

Targeting the AMPK pathway for the treatment of Type 2 diabetes

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Abstract

Type 2 diabetes is one of the fastest growing public health problems worldwide resulting from both environmental and genetic factors. It is characterized by the abnormal glucose and lipid metabolism due in part to resistance to the actions of insulin in skeletal muscle, liver and fat. This may result from inadequate adaptation to environmental changes (e.g., imbalance between energy intake and energy expenditure). AMP-activated protein kinase (AMPK), a phylogenetically conserved serine/threonine protein kinase, acts as an integrator of regulatory signals monitoring systemic and cellular energy status. The growing realization that AMPK regulates the coordination of anabolic (synthesis and storage of glucose and fatty acids) and catabolic (oxidation of glucose and fatty acids) metabolic processes represents an attractive therapeutic target for intervention in many conditions of disordered energy balance. Recent evidences that pharmacological activation of AMPK improves blood glucose homeostasis, lipid profile and blood pressure in insulin-resistant rodents, make this protein kinase a novel therapeutic target in the treatment of type 2 diabetes. Consistent with these results, physical exercise and two major classes of antidiabetic drugs (biguanides and thiazolidinediones) have recently been reported to activate AMPK. In the present review, we update these topics and discuss the concept of targeting AMPK pathway for the treatment of type 2 diabetes.

Introduction

The increased prevalence of obesity and type 2 diabetes, with the attendant increase in morbidity and mortality, pose a substantial therapeutic challenge. Type 2 diabetes is a complex polygenic disease with a strong genetic component, as indicated by the high prevalence in certain ethnic groups and by studies of identical twins. Nevertheless, the rapid increase in the prevalence of obesity-associated disease conditions, including type 2 diabetes, in worldwide populations suggests the contribution of environmental factors. A widely accepted explanation lays on the frequent consumption of processed foods with a high-calorie content and the reduction in physical exercise due to sedentary lifestyle in modern urban environment. Disruption of energy balance has led to an increased prevalence of these conditions (1). Type 2 diabetes is characterized by altered lipid and glucose metabolism (fasting or postprandial hyperglycemia, dyslipidemia) as a consequence of combined insulin resistance in skeletal muscle, liver and adipose tissue and relative defects of insulin secretion by β -cells that may arise due to an imbalance between energy intake and expenditure (2). Insulin resistance occurs when a normal dose of hormone is unable to elicit its metabolic responses. Insulin is the primary anabolic hormone that stimulates uptake and storage of fuel substrates, while inhibiting substrate production in peripheral tissues. It lowers blood glucose levels by facilitating glucose uptake, mainly into skeletal muscle and fat tissue, and by inhibiting endogenous glucose production in the liver. Peripheral insulin resistance is associated with lipid partitioning in specific compartments, i.e. muscle and liver, more than with obesity per se. Usually, after an asymptomatic period of insulin resistance, hyperglycemia appears when pancreatic β -cells fail to secrete sufficient amounts of insulin to meet the metabolic demand. In the natural history of type 2 diabetes, pancreatic β -cells initially compensate for insulin resistance by hypersecretion of insulin, but with time, progressive β -cell failure leads to insulin deficiency and hyperglycemia ensues. Progression in diabetes leads to the development of chronic complications such as blindness, kidney failure, neuropathy, cardiovascular diseases and amputations. Thus novel ways to prevent and treat type 2 diabetes are urgently needed.

Non-pharmacological approaches including diet modification, weight control, regular exercise and patient education are used as first-line therapy for the management of type 2 diabetes and remain important for optimization of metabolic control. When lifestyle modification fails to achieve or sustain adequate glycemic control, insulin or oral anti-diabetic agents are typically used to manage the disease. Treatment options with oral agents are quite diverse, including metformin (Glucophage®) (inhibition of hepatic glucose production), thiazolidinediones (TZDs) (insulin sensitizers), α -glucosidase inhibitors (inhibition of gut glucose absorption) and sulphonylureas (β -cell secretagogues). Several new drugs with glucose-lowering efficacy offering certain advantages have recently become available, such as injectable glucagon-like peptide-1 (GLP-1) receptor agonists and oral dipeptidyl peptidase-IV (DPP-IV) inhibitors. Both of them are promising not only to normalize fasting and postprandial glucose levels but also to improve β -cell functioning and mass by stimulating neogenesis. Indeed, β -cell plasticity enables these cells to adapt their number and volume (β -cell mass) and their function to the increased secretory demand linked to insulin resistance. The currently available classes of oral agents differ in mechanism

and duration of action, the degree to which they lower blood glucose and their side-effect profile. Although in recent years the emphasis on initial therapy has been shifting from insulin secretagogues to insulin sensitizers, their mechanisms of action are still incompletely understood. Exciting recent developments have shown that AMP-activated protein kinase (AMPK), a phylogenetically conserved serine/threonine protein kinase, is one of the probable target of major antidiabetic drugs, metformin and TZDs, and of insulin sensitizing adipokines (e.g., adiponectin). Evidence accumulated over the past few years indicates that AMPK acts as an integrator of regulatory signals monitoring systemic and cellular energy status, thus providing powerful validation of the concept of targeting the AMPK pathway for the treatment of type 2 diabetes.

Managing type 2 diabetes by targeting AMPK pathway: an emerging concept

Lifestyle intervention strategies to prevent and control type 2 diabetes

During the past two decades, type 2 diabetes has reached pandemic proportions in association with rising levels of obesity and inactivity. Although physical activity is known to produce multiple health benefits, many people are considered to be relatively physically inactive. Current guidelines recommend practical, regular and moderate regimens of physical activity. The multiple metabolic adaptations that occur in response to physical activity can improve glycemic control for individuals with type 2 diabetes or delay the onset of the disease. This is supported by observational studies and clinical trials of diet, drugs or exercise, in persons at high risk for type 2 diabetes (3–5). The Diabetes Prevention Program (DPP) Research Group conducted a large, randomized clinical trial involving adults in the United States who were at high risk for the development this disease (5). Eligible participants were randomly assigned to one of three interventions: standard lifestyle recommendations plus metformin at a dose of 850 mg twice daily, standard lifestyle recommendations plus placebo twice daily, or an intensive program of lifestyle modification (low-calorie, low-fat diet and physical activity of moderate intensity). The incidence of diabetes was reduced by 58% with the lifestyle intervention and by 31% with metformin, as compared with placebo after 3 years of follow-up. These effects were similar in men and women. In this study, the lifestyle intervention was particularly effective (and more than metformin treatment), with one case of diabetes prevented per seven persons treated for three years.

In addition to impacting development of type 2 diabetes, regular physical activity participation is associated with numerous health benefits for the individual with diabetes. Physical activity appears to have an independent and beneficial effect on insulin action, glycemic control and metabolic abnormalities associated this disease (6). Regular physical activity leads to a number of beneficial physiological changes that favourably affect muscle and liver insulin sensitivity, muscle glucose uptake and utilization and overall glycemic control (7). In addition, performed activity can improve lipid profile, decrease body weight and percentage of body fat, lower blood pressure, positively affect thromboembolic state and thus reduce overall cardiovascular disease risk (8, 9). These beneficial effects are partly explained by AMPK activation during physical activity not only in skeletal muscle but also in liver and adipose tissue (10). Indeed, in type 2 diabetes patients, improvement in glycemic control during physical activity is linked to enhanced glucose transport in skeletal muscle and reduction in hepatic glucose production (11). Interestingly, these effects are insulin-independent. This probably explains that, despite whole-body insulin resistance, AMPK can be fully activated by physical training in type 2 diabetes patients as compared to healthy controls (12). In addition to this acute metabolic effect on glucose disposal, repeated physical activity improves insulin action in skeletal muscle from obese and insulin-resistant individuals (13). This improvement parallels an increase in the oxidative capacity of skeletal muscle by up-regulating lipid oxidation and the expression of proteins involved in mitochondrial biogenesis. Both of these mechanisms are linked to AMPK activation that seems to be a crucial key for metabolic adaptation to physical activity (see below). Use of AMPK agonists could be a new strategy to increased endurance without exercise in physically inactive type 2 diabetes patients.

Management of β -cell mass and function in type 2 diabetes

Clinical studies demonstrated that TZDs improve β -cell function for a long time both as monotherapy and in combination with metformin or sulfonylurea (14). They may also improve insulin processing, as demonstrated by a reduction in the proinsulin/total immunoreactive insulin ratio, (an indicator of β -cell dysfunction) whereas sulfonylureas did not (15). More importantly, TZDs can prevent or delay type 2 diabetes in patients with impaired fasting glucose. This has been clearly demonstrated in different clinical studies, such as TRIPOD (Troglitazone in Prevention of Diabetes) (16), PIPOD (Pioglitazone in Prevention of Diabetes) (17) in women with prior gestational diabetes mellitus, DPP (5) and DREAM (Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication) (18). In consequence, these clinical trials support the idea that early detection and proper preventive measures, including TZDs treatment, can prevent the progression of high-risk patients from developing DM2. Interestingly, metabolic and insulin-sensitizing effects of TZDs have been shown to be in part mediated through adiponectin-dependent activation of AMPK since activation of AMPK by rosiglitazone treatment is diminished in adiponectin KO mice (19). TZDs can markedly enhance the expression and secretion of adiponectin in humans and rodents in vitro and in vivo, through the activation of its promoter and also antagonize the suppressive effect of TNF- α on the production of adiponectin (20).

Management of cardiovascular diseases in type 2 diabetes

Cardiovascular disease (CVD) is the main cause of morbidity/mortality in diabetes. As suggested by the results of the clinical trial ACCORD (Action to Control Cardiovascular Risk in Diabetes), there is no evidence that normoglycemia can, by itself, lower the CVD in type 2 diabetic patients (21). Thus, it is necessary to develop new strategies to reduce CVD in this population. Metformin has cardioprotective effects on lipids, thrombosis and blood flow. It was reported that metformin was associated with a reduction by 32% in any diabetes related endpoint and in myocardial infarction by 39% (22). The figures for macrovascular complications compare favourably for those described for other cardioprotective agents such as angiotensin converting enzyme (ACE) inhibitors and statins. The beneficial effect of metformin on CVD is independent of changes in insulin resistance or glucose control suggesting a direct effect on vessels. Studies in vitro or in type 2 diabetes rodents models clearly demonstrated that metformin normalizes endothelial function (23). Similarly, adiponectin has protective effect on endothelial functions and CVD and type 2 diabetes mellitus are associated with low plasma concentration of adiponectin (24). In isolated rat hearts, adiponectin protects from myocardial contractile dysfunction and limits infarct size in following ischaemia and reperfusion by a mechanism involving activation of AMPK and production of NO (25). In humans, clinical trials concerning use of recombinant adiponectin during myocardial infarction are not available. Nevertheless, the increase of blood adiponectin levels to normal range after a cardiovascular event could be a new therapeutic target. To this aim, the association of drugs classically used for the prevention of recurrence of CVD and some dietary modifications could be useful to restore adiponectin levels in the normal range. This has been described recently in a double-blind, placebo-controlled, parallel trial where 44 patients who survived myocardial infarction and received statin therapy for at least 6 months were randomised to receive either 3 × 85 mg/day of chokeberry flavonoid extract or placebo for a period of 6 weeks (26). A significant increase in adiponectin levels and also a reduction in inflammatory markers were found when flavonoid extract was used. These beneficial effects, regardless of statins, are potentially interesting for secondary prevention of ischaemic heart disease.

Structure and regulation of AMPK

AMP-activated protein kinase (AMPK) plays an important role in the regulation of cellular and whole-body energy homeostasis. AMPK has been described as a “metabolic master switch” that mediates the cellular adaptation to nutritional and environmental variations depleting intracellular ATP levels, including heat shock, hypoxia, starvation or prolonged exercise. Regardless, the result of AMPK activation is the inhibition of energy-consuming biosynthetic pathways (such as fatty acid synthesis in liver and adipocytes, cholesterol synthesis in liver, protein synthesis in liver and muscle and insulin secretion from β -cell) and the activation of ATP-producing catabolic pathways (such as fatty acid uptake and oxidation in multiple tissue, glycolysis in heart and mitochondrial biogenesis in muscle). AMPK can also modulate transcription of specific genes involved in energy metabolism, thereby exerting long-term metabolic control.

AMPK is composed of three different subunits α , β and γ . Homologues of these subunits have been identified in mammals, *Drosophila*, worm, yeast, plants and primitive protozoan, with a high degree of conservation, suggesting the important role of AMPK in regulation of metabolic homeostasis. In mammals, the heterotrimeric complexes combine catalytic α subunit ($\alpha 1$ or $\alpha 2$), with β ($\beta 1$ or $\beta 2$) and γ ($\gamma 1$, $\gamma 2$ or $\gamma 3$) regulatory subunits encoded by separate genes yielding to 12 heterotrimeric combinations (Figure 1). In addition, the $\gamma 2$ and $\gamma 3$ genes also give rise to short and long splice variants adding to the diversity. Differences in level expression and tissue distribution of the three subunits types were also described. The catalytic $\alpha 1$ subunit is strongly expressed in the kidney, the lung and the adipose tissue, whereas catalytic $\alpha 2$ subunit is predominantly found in heart and skeletal muscles. The regulatory $\beta 1$ subunit is preferentially expressed in the liver and $\beta 2$ in the skeletal muscle. The regulatory $\gamma 1$ and $\gamma 2$ subunits have broad tissue distribution whereas $\gamma 3$ seems highly specific to glycolytic skeletal muscle. Moreover, despite the possible multiple combinations of the different subunits, only three are found in human skeletal muscle ($\alpha 2\beta 2\gamma 1$; $\alpha 2\beta 2\gamma 3$ and $\alpha 1\beta 2\gamma 1$) with different levels of expression and activation during exercise (27, 28). The α subunit contains a serine/threonine protein kinase catalytic domain in the N-terminal part, typical of the protein kinase superfamily (29). The catalytic domain has a site of phosphorylation at threonine residue Thr172 within the activation loop (T-loop) which is the key site for AMPK activation by upstream kinases (30, 31). In the extreme C-terminus part, a region of ~150 amino acid residues is required for association with β and γ subunits, whereas the central part seems to have an inhibitory function (32, 33). Moreover, localization of $\alpha 2$ -containing complexes both in the nucleus and the cytoplasm implies a direct control of genes expression via phosphorylation of transcription factors (34). The β subunit appears to stabilize the interaction between α and γ subunits through its binding domain in the C-terminal part (35, 36). In addition, the presence of a glycogen-binding domain in the N-terminal part corroborates the fact that AMPK binds glycogen granules and regulates glycogen metabolism (37, 38). The high variable γ subunits are characterized by the presence of four cystathionine- β synthase (CBS) domains organized in tandem pairs to generate two binding sites for AMP or ATP, bound in a mutually exclusive manner, called Bateman domains (39). Glycogen storage disorders and a related hereditary heart disease have been described consecutively to mutations in these domains for the $\gamma 3$ and $\gamma 2$ genes, respectively (40, 41). Recently, in addition to the yeast homologue Snf1 (42) and mammalian glycogen-binding site of $\beta 1$ subunit (43), crystal structure of a trimeric complex containing the C-terminal domains of $\alpha 1$ and $\beta 2$ with full-length $\gamma 1$ was resolved, in presence of AMP or Mg-ATP. The results confirm the binding of either AMP or ATP on two known sites of the γ subunit and reveal a third site with tightly bound AMP. Under physiological conditions, AMPK interacts predominantly with Mg-ATP to form inactive complexes which are more abundant than complexes with AMP. These studies also suggest that binding of phosphorylated α and/or β subunit(s) would be possible in the presence of AMP but not when ATP is

bound to the γ subunit (44). In addition to the multiple combinations of the three different subunits in the heterotrimeric complex, as well as their tissue-specific expression, the various regulations (i.e. phosphorylation, binding of AMP/ATP and glycogen) and subcellular localization may constitute a very fine and specific regulation of the metabolic tracts by AMPK.

Regulation of AMPK activity involves both direct allosteric activation by AMP and reversible phosphorylation of AMPK α subunit on Thr172 by upstream kinases (Figure 2). The combination of the allosteric and phosphorylation effects causes >1000-fold increase in kinase activity (45) allowing to respond to small changes in cellular energy status in a highly sensitive manner. Under conditions of high cellular energy demands, intracellular ATP is reduced, AMP levels rise and the AMP/ATP ratio forms a very sensitive indicator of cellular energy status (46). Binding of AMP to the regulatory γ -subunit of AMPK promotes allosteric activation, phosphorylation of Thr172 by upstream kinases and inhibition of dephosphorylation of Thr172 by protein phosphatases. There are at least two protein kinases capable of phosphorylating Thr172 *in vivo*, LKB1 (47–49) and Ca²⁺/calmodulin-dependent kinase kinase (50–52), especially the β isoform (CaMKK β). LKB1, originally identified as a tumour suppressor, exists as a complex with two accessory subunits, STRAD and MO25. The LKB1 complex is supposed to be constitutively active and to promote activation by the AMP-dependent pathway (49) but recent studies indicate that cytosolic localization and activity of LKB1 can be governed by LKB1 acetylation status in the liver (53). An alternate pathway for AMPK activation involves CaMKK β which responds to changes in cytoplasmic Ca²⁺ levels, indicating that AMPK may be activated in the absence of increase levels of AMP (50–52). Phosphorylation and activation of AMPK can be reversed by protein phosphatases. Binding of AMP to AMPK induces a conformational change in the kinase domain that protects AMPK from dephosphorylation of Thr-172 (54), probably catalysed by a form of protein phosphatase-2C (55).

Mimicking the beneficial effects of physical exercise

Although appropriate diet and exercise regimes should therefore be the first choice of treatment and prevention of type 2 diabetes, there are patient groups for whom such regimes are not appropriate for other medical reasons, or where compliance is difficult because of social factors or poor motivation. In these cases, drugs acting on the signaling pathways that induce the favourable changes in whole body metabolism are attractive candidates for treatment and prevention. It is now well established that muscle contraction is a prototypical AMPK activator (56). It has been suggested that AMPK activation may recapitulate some of the exercise-induced adaptations and is likely to mediate beneficial effects of exercise on insulin sensitivity and glucose transport in skeletal muscle (57). Activation of AMPK with the pharmacological compound AICAR (5-Aminoimidazole-4-carboxamide-1- β -D-ribose nucleoside, metabolized to ZMP which is an analog of AMP) increases running endurance in untrained mice suggesting that AMPK agonists are exercise mimetics (58). Furthermore, AICAR upregulates genes linked to oxidative metabolism, angiogenesis and glucose sparing (58). Thus, it is expected that part of the effect of physical activity in preventing the development of diseases related to a sedentary lifestyle is due to activation of AMPK. However, although the activation of AMPK in skeletal muscle can lead to the stimulation of glucose transport, contraction-stimulated glucose transport is normal or partly affected in transgenic mouse models overexpressing dominant negative form of AMPK α 2, in skeletal muscle and in whole body AMPK α 1 and AMPK α 2 knock-out (KO) mice (59–62). These results suggest that muscle contraction leads to the activation of multiple redundant signaling pathways and that inhibition of only one is not sufficient to alter contraction-induced glucose transport. Conversely, activation of AMPK with agents that elicit or mimic the effects of contraction (changes in AMP:ATP ratio, intracellular Ca²⁺ levels and reactive oxygen species) is responsible for increase in muscle glucose transport. Studies with AMPK activators in animal models of type 2 diabetes have provided promising results. The first evidence came from *in vivo* treatment with the pharmacological compound AICAR of various animal models of insulin resistance, causing improvement in most, if not all, the metabolic disturbances of these animals (63–67). Interestingly, long-term AICAR administration prevents the development of hyperglycemia in Zucker diabetic fatty (ZDF) rats, improves peripheral insulin sensitivity in skeletal muscle and delays β -cell dysfunction associated with type 2 diabetes (66). In addition, ablation of AMPK α 2 specifically in skeletal muscle, exacerbates the development of insulin resistance and glucose intolerance caused by high-fat feeding (68). Finally, it was reported that the insulin sensitizing effects of AICAR were diminished by inhibition of AMPK in C2C12 myotubes (69). Repetitive pharmacological activation of AMPK *in vivo* results in expression of specific muscle proteins mimicking some of the effects of exercise training such as increased glucose uptake and mitochondrial biogenesis (Figure 3). In rodent, AICAR or chronic intake of the creatine analogue β -guanidinopropionic acid (β -GPA) increases the expression of genes encoding glucose transporter GLUT4, hexokinase II and markers of mitochondrial biogenesis (70–73). A recent report reveals that peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) expression is required for AMPK-dependent activation of gene expression, including PGC-1 α itself, GLUT4 and mitochondrial genes (74). Interestingly, these gene expression effects are abolished in AMPK α 2 KO mice and transgenic mice overexpressing a kinase-dead AMPK α 2 mutant in skeletal muscle (mAMPK-KD mice) (75, 76).

Recent studies support the critical role of AMPK in the metabolic adaptation of skeletal muscle to exercise training. Over-expression of an active AMPK γ 1R70Q mutant in skeletal muscle significantly rises the relative proportion of type IIa/x fibers, the expression of PGC-1 α and the activity of mitochondrial markers in sedentary transgenic animals compared to their sedentary controls, without any further increase with exercise training (77). Moreover, AMPK α 2 KO mice have a disturbed muscle energy balance during exercise, as indicated by a reduced ATP content (76). Physical training also increases circulating adiponectin and mRNA expression of its receptors in

muscle, which may mediate the improvement of insulin resistance in response to exercise by activation of AMPK (78). Interestingly, metformin treatment of subjects with type 2 diabetes significantly increases AMPK activity in skeletal muscle and this stimulation is accompanied by enhanced peripheral glucose disposal (79 , 80). Metformin is also able to restore glucose uptake stimulation in insulin-resistant cardiomyocytes, suggesting that AMPK activation could be a potential therapeutic approach to treat insulin resistance in diabetic hearts (81). Finally, activation of AMPK in response to physical exercise is also observed in extra-muscular tissues such as liver and adipose tissue and might account for additional metabolic benefits (10).

Mimicking the beneficial effects of calorie/dietary restriction

Calorie intake is an important determinant of health. Excessive calorie intake and subsequent abdominal obesity increase the risk of developing chronic disease such as type 2 diabetes, cardiovascular complications and premature mortality. In overweight and obese humans, calorie restriction (CR) with adequate nutrition improves glucose tolerance and insulin action and reduces mortality for type 2 diabetes and cardiovascular diseases (82 –85). Even if calorie restriction produces a metabolic profile desirable for treating type 2 diabetes, it is unlikely that such restriction will be widely adopted because of the difficulty in maintaining long-term low calorie intake in modern society. There is an increased interest in developing pharmacological agents acting as “calorie-restriction” mimetics. Such agents could provide the beneficial metabolic, hormonal and physiological effects of calorie restriction without altering dietary intake or experiencing any potential adverse consequences of excessive restriction. The plant-derived polyphenolic compounds, such as resveratrol (RSV) present in grapes, peanuts and several other plants, recently held great attention for their role in mimicking the effects of calorie restriction. This was evidenced by the findings that RSV can delay the aging process in lower eukaryotes (86). In rodents, RSV prevents the deleterious effects of excess calorie intake on insulin resistance and metabolic disorders (87 –91). RSV acts as a potent activator of the NAD(+)-dependent deacetylases sirtuins including SIRT1, one of the seven mammalian sirtuin genes. SIRT1 has been suggested to prime the organism for metabolic adaptation to insulin resistance, increasing hepatic insulin sensitivity and decreasing whole-body energy requirements (91 , 92). It is also involved in insulin secretion (93 , 94) and lipid mobilization (95). Interestingly, the polyphenols RSV and epigallocatechin-3-gallate (EGCG) were recently identified as potent activators for AMPK in vitro and in vivo (88 , 96 , 97). Furthermore, it has been demonstrated that SIRT1 functions as an upstream regulator of the LKB1/AMPK signaling axis in response to RSV activation in hepatocytes (98). SIRT1 promotes LKB1-dependent AMPK stimulation through the direct deacetylation and activation of LKB1 (53). It should be noted that the regulation of LKB1/AMPK signaling by SIRT1 is probably tissue-specific as resveratrol-stimulated AMPK activation is independent of SIRT1 in neurons (99). This is consistent with in vivo data suggesting that resveratrol may act on additional sirtuins than SIRT1 or on different targets (92). Nevertheless, understanding the role of AMPK in the action of polyphenols will provide valuable information to aid decisions about whether these compounds might be used as additives in foods or beverages to promote health and attenuate, or delay, the onset of various diseases, including cardiovascular disease and diabetes.

Role of AMPK in the control of glucose homeostasis

Glucose homeostasis is maintained by a balance between glucose production and glucose uptake by peripheral tissues. Elevated hepatic glucose production (HGP) is a major cause of fasting hyperglycemia in diabetic subjects (2). The importance of AMPK in the control of glucose output by the liver is emphasized by findings showing that pharmacological activation of AMPK leads to inhibition of HGP in vitro and in vivo . It has been first shown that systemic infusion of AICAR in normal and insulin-resistant obese rats led to the inhibition of hepatic glucose production (HGP) (63). Similarly, it was reported that AMPK activation by metformin in cultured rat hepatocytes mediates the inhibitory effects of the drug on hepatic glucose production (100). There is now good in vivo evidence from studies of mice deficient in the upstream kinase LKB1 in the liver that the blood-lowering effect of metformin is mediated by activation of the LKB1/AMPK axis (101). It has been also reported that short-term hepatic expression of a constitutively active form of the $\alpha 2$ catalytic subunit (AMPK $\alpha 2$ -CA) led to mild hypoglycemia in normal mice (102 , 103) and abolished hyperglycemia in diabetic ob/ob and STZ-induced diabetic mice (102). This hypoglycemic effect of AMPK activation is consistent with the abolition of HGP, as suggested by the down-regulation of gluconeogenic gene expression (e.g., phosphoenolpyruvate carboxykinase [PEPCK] and glucose-6-phosphatase [G6Pase]) and inhibition of glucose production in hepatocytes expressing AMPK-CA or treated with AICAR (102 –104). Inhibition of gluconeogenesis by AMPK is achieved at least to a large extent via the regulation of a transcriptional coactivator, transducer of regulated CREB activity 2 (TORC2) (105). TORC2 mediates CREB-dependent transcription of PGC1 α and its subsequent gluconeogenic targets PEPCK and G6Pase. TORC2 is regulated by multiple signaling pathways in response to changes in glucagon and insulin levels or intracellular energy status. AMPK activation causes TORC2 phosphorylation and sequesters the coactivator in the cytoplasm, thus blunting the expression of the gluconeogenic program (Figure 4). A physiological link has been established between the potent effects of various circulating adipocyte-derived hormones circulating levels and hepatic AMPK activity in the maintenance of blood glucose levels. Low blood glucose levels and reduced HGP in mice lacking resistin are likely related, at least in part, to activation of AMPK and decreased expression of gluconeogenic enzymes in the liver (106). Administration of adiponectin is known to reduce both blood glucose levels and expression of gluconeogenic genes. It has been shown that these effects required AMPK activation at least in the liver (107). According to this result, adiponectin failed to regulate HGP in liver-specific AMPK $\alpha 2$ KO mice (108).

Skeletal muscle is the main site for glucose disposal in the body. Insulin increases glucose uptake in the muscle by stimulating the translocation of glucose transporter GLUT4 from intracellular vesicles to the cell surface. (Figure 3). It has been shown that muscular AMPK activation stimulates muscle glucose uptake either by exercise or by AICAR and this occurs through a distinct mechanism than the insulin-signaling pathway. AICAR can effectively increase glucose transport and GLUT4 translocation in skeletal muscle, not only in lean subjects but also in type 2 diabetes subjects (109). An AMPK-dependant increase in glucose transport is therefore observed in insulin-resistant skeletal muscle, both in rodents and humans, providing evidence that the AMPK pathway can be activated in this case (46). Stimulation of AMPK in the muscle could be an efficient method to increase glucose uptake in an insulin-independent manner, thus bypassing defective insulin signaling, such as one observed in type 2 diabetes patients. Although the upstream stimuli that activate AMPK are relatively well known (46), the signaling mechanisms downstream of AMPK which regulate muscle glucose transport are not so well understood. It has been recently discovered that the Akt substrate of 160kDa (AS160/TBC1D4), downstream target of Akt, played a major role in regulating insulin-stimulated glucose uptake. Another possible downstream effector of AMPK in the regulation of muscle glucose transport is an AS160/TBC1D4 homolog, TBC1D1 (110). AS160/TBC1D4 is a Rab-GTPase activating protein that regulates the translocation of GLUT4 from intracellular vesicles to the plasma membrane by maintaining small G-proteins, known as Rab, in a GDP-bound state. The phosphorylation of AS160 at specific sites is thought to disrupt GTPase activity and allow the release of AS160 inhibition on GLUT4 vesicle intracellular localization, thus initializing the vesicle translocation (110). TBC1D1 is also a Rab-GTPase activating protein and its phosphorylation is enhanced in mouse skeletal muscle myotubes following treatment with AICAR (111). In a similar way, exercise and AICAR-induced AMPK activation caused AS160/TBC1D4 phosphorylation in skeletal muscle like insulin (28). Furthermore, AS160 phosphorylation as well as glucose uptake is increased in skeletal muscle following a resistance exercise in humans. A positive correlation between increased AS160/TBC1D4 phosphorylation and muscle glucose uptake during post-exercise recovery has been observed, as well as increased muscle AMPK α 2 activity (112). Taken together, these results show that AMPK-induced muscle glucose uptake stimulation is mediated by AS160/TBC1D4. This represents a point of convergence connecting insulin, contraction and AMPK-stimulated glucose transport. It has been observed that exercise-induced AMPK activation in muscle is diminished in both obese non-diabetic and obese type 2 diabetes subjects, but maintained in lean type 2 diabetes patients (12 , 113). This suggests that dysregulation of the AMPK pathway may be more associated with obesity rather than with type 2 diabetes per se . This reflects impaired adaptability to utilize lipid and carbohydrate fuels and to transition between them, also referred as metabolic inflexibility (114), observed in obese and insulin resistant people. It is therefore likely that obese patients may require a more intense exercise protocol to achieve the same benefits than in lean individuals (113). Consistently, in obese type 2 diabetes subjects, AS160 phosphorylation is blunted in skeletal muscle following moderate intensity exercise (113).

Several cytokines have been shown to stimulate glucose transport in muscle in an AMPK-dependent manner. Leptin is an adipokine recently shown to improve insulin action in muscle of patients with lipodystrophy, although the role of AMPK activation as mediator is not studied (115). Leptin is also known to stimulate glucose uptake in peripheral tissue (116 , 117). It has been shown that leptin selectively stimulated AMPK α 2 phosphorylation and activation in skeletal muscle, further confirming the role of AMPK in leptin-mediated stimulation of glucose transport (118). Adiponectin also increases glucose transport in both lean and obese skeletal muscle, although the effect is less significant in the latter (119). This suggests a possible development of adiponectin resistance related to metabolic inflexibility observed in skeletal muscle from obese individuals. Interleukin-6 (IL-6) is a proinflammatory cytokine that activates AMPK and is thought to modify insulin sensitivity. Recently, it has also been recognized as a “myokine”, due to its release from the skeletal muscle during prolonged exercise (120). Muscle strips obtained from healthy young men treated with IL-6 show an increased glucose uptake concomitant with AMPK phosphorylation (121). It has been shown that IL-6-stimulated glucose transport is mediated via the LKB1/AMPK/AS160 pathway. However, the same study also shows that IL-6 has a dual effect: short term IL-6 treatment is additive to insulin on activating glucose transport and AS160 phosphorylation, resulting in an improved glucose tolerance and insulin sensitivity in mice, whereas chronic exposure produces insulin resistance both in vitro and in vivo (122). It has been shown that AMPK participates in the regulation of IL-6 release from oxidative muscle. It has been also suggested that AICAR, in addition to activating AMPK, suppresses IL-6 release by an AMPK independent mechanism (123). Collectively these results demonstrate that AMPK plays a major role in glucose homeostasis by modulating glucose transport in skeletal muscle. Thus, AMPK could have a significant role in the modification of glucose muscle metabolism, thus establishing the AMPK pathway as an attractive target for the treatment of type 2 diabetes.

Role of AMPK in the control of lipid metabolism

Both insulin resistance and type 2 diabetes are characterized by dyslipidemia, which is an important and common risk factor for cardiovascular disease. Diabetic dyslipidemia is a cluster of potentially atherogenic lipid and lipoprotein abnormalities that are metabolically interrelated. AMPK coordinates the changes in the hepatic lipid metabolism and, so, regulates the partitioning of fatty acids between oxidative and biosynthetic pathways. Thus, once activated, AMPK phosphorylates and inactivates a number of metabolic enzymes involved in ATP-consuming cellular events, such as 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) and acetyl-CoA carboxylase (ACC), key enzymes in hepatic cholesterol and fatty acid synthesis (Figure 4). In addition, AMPK suppresses expression of lipogenesis-associated genes such as fatty acid synthase, pyruvate kinase and ACC (102 , 124 –127). ACC is an important rate-controlling enzyme for the synthesis of malonyl-CoA, which is both a critical precursor for the biosynthesis of fatty acids and a potent

inhibitor of mitochondrial fatty acid oxidation. Inhibition of ACC by AMPK leads to a drop in malonyl-CoA content and a subsequent decrease in fatty acid synthesis and increase in fatty acid oxidation, thus reducing excessive storage of triglycerides. Malonyl CoA decarboxylase (MCD), an enzyme involved in the turnover of malonyl-CoA, has been shown to be activated by AMPK in response to energy depletion, resulting in reduced malonyl CoA levels and increased fatty acid oxidation (128). Consistently, overexpression of AMPK α 2-CA in the liver, treatment with AICAR or metformin in lean and obese rodents increase plasma β -hydroxybutyrate levels, suggesting elevated hepatic lipid oxidation, concomitantly with a decrease in plasma triglyceride levels (63 , 100 , 102). Conversely, liver-specific AMPK α 2 deletion leads to increased plasma triglyceride levels and enhances hepatic lipogenesis (108). These data emphasizes the critical role for AMPK in the control of hepatic lipid deposition via decreased lipogenesis and increased lipid oxidation, thus improving lipid profile in type 2 diabetes. In addition, AMPK also emerged as a key player in the regulation of fatty acid oxidation in skeletal muscle (Figure 3). In rodent skeletal muscle, stimulation of AMPK by AICAR increased the oxidation of palmitate (129 , 130). Leptin, an adipocyte-secreted hormone that plays a pivotal role in regulation of energy expenditure, was found to increase fatty acid oxidation in skeletal muscle by activating AMPK (118). Leptin activates AMPK by a dual effect, i.e. early and transient activation of AMPK by leptin directly at the level of muscle and a more sustained activation mediated through the hypothalamic-sympathetic nervous system axis and α -adrenergic receptors in muscle (118). Mice harboring a gain-of-function of the γ 3 subunit (R225Q) in skeletal muscle demonstrate lower intramuscular TG content, increased lipid oxidation and protection against diet-induced insulin resistance (131). As in the liver, AMPK enhances fatty acid oxidation by inactivating ACC, thereby reducing the synthesis of malonyl CoA. However, recent studies using mAMPK-KD mice indicate that AMPK-independent pathways can regulate skeletal muscle fatty acid oxidation (132). Furthermore, incubation of isolated rat muscle with both AICAR and electrical stimulation result in higher fatty acid oxidation than for each condition alone (133). In human skeletal muscle, the connection between changes in AMPK activation and fatty acid oxidation during exercise is not clear (134 , 135).

Management of fatty liver disease by AMPK activation

One of the critical complications of type 2 diabetes is nonalcoholic fatty liver disease (NAFLD), a disorder of triacylglycerol accumulation in the liver that has potential to develop into end stage liver failure. It has been proposed that steatosis primed the liver to progress to more severe liver pathologies when individuals were exposed to subsequent metabolic and/or environmental stresses. Insulin resistance is a major feature of NAFLD and studies in humans and various animal models suggest that efforts to enhance insulin sensitivity might improve fatty liver disease. The efficacy of insulin-sensitizer metformin as a treatment for this disease is confirmed in obese ob/ob mice, which develop hyperinsulinemia, insulin resistance and fatty livers (136). Similarly, adiponectin treatment restores insulin sensitivity and decreases hepatic steatosis by lowering the triglyceride content in the liver of obese mice (137). Metabolic improvement of adiponectin is linked to an activation of AMPK in the liver that decreases fatty acid biosynthesis and increases mitochondrial fatty acids oxidation (138). This is confirmed by a decrease in liver triglycerides content in lean and obese rodents during AICAR infusion (63) and treatment with small-molecule AMPK activators (139). Increased intracellular fat content in liver associated with insulin resistance leads to the hypothesis that a mitochondrial dysfunction in substrate oxidation is a primary defect in insulin resistant. It was recently demonstrated that activation of AMPK by RSV protected against lipid accumulation in the liver of diabetic mice (88), in association with increased mitochondrial number (87) and SIRT1-dependent deacetylation of peroxisome proliferator-activated receptor coactivator (PGC)-1 α , a master regulator of mitochondrial biogenesis (87 , 140). Hepatocytes deleted for both AMPK catalytic subunits have reduced mitochondrial biogenesis as suggested by decreased transcript and protein expression of key mitochondrial constituents such as PGC-1 α , cytochrome c oxidase I (COX I), COX IV and cytochrome c genes (141). These results emphasize the importance of AMPK in the regulation of cellular energy homeostasis through the control of adaptive mitochondrial function. However, the role of the AMPK system in the treatment of fatty liver diseases remains to be clearly established in humans. Its importance is strongly indicated by recent studies with AICAR infusion in type 2 diabetic patients. This study reported that AICAR infusion resulted in significant decline in circulating plasma non-esterified fatty acids (NEFA) levels, suggesting stimulation of hepatic fatty acid oxidation and/or reduction in whole body lipolytic rate (142).

Role of AMPK in the regulation of β -cell function

Insulin resistance and insulin secretion defects are major risk factors for type 2 diabetes (143). The pathogenesis of type 2 diabetes is associated with different degrees of β -cell failure relative to varying degrees of insulin resistance. A progressive decrease of β -cell function leads to glucose intolerance, which is followed by type 2 diabetes that inexorably aggravates with time (144). According to the glucolipotoxicity hypothesis (145), chronic high glucose dramatically influences β -cell metabolism and results in an increase of cytosolic fatty acyl-CoA partitioning toward potentially toxic cellular products (e.g., diacylglycerol, ceramide and lipid peroxides). This leads to impaired insulin secretory response to glucose and ultimately in apoptosis. Evidence from recent literature clearly demonstrated that changes in AMPK signaling are important in the pathogenesis of both β -cell glucolipotoxicity and type 2 diabetes. Metformin, TZDs and AICAR treatments favor fatty acid β -oxidation and prevent glucolipotoxicity-induced insulin secretory dysfunction in β -cells (146 –148). These results have been confirmed by using adenovirus-mediated over-expression of AMPK α 1-CA in β -cell (149). A recent study reported that AICAR dose-dependently improves β -cell function without changing intracellular TG levels and may act through reducing

apoptosis induced by prolonged hyperglycemia. However, the role of AMPK in the control of β -cell death remains controversial (150–155).

In β -cells, AMPK activity is rapidly decreased by elevations in glucose concentration over the physiological range indicating that AMPK could play a role in insulin release acting as a fuel sensor (156–158). Activation of AMPK by AICAR, metformin, TZDs and berberine or by overexpression of AMPK α 1-CA markedly reduces glucose-stimulated insulin secretion in β -cell lines and in rodent and human islets (149, 156, 159, 160). Conversely, overexpression of a dominant-negative form of AMPK stimulates insulin release at low glucose concentrations (157, 161). These data suggest that activation of AMPK inhibits insulin release to maintain glucose homeostasis. Thus, inhibition rather than activation of AMPK would be desirable for the treatment of type 2 diabetes in order to reverse the decline in glucose-induced insulin secretion. Surprisingly, it has been reported that agents that suppress insulin secretion, such as diazoxide, improve glucose tolerance and β -cell function (162). This effect is thought to be mediated via hyperpolarization of β -cells, thereby providing β -cell rest by reducing insulin release and could protect against the negative effects of overstimulation. One important point to take into account in this context is the role of pancreatic AMPK during the adaptation of insulin secretion in front of various degrees of insulin resistance during the onset of type 2 diabetes. First, AMPK-mediated suppression of insulin release could directly counteracts glucolipotoxicity, which may prevent functional exhaustion of β -cells in prediabetic states. A decrease in β -cell mass is likely to play a role in the pathogenesis of human type 2 diabetes (163) as it does in rodent models of the disease (164). Second, inhibition of insulin release would reduce the pathological basal hyperinsulinemia and markedly increase insulin sensitivity and hence improve β -cell function and mass. Consistently, systemic AICAR infusion in prediabetic Zucker fatty rats prevents the development of hyperglycemia and preserved β -cell mass (66).

Management of cardiovascular diseases by AMPK activation

Type 2 diabetes is associated with an increased risk of cardiovascular disease and coronary heart disease mortality. The high energy demands of the heart are primarily met by the metabolism of fatty acids and glucose, both processes being regulated by AMPK. Indeed, AMPK stimulates glycolysis and sustains energy supply during ischemic stress. Promotion of glucose oxidation or inhibition of fatty acid oxidation in ischemic/reperfused hearts could be a promising novel therapeutic approach to myocardial ischemic conditions. Such a mechanism has been demonstrated during the phenomenon called ischemic preconditioning. Brief episodes of myocardial ischemia render the heart more resistant to subsequent ischemic episodes (165). Ischemic preconditioning is known to induce endogenous protective mechanisms in the heart. It activates AMPK in a PKC-dependent manner and promotes glucose utilization in myocardial cells, supporting resistance toward ischemic consequences (166). Thus, AMPK activators could be of particular interest for the management of myocardial ischemia. Attractively, it has been reported that adiponectin protects the heart from ischemia by activating AMPK and increasing the energy supply to heart cells (167). In addition, it has also been reported that adiponectin attenuates cardiac hypertrophy through activation of AMPK signaling pathway (168, 169).

The presence of endothelial cell dysfunction in patients with type 2 diabetes, as manifested by impaired vascular relaxation or increase in circulating vascular cell adhesion molecules is thought to be one component of the inflammatory process initiating atherogenesis. In this respect, metformin has been proposed to improve endothelium function in diabetes by favoring phosphorylation of endothelial NO synthase (eNOS) by AMPK activation (23). Metformin was also shown to relax endothelium-denuded rat aortic rings pre-contracted with phenylephrine, showing that AMPK can induce vasorelaxation in an endothelium- and NOS-independent manner (170). Thus, vascular AMPK could be involved in the metabolic regulation of vascular tone. AMPK activation in response to hypoxia or metabolic challenge can induce vasorelaxation of big vessels (171, 172), thereby favoring blood flow. Interestingly, AMPK-dependent adiponectin vascular effects have been demonstrated for angiogenic repair in an ischemic hind limbs model (173).

Conclusion and medical perspectives

Because of its global favourable effects on energy metabolism pathways, it is tempting to consider AMPK as a potential therapeutic target in the prevention and the treatment of type 2 diabetes and insulin resistance. A large number of AMPK activators have been employed with promising results in diabetic animal models and these encouraging results have provided the rationale for the development of new pharmacological (e.g., A-769662) but also nutritional (e.g., polyphenols) AMPK activators. AMPK activation in the liver and skeletal muscle entails metabolic changes that are beneficial for the diabetic patients with inhibition of HGP and stimulation of glucose uptake in skeletal muscle which help to maintain glycemia. However, some of the effects of AMPK activation in other organs or tissues should be carefully evaluated. The widespread cellular functions of AMPK make its selective targeting in therapeutics a difficult one, with simultaneous advantageous and deleterious consequences being possible. Thus, care should be taken that other metabolic effects due to AMPK activation could be detrimental for diabetic patients. One of the major caveats in the use of AMPK activators is their possible role in the regulation of food intake. Stimulation of AMPK expressed in specific nuclei of the hypothalamus has been shown to increase food intake (174). Thus, the ideal AMPK activators will be administrated by the oral route, activate AMPK at low concentration and be effective in specific target organs, such as the liver and skeletal muscle but not the hypothalamus, and will also have minimal off-targets. An emerging concept is the use of tissue-specific pharmacological activation of AMPK that could be achieved through isoform-specific

activation of AMPK. Interestingly, the small AMPK activator A-769662 is mainly targeted to the liver (175) and treatment of animal models with this compound recapitulates many of the effects expected for a specific hepatic AMPK activation (102). Similarly, it appears that the major effect of intravenous AICAR infusion in type 2 diabetic patients is restricted to the liver with inhibition of hepatic glucose output and decreased blood glucose levels (142). Curiously, AMPK activation is not evident in muscle biopsies from healthy volunteers (176) or diabetic patients (142) in response to AICAR infusion. These human data raise the question about the effective dose in producing a detectable AMPK activation in skeletal muscle and the benefit in term of glucoregulation in diabetic patients.

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Abbreviations

ACC : acetyl CoA carboxylase

AICAR : 5-aminoimidazole-4-carboxamide riboside

AMPK : AMP-activated protein kinase

HGP : hepatic glucose production

HMG-CoA : hydroxy-3-methylglutaryl-CoA

KO : knockout

PGC-1 α : peroxisome proliferator-activated receptor- γ coactivator-1 α

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Figure 1

Structure of AMPK

The mammalian AMPK α ($\alpha 1$ and $\alpha 2$), AMPK β ($\beta 1$ and $\beta 2$) and AMPK γ ($\gamma 1$, $\gamma 2$ short form, $\gamma 2$ long form and $\gamma 3$) subunits are shown. The α -subunits contains the Thr172 residue that must be phosphorylated (P) by upstream kinases for activity and an autoinhibitory sequence domain that inhibits the activity of the kinase domain. The C-terminal domain is required for binding the β - and γ -subunits. The β -subunits contains central glycogen-binding domains and C-terminal domain that is required for binding the α - and γ -subunits. The three γ -subunit isoforms have variable N-terminal domains and four conserved cystathionine beta-synthase motifs (CBS1–4). The CBS motifs act in pairs to form two Bateman domains that bind AMP or ATP.

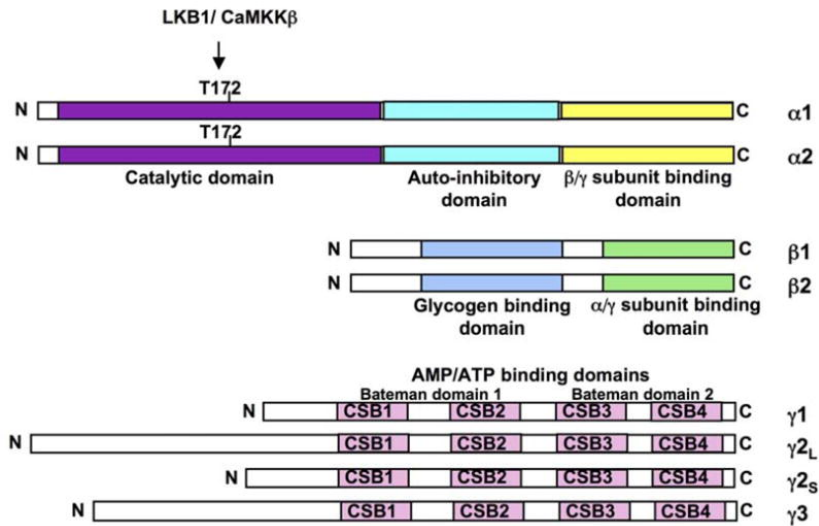


Figure 2

Regulation of AMPK activation

AMPK is activated by phosphorylation of Thr 172 catalysed by LKB1:STRAD:MO25 complex in response to increase in the AMP/ATP ratio and by CaMKK β in response to elevated Ca^{2+} levels. Thr172 is dephosphorylated by PP2C protein phosphatase switching active AMPK to the inactive form.

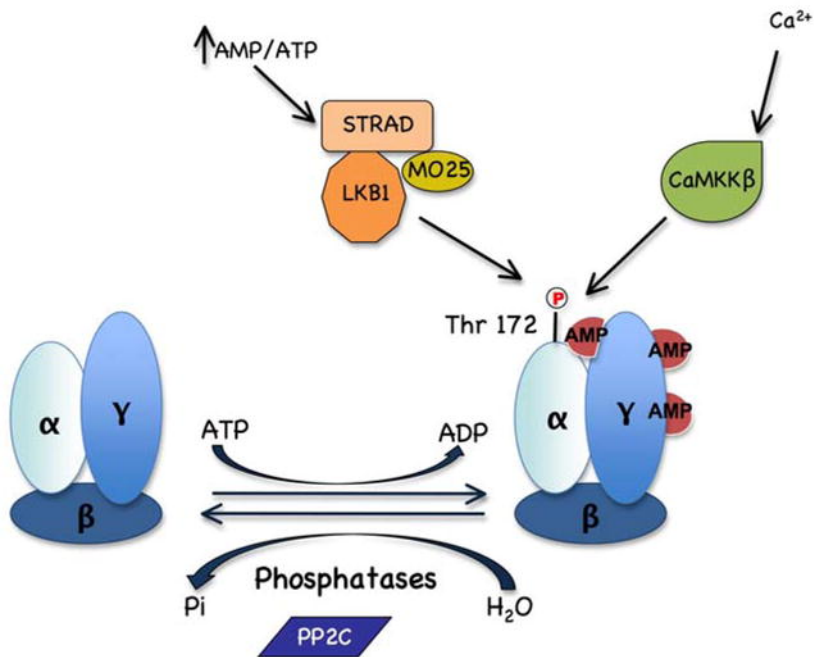
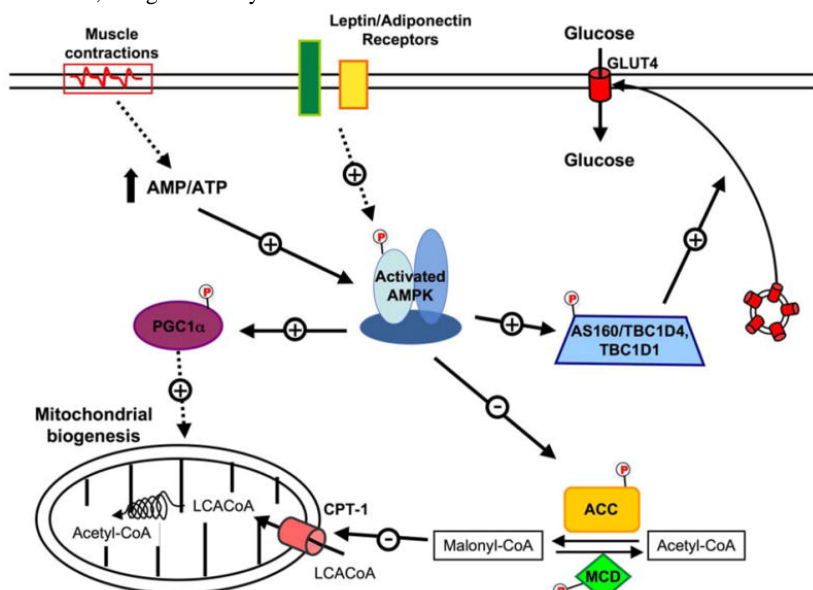


Figure 3**AMPK and the regulation of skeletal muscle metabolism**

Proposed model for the role of AMPK in the regulation of lipid and glucose metabolism in skeletal muscle. AMPK activity may be increased by an altered energy nucleotide or by hormonal action. This activation of AMPK may result in an increase in glucose transport as well as an increase in fatty acid oxidation. ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; AS160, Akt substrate of 160kDa; CPT1- α , carnitine palmitoyl transferase-1; Glut4, glucose transporter 4; MCD, malonyl-CoA decarboxylase; PGC1 α , PPAR γ co-activator 1 α ; LCACoA, Long Chain acyl CoAs.

**Figure 4****AMPK and the regulation of hepatic metabolism**

Activation of AMPK leads to the inhibition of cholesterol synthesis by the phosphorylation of HMG-CoA reductase. By inhibiting ACC and activating MCD, AMPK increases fatty acid oxidation via the regulation of malonyl CoA levels, which is both a critical precursor for biosynthesis of fatty acids and a potent inhibitor of CPT-1, the shuttle that controls the transfer of LCACoA into the mitochondria. AMPK inhibits hepatic glucose production via the phosphorylation of TORC2 and inhibition gene expression for key gluconeogenic enzymes, G6Pase and PEPCK, and for the transcriptional co-activator PGC-1 α . ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; CPT1- α , carnitine palmitoyl transferase-1; G6Pase, glucose-6-phosphatase; LCACoA, Long Chain acyl CoAs; MCD, malonyl-CoA decarboxylase; PEPCK, phosphoenolpyruvate carboxykinase; PGC1 α , PPAR γ co-activator 1 α ; TORC2, transducer of regulated CREB activity 2.

