila* (127). Indeed, expression of an MRLC mutant containing phosphomimetic residues replacing the residues phosphorylated by AMPK rescued both cell polarity and mitosis defects in AMPK- and LKB1-null fly mutants (127). These findings uncovered a link between energy status and cell structures, revealing new pathophysiological functions for AMPK signaling pathway.

****Caenorhabditis elegans***

Senescence in *C. elegans* is associated with changes in cellular AMP/ATP ratio suggesting a link between genes known to affect lifespan and the control of energy metabolism in worms (131). Over-expression of the *C. elegans* AMPK α catalytic subunit increases lifespan (131). The generation of mutants in *C. elegans* has also highlighted the role of AMPK in the regulation of lifespan in response to environmental stress and insulin-like signaling (132). Environmental stresses such as starvation, heat shock or mutations that inactivate genes involved in insulin signaling extend life span only in worms expressing *aak-2*, which is one of the two catalytic subunits of AMPK in *C. elegans* (131, 133). It has been recently demonstrated that AMPK is required for mediating lifespan extension by dietary restriction in worms via the phosphorylation of the FOXO transcription factor DAF-16 (134). Interestingly, AMPK also phosphorylates human FOXO3 in mammalian cells, indicating that the regulation of FOXO by AMPK may be conserved (135). Thus, these studies raise the intriguing possibility that AMPK may also be implicated in the increase in life span induced by caloric restriction in mammals. Indeed, resveratrol, a polyphenol that activates AMPK, has been shown to mitigate effects of high-calorie and high-fat diets in mice by improving lifespan (22).

Action of anti-diabetic drugs on the AMPK pathway

Since it has been shown that AMPK could be involved in the anti-diabetic effect of metformin (136), numbers of studies have used AMPK activators to investigate putative treatments against insulin resistance and diabetes. For example, it has been shown that AMPK activation by oligomycin or metformin was able to re-establish a normal insulin-sensitivity in insulin-resistant cardiomyocytes (137). In this last study, the putative role of AMPK was re-enforced by the use of a constitutively active form of AMPK that mimicked the effects of AMPK activators. But caution is required when interpreting findings on the sole basis of treatment with AICAR, oligomycin or metformin as these widely used pharmacological AMPK

activators are not necessarily completely specific for AMPK activation. It has been recently demonstrated using mice lacking both $\alpha 1$ and $\alpha 2$ catalytic subunits in the liver that AICAR and metformin have detrimental effects on energy metabolism by their inhibition of the mitochondrial respiratory-chain complex I independently of AMPK activation (23, 24). These results suggest that AICAR and metformin might activate AMPK indirectly by increasing cellular AMP/ATP ratio. Significant changes in the cellular AMP/ATP ratio are indeed readily detected after treatment of cultured hepatocytes with AICAR and metformin (23, 24) and heart perfused with metformin (138). Unlike these non-specific AMPK activators, a direct activator A-769662 has been recently identified exerting its cellular effects by direct allosteric activation of AMPK activity and not by altering the AMP/ATP ratio (139). In addition to its allosteric activation of AMPK, this compound also inhibited the dephosphorylation of Thr 172 within the α subunit (140). Its specificity as a direct pharmacological activator of AMPK has been recently tested on AMPK KO cellular systems validating its use as a valuable experimental tool to map new regulatory elements of the AMPK signaling pathway (141).

Relationship between adiponectin and AMPK pathways: insights from KO models

Abundant literature has suggested that adiponectin functions as a protecting factor against the development of insulin resistance and diabetes, in part through the activation of AMPK (29, 142). Therefore, deletion of adiponectin gene or respective receptors could provide valuable mice models with impairment of AMPK activation. Comparison of these models with AMPK KO mice could raise new interesting data on the consequences of global reduction in AMPK activation. Surprisingly, deletion of adiponectin gene is not sufficient to provoke major metabolic disorders when fed a standard chow diet (143, 144). However, adiponectin KO mice exhibit severe diet-induced insulin resistance when fed a high fat/sucrose diet (145). In this model, as long as calorie overloading persists, hypoadiponectinemia and concomitant increased in TNF- α levels may drive and aggravate insulin resistance. Contrasting with adiponectin KO mice, adiponectin receptor-1 KO mice (AdipoR1 KO mice) showed impaired glucose tolerance, increased HGP and insulin resistance when fed a regular chow diet (146, 147). Abrogation of adiponectin-induced hepatic AMPK activation in AdipoR1 KO mice suggested a strong relationship between AMPK and adiponectin pathway mediated through adiponectin R1 receptor in the liver (147). According to this result, adiponectin failed to regulate hepatic glucose production in liver-specific AMPK $\alpha 2$ KO mice (26). Regarding

adiponectin receptor-2 KO mice (AdipoR2 KO mice), opposite phenotypes have been published. While one strain of AdipoR2 KO is characterized by a resistance to high-fat induced obesity and increased glucose tolerance (146), another strain of AdipoR2 KO mice exhibits glucose intolerance and insulin resistance (147). AdipoR2 KO mice did not show changes in adiponectin-induced phosphorylation of AMPK in the liver or skeletal muscle (146, 147). Thus, changes in AMPK phosphorylation cannot explain different insulin-responsive phenotype observed in these two AdipoR2 KO models. Alternatively, AdipoR2 deletion resulted in decreased activity of PPAR α signaling pathways in the liver which may contribute to reduced insulin sensitivity in these mice (147). These different KO mice models indicates that functional differences exist in adiponectin-signaling pathways in the liver, AdipoR1 being tightly linked to activation of AMPK pathways whereas AdipoR2 being more associated with the activation of PPAR- α pathways (146, 147). However, the link between adiponectin and AMPK is more convincing in heart pathologies like hypertrophy. Indeed, it has been shown that pressure overload in adiponectin KO mice resulted in enhanced hypertrophy associated with a decreased AMPK signaling (148). In cardiomyocytes, adiponectin was able to induce AMPK activation concomitantly to the inhibition of an agonist-stimulated hypertrophy (148). The fact that the expression of a dominant-negative form of AMPK reversed these effects revealed a clear implication of the AMPK signaling in the putative adiponectin role as treatment against cardiac hypertrophy.

Perspectives

While the primary targets for AMPK have been involved mainly in the control of energy metabolism (16), new evidence indicates that AMPK also participates in the control of non-metabolic processes such as regulation of mitotic cell division, cell growth, progression through the cell cycle and organization of the cytoskeleton. Thus, AMPK signaling pathway has evolved into a highly complex system which integrates multiple signals from cellular environment to ensure that all highly energy-consuming cellular functions only proceed if cells have sufficient metabolic resources. Thus, the key role of AMPK in maintaining energy balance places it as an ideal therapeutic target for the treatment of derangements of energy metabolism that occur in conditions like obesity, insulin resistance, type 2 diabetes and the metabolic syndrome but also cancer. Different specific and non-specific AMPK activators have been employed with promising results in animal models with obesity or type 2 diabetes (16, 139), but, on the other hand, caution should be exercised for the design of clinical trials.

Difficulties in clinical applications may be multifactorial. Experimental animal models are not fully reliable and reproduce at least some aspects of human disease. Expression and activation pattern of AMPK isoforms differs between rodent and human muscle and between muscle fiber types (149, 150). Furthermore, sex difference in muscle AMPK activation has been observed in human, probably due to sex specific muscle morphology (higher proportion of type 1 muscle fibers in women) (151). Inclusion of these criteria should be accurately discussed to better define the population most likely to benefit from AMPK-based therapeutic strategies.

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Send correspondence to: Benoit Viollet, Institut Cochin, Dpt Endocrinology, Metabolism and Cancer 24 rue du Faubourg Saint-Jacques 75014 Paris, France, Tel: 33 1 44 41 24 08, Fax 33 1 44 41 24 21, E-mail: viollet@cochin.inserm.fr

Running title: AMPK animal models

Figure legends:

Figure 1: AMPK α 1 α 2_{LS}^{-/-} mice are resistant to AICAR hypoglycemic effect. AMPK α 1 α 2_{LS}^{-/-} mice deleted for both AMPK catalytic subunit in the liver were obtained by crossing liver-specific AMPK α 2^{-/-} mice with AMPK α 1^{-/-} mice. AICAR tolerance test were performed on overnight fasted mice injected intraperitoneally with 0.25 g/kg of AICAR and tail blood was collected at 0, 20, 40, 60 and 80 min for determination of glucose concentration using a glucometer. **P < 0.001, ***P < 0.0001 vs. control mice by unpaired, two-tailed Student t test.

Figure 2: Effect of hepatic AMPK α 2-CA expression on glycolytic and lipogenic gene expression in the liver of 6hours fasted *ob/ob* mice. Adenovirus expressing AMPK α 2-CA (Ad α 2-CA) or β -gal (Ad β -gal) were injected in *ob/ob* and control mice and gene expression was analyzed 48 hours post-injection. Each value indicates the amount of mRNA with respect to that in 6 hours fasted control C57Bl6J mice, arbitrarily defined as 1. Fold increase is indicated below each graph column. *P < 0.05, **P < 0.01, #P < 0.005, ##P < 0.001 vs. control mice and \$P < 0.05, \$P < 0.005, \$\$P < 0.001 vs. *ob/ob* Ad β -gal-injected mice. L-PK, L-pyruvate kinase; GK, glucokinase; S14, Spot 14; SREBP-1, sterol regulatory element-binding protein-1; ChREBP, carbohydrate response element-binding protein; ACC, acetyl CoA-carboxylase; FAS, fatty acid synthase; SCD-1, stearoyl-CoA desaturase-1; GPAT, glycerol-3-phosphate acyltransferase.

Figure 3: Wheel-running parameters for wild type (Control), and AMPK α 2 (AMPK α 2^{-/-}) deficient mice. Parameters are averaged per week. (A) distance per day in km. (B) maximal running speed in m per min. (C) number of runs per day. (D) distance per run in meters. Running wheels were connected to a DC generator allowing slight loading of the wheel (27.10^{-3} N m at mean maximal speed) and continuous recording of the output voltage of the DC generator on a PC computer. Instantaneous speed was calculated from the voltage and the resistive load on the DC generator and allowed calculating the distance. These daily values were averaged over each week for each animal. Total distance divided by number of times (number of runs) gave the distance per run.

Figure 4: Role of AMPK α 2 in skeletal muscle mitochondrial function. Substrate utilization by mitochondria were determined in permeabilized fibers of soleus (A) and plantaris (B) muscles of sedentary (SED) and active (ACT) AMPK α 2^{-/-} (KO) mice and littermate (CT) controls. Respiration rates were measured during the cumulative addition of substrates in saponin-skinned cardiac fibers of control and AMPK α 2^{-/-} mice. VO₂: rate of O₂ consumption in $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g dw}^{-1}$. G3P: glycerol-3-phosphate, Mal: malate, Oct: octanoyl-carnitine, Pyr: pyruvate, Glu: glutamate. * p<0.05 vs. control mice.

Figure 5: Control of cardiac metabolism during normoxia (A), ischemia (B) and reperfusion (C). Under normoxic condition, ATP production comes from fatty acids and glucose oxidation. Fatty acids is the privileged substrate (70%) used by the heart, its β -oxidation inhibiting glucose oxidation via the Randle cycle. Under ischemic condition, glycolysis becomes the sole ATP-providing pathway. AMPK plays the role of fuel gauge

during this phase. Being activated by the resulting increase in AMP/ATP ratio, AMPK is able to promote Glut-4 translocation and PFK-1 stimulation via PFK-2 activation. By stimulating glycolysis, AMPK is expected to be protective for the heart during ischemia. During early reperfusion, the still existing activated AMPK phosphorylates and inactivates ACC, inducing fatty acid entry into mitochondria. This favors fatty acid oxidation to become again the principal source for ATP production (80-90%). The concomitant stimulation of glycolysis and fatty acid oxidation should induce the uncoupling of glycolysis and glucose oxidation, and, so, the detrimental production of protons.

Figure 6: Effect of AMPK α 2 or LKB1 deletion on glycogen content and lactate production in normoxic and ischemic hearts. Hearts were from control (+/+) and knock-out (-/-) mice and perfused under normoxic (open bars) or ischemic (solid bars) conditions. Values are the means \pm SE of at least 5 hearts. *P < 0.05 vs. control mice .

Figure 7: Proposed model for the role of AMPK in the hypothalamic control of food intake. The activation of AMPK in the hypothalamus during starvation and its inactivation upon refeeding are mediated by nutritional changes in circulating hormones and nutrients. Ghrelin and adiponectin, which stimulate food intake, activate hypothalamic AMPK, whereas glucose, insulin, leptin and presumably resistin, which are known to inhibit food intake, inhibit it. Once activated, AMPK phosphorylates and inactivates ACC. The expected consequences are elevated malonyl-CoA concentration, leading to subsequent inhibition of mitochondrial fatty acid oxidation, and increased level of LCFA-CoA. The hypothalamic integration of these signals results in opposite changes in orexigenic/anorexigenic neuropeptides expression and subsequent modification of food intake. The underlying mechanism(s) upstream and downstream AMPK remain to be identified. ACC, acetyl-CoA carboxylase; ACS, acyl-CoA synthetase; AMPK, AMP-activated protein kinase; CPT-1, carnitine palmitoyl transferase 1; FAS, fatty acid synthase; MCD, malonyl-CoA decarboxylase; mTOR, mammalian target of rapamycin.

Table 1A: Genetic animal models for the study of AMPK signaling pathway.

Animal model	Tissue specificity	Remarks	Reference
<i>Knock-out mice</i>			
AMPK α 1	whole-body		(17)
AMPK α 2	whole-body		(18)
AMPK β 1	whole-body		(B. Kemp)*
AMPK β 2	whole-body		(B. Kemp)*
AMPK γ 3	whole-body		(65)
Alfp α 2	liver	use of Alfp-Cre	(26)
α 1KOAlfp α 2	whole-body α 1 and liver α 2	use of Alfp-Cre	(24)
AgRP α 2	hypothalamus (AgRP neurons)	use of AgRp-Cre	(124)
POMC α 2	hypothalamus (POMC neurons)	use of POMC-Cre	(124)
α 1KOPOMC α 2	whole-body α 1 and POMC α 2	use of POMC-Cre	(124)
Muscle/heart LKB1	skeletal and cardiac muscle	use of MCK-Cre	(7, 8, 152)
<i>Transgenic mice</i>			
AMPK α 1R45A	skeletal and cardiac muscle	MCK promoter/ AMPK-DN	(64)
AMPK α 2D157A	skeletal and cardiac muscle	MCK promoter/ AMPK-DN	(64)
AMPK α 2R45A	skeletal and cardiac muscle	MCK promoter/ AMPK-DN	(19)
AMPK α 2D157A	heart	α -MHC promoter/ AMPK-DN	(85)
AMPK γ 1R70Q	skeletal muscle	α -actin promoter/ AMPK-CA	(77)
AMPK γ 2	heart	α -MHC promoter	(87, 88)
AMPK γ 2R302Q	heart	α -MHC promoter/ AMPK-CA	(87)
AMPK γ 2N488I	heart	α -MHC promoter/ AMPK-CA	(88, 89)
AMPK γ 2T400N	heart	α -MHC promoter/ AMPK-CA	(91)
AMPK γ 2R531G	heart	α -MHC promoter/ AMPK-CA	(90)
AMPK γ 3	skeletal and cardiac muscle	MCK promoter	(65, 76)
AMPK γ 3R225Q	skeletal and cardiac muscle	MCK promoter/ AMPK-CA	(65, 76)
<i>Adenovirus-mediated gene transfer</i>			
Liver AMPK α 2-CA	liver	Ad AMPK α 2-CA infection	(21)
Neuron AMPK α 1-CA	hypothalamus	Ad AMPK α 1-CA infection	(107)
Neuron AMPK α 1+ α 2-DN	hypothalamus	Ad AMPK α 1+ α 2-DN infection	(107)
Liver LKB1 KO	liver	Ad Cre infection	(27)

Ad, adenovirus; Alfp, albumin α -fetoprotein; AgRP, agouti-related protein; Cre, Cre recombinase; MCK, muscle creatine kinase; POMC, pro-opiomelanocortin; α -MHC, α -myosin heavy chain; AMPK α 1-CA, AMPK α 1 constitutively active form; AMPK α 2-CA, AMPK α 2 constitutively active form; AMPK α 1+ α 2-DN, AMPK α 1 and AMPK α 2 dominant negative forms.

* Bruce Kemp, personal communication.

Table 1B: Validation of AMPK pathways in transgenic and knock-out mouse models.

Organ/tissue	Pathway/function	Animal model	Reference
<i>Heart</i>	Glycogen storage cardiomyopathy	Tg AMPK γ 2R302Q	(87)
		Tg AMPK γ 2N488I	(88, 89)
		Tg AMPK γ 2R531G	(90)
		Tg AMPK γ 2T400N	(91)
	Ischemic/post-ischemic tolerance	AMPK α 2 KO	(82, 84)
		Tg AMPK α 2R45A	(83)
		Tg AMPK α 2D157A	(85)
	Glucose transport, glycogen metabolism, glycolysis	AMPK α 2 KO	(82, 84)
		Tg AMPK α 2R45A	(83)
		Tg AMPK α 2D157A	(85)
	Mitochondrial biogenesis/ function	Tg AMPK α 2R45A	(95)
		AMPK α 2 KO	(95)
<i>Skeletal muscle</i>	Glucose transport	Tg AMPK α 2R45A	(6, 19)
		Tg AMPK α 2D157A	(85)
		AMPK α 2 KO	(6)
	Contraction/exercise tolerance	Tg AMPK α 2R45A	(19)
		Tg AMPK α 2D157A	(43, 51)
		Tg AMPK γ 1R70Q	(51, 77)
		Tg AMPK γ 3R225Q	(65, 71)
	Glycogen metabolism	Tg AMPK γ 3R225Q	(65, 76)
		Tg AMPK γ 1R70Q	(77)
		AMPK γ 3 KO	(65)
	Mitochondrial biogenesis/ function	Tg AMPK α 2R45A	(52)
		AMPK α 2 KO	(44, 45, 153)
<i>Liver</i>	Hepatic glucose production	Alf α 2 KO	(26)
	Mitochondrial biogenesis/ function	α 1KOAlf α 2	(23)
<i>Blood vessels</i>	eNOS activation	AMPK α 1 KO	(96)
	Vasorelaxation	AMPK α 1 KO	(102)
<i>Adipose tissue</i>	Lipolysis	AMPK α 1 KO	(37)
	Fat mass/ morphology	AMPK α 1 KO	(37)
		AMPK α 2 KO	(38)
		Liver AMPK α 2-CA	(21)
<i>Brain</i>	Food intake	AgRP α 2	(124)
		POMC α 2	(124)
		Neuron AMPK α 1-CA	(107)
		Neuron AMPK α 1/ α 2-DN	(107)
	Neuroprotection/stroke	POMC α 2	(126)

Tg, transgenic mice; KO, knock-out mice; AMPK α 1-CA, AMPK α 1 constitutively active form AMPK α 2-CA, AMPK α 2 constitutively active form; AMPK α 1+ α 2-DN, AMPK α 1 and AMPK α 2 dominant negative forms.

Table 2: Body weight, food intake, 24h-respiratory quotient and energy expenditure in AMPK α 1-/- and AMPK α 2-/- mice during fasted and fed periods.

	24h-FASTED		FED	
	control	AMPKα1-/-	control	AMPKα1-/-
Body weight (g)	30 \pm 1	31 \pm 1	30 \pm 1	31 \pm 1
Food intake (g/day)	-	-	4.7 \pm 0.1	4.8 \pm 0.1
Energy expenditure (mean/24H)	168 \pm 6	175 \pm 5	245 \pm 11	246 \pm 7
24h-respiratory quotient (VCO ₂ /VO ₂)	0.77 \pm 0.01	0.77 \pm 0.01	0.98 \pm 0.03	0.96 \pm 0.03
	control	AMPKα2-/-	control	AMPKα2-/-
Body weight (g)	32 \pm 1	31 \pm 1	31 \pm 1	31 \pm 1
Food intake (g/day)	-	-	3.8 \pm 0.1	3.7 \pm 0.1
Energy expenditure (mean/24H)	207 \pm 6	205 \pm 3	259 \pm 6	264 \pm 8
24h-respiratory quotient (VCO ₂ /VO ₂)	0.88 \pm 0.01	0.89 \pm 0.01	0.93 \pm 0.01	0.94 \pm 0.01

Values are means \pm S.E.M.

Table 3: Mitochondrial respiration on control and AMPK \square 2-/- muscles. Values are means \pm S.E.M. Oxygen consumption rates in the absence (V_0) and presence of 2mmol/l ADP (Vglu+mal respiration through complex I with glutamate+malate; Vsuc respiration through complex II with succinate). Michaelis-Menten constant of respiration rate for ADP (μ mol/l) without (K_m) or with (K_{mCr}) 20mmol/l creatine. ACR: acceptor control ratio (Vglu+mal/ V_0). * $p < 0.05$ versus control mice.

	Soleus		Plantaris	
	CT	AMPK \square 2-/-	CT	AMPK \square 2-/-
Animals (n)	9	8	9	8
Fibers (n)	9	8	18	16
V_0 (μ mol O ₂ .min ⁻¹ .g ⁻¹ dw)	1.8 \pm 0.5	1.3 \pm 0.2	1.5 \pm 0.2	1.7 \pm 0.3
Vglu+mal (μ mol O ₂ .min ⁻¹ .g ⁻¹ dw)	9.8 \pm 1.4	9.9 \pm 1.4	7.8 \pm 0.6	7.7 \pm 0.9
Vsuc (μ mol O ₂ .min ⁻¹ .g ⁻¹ dw)	6.3 \pm 1.7	7.3 \pm 1.3	4.6 \pm 0.3	5.5 \pm 0.7
K_{mADP} (μ M)	246 \pm 54	224 \pm 57	233 \pm 30	406 \pm 61
$K_{mADP+Cr}$ (μ M)	98 \pm 19	80 \pm 13	93 \pm 12	126 \pm 20
ACR	7.7 \pm 1.6	8.5 \pm 1.2	6.8 \pm 1.1	4.6 \pm 0.3

Fig1.jpg

Figure 1

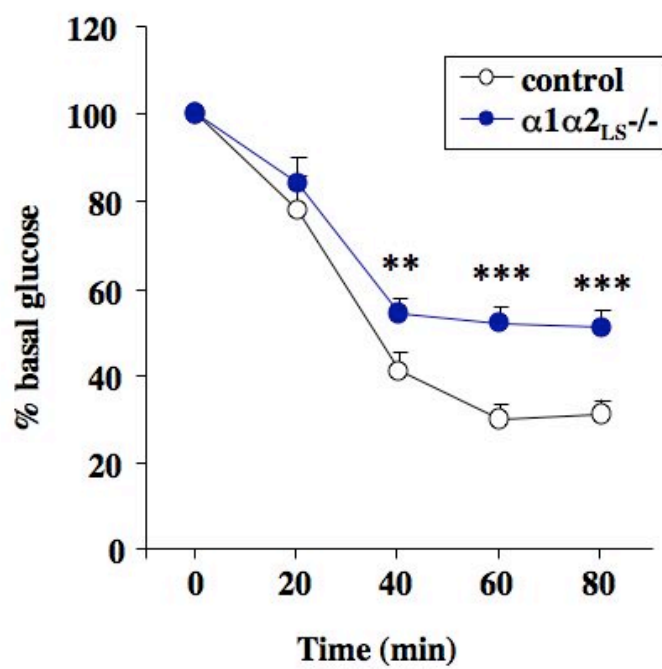


Fig2.jpg

Figure 2

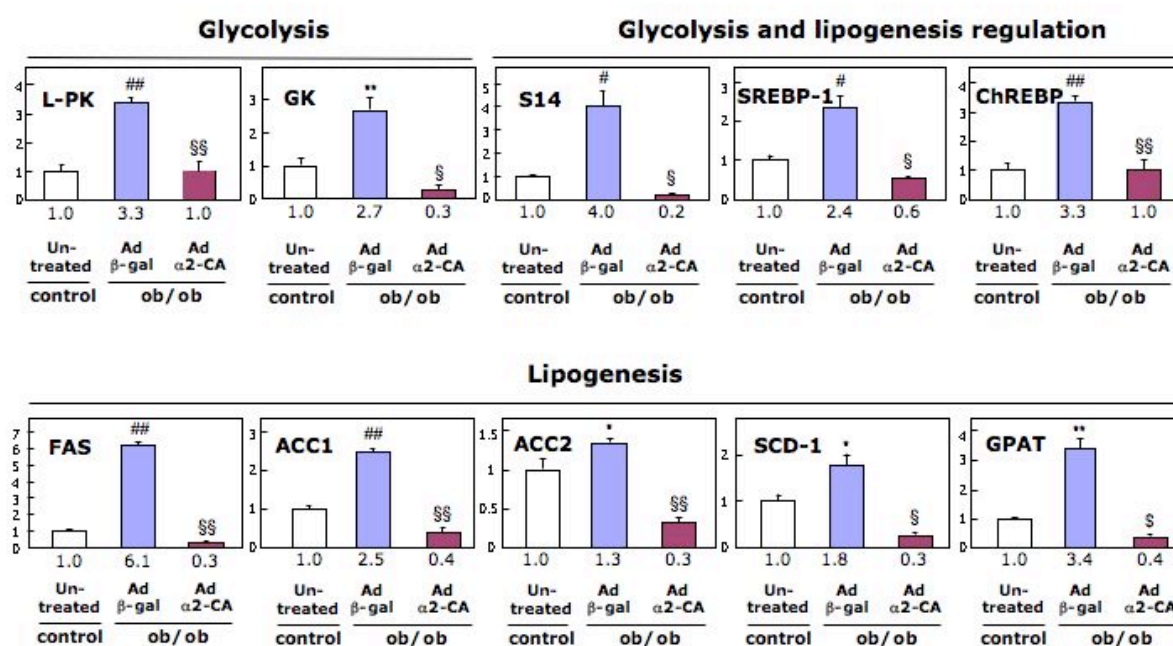


Fig3.jpg

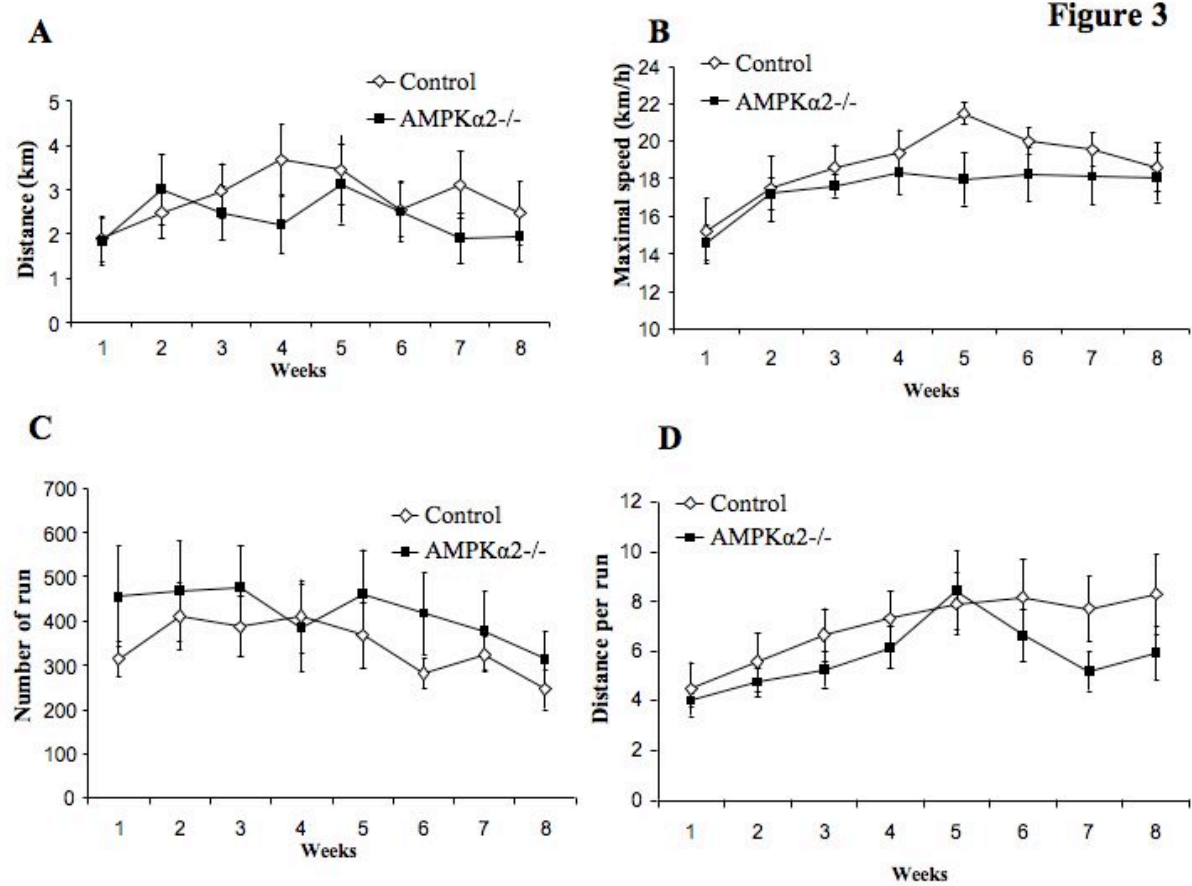


Fig4.jpg

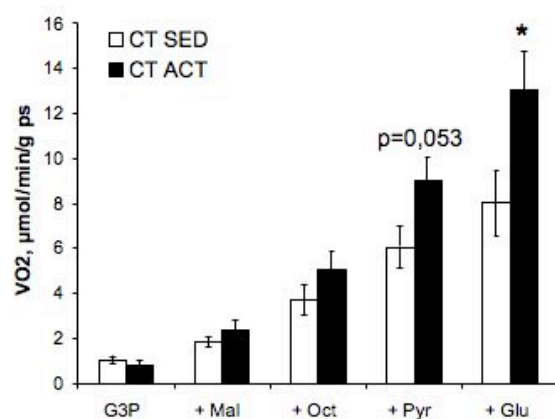
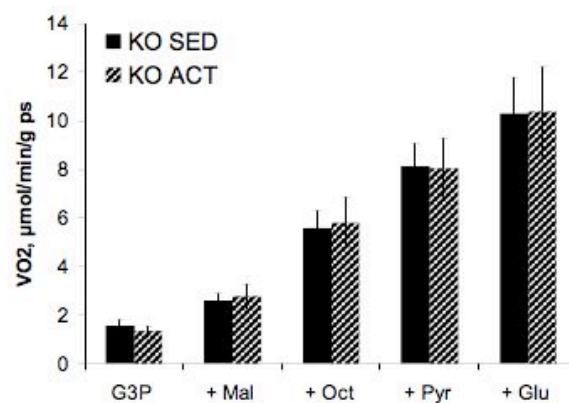
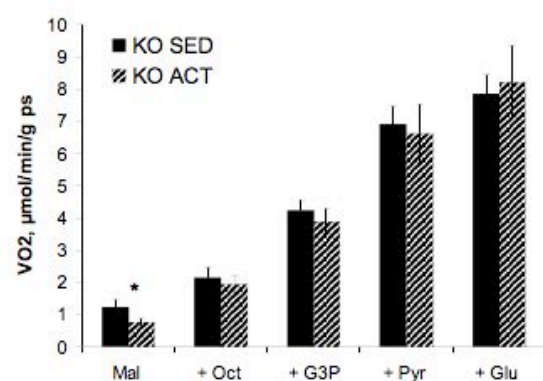
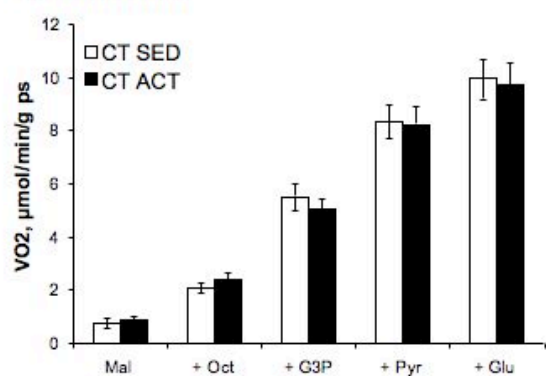
A Soleus**Figure 4****B Plantaris**

Fig5A.jpg

Figure 5A

Aerobic heart

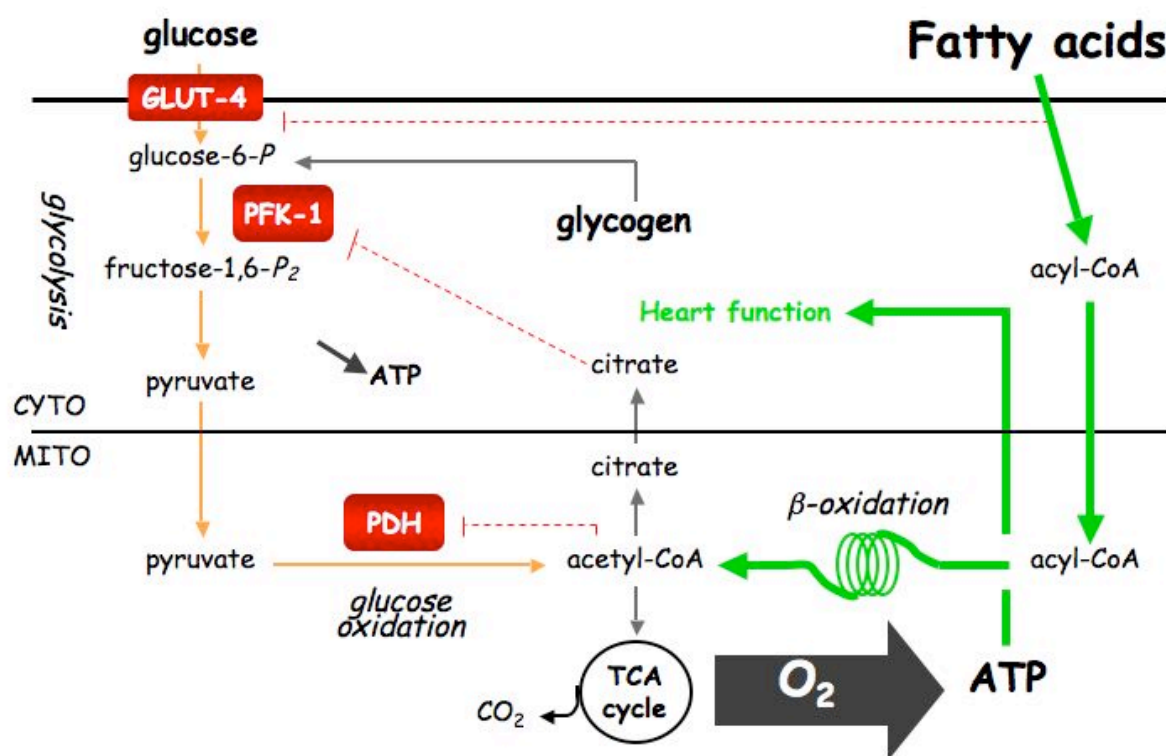


Fig5B.jpg

Figure 5B

Ischemic heart

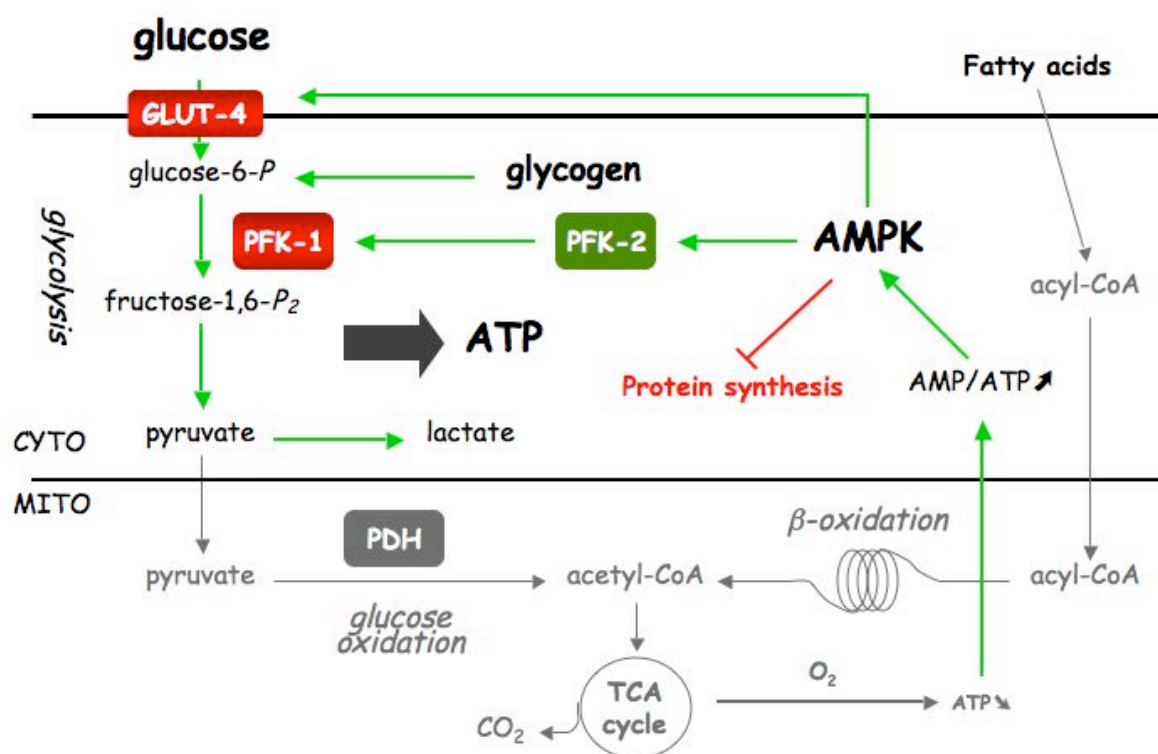


Fig5C.jpg

Figure 5C

Reperfused heart

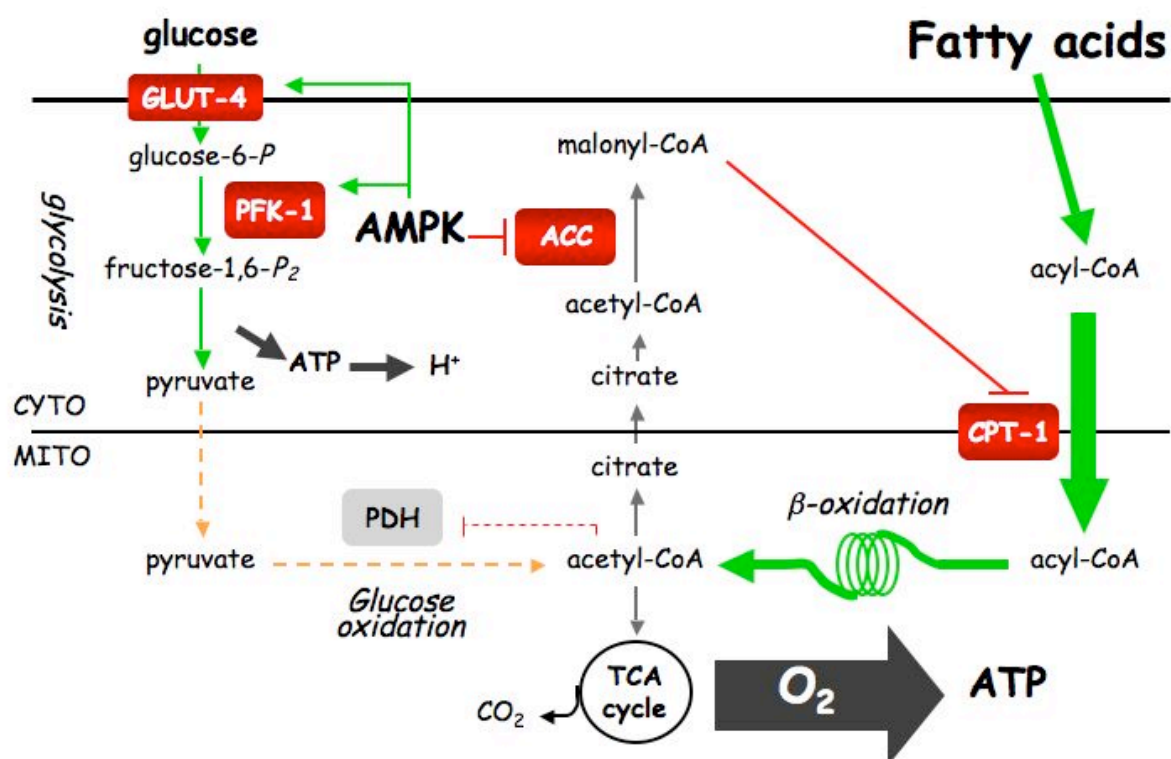


Fig6.jpg

Figure 6

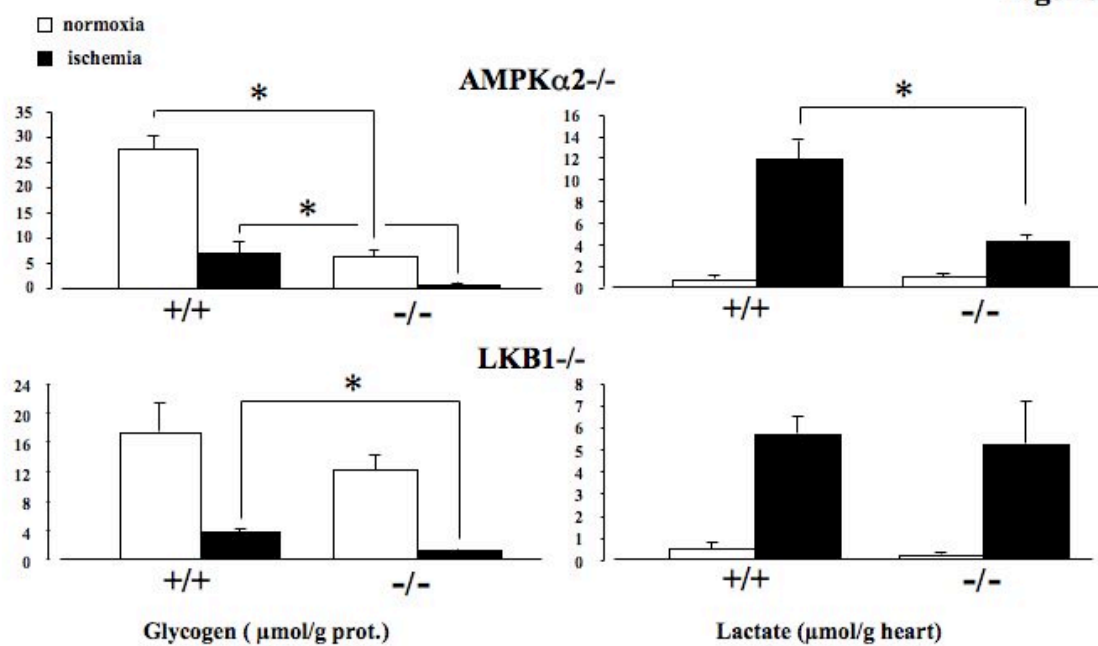


Fig7.jpg

Figure 7

