

Adenosine Monophosphate-Activated Protein Kinase (AMPK) as a New Target for Antidiabetic Drugs: A Review on Metabolic, Pharmacological and Chemical Considerations

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■ Abstract

In view of the epidemic nature of type 2 diabetes and the substantial rate of failure of current oral antidiabetic drugs the quest for new therapeutics is intensive. The adenosine monophosphate-activated protein kinase (AMPK) is an important regulatory protein for cellular energy balance and is considered a master switch of glucose and lipid metabolism in various organs, especially in skeletal muscle and liver. In skeletal muscles, AMPK stimulates glucose transport and fatty acid oxidation. In the liver, it augments fatty acid oxidation and decreases glucose output, cholesterol and triglyceride synthesis. These metabolic effects induced by AMPK are associated with lowering blood glucose levels in hyperglycemic individuals. Two classes of oral antihyperglycemic drugs (biguanidines and thiazolidinediones) have been shown to exert some of their therapeutic effects by directly or indirectly activating AMPK. However, side effects and an acquired resistance to these drugs emphasize the need for

the development of novel and efficacious AMPK activators. We have recently discovered a new class of hydrophobic D-xylose derivatives that activates AMPK in skeletal muscles in a non insulin-dependent manner. One of these derivatives (2,4;3,5-dibenzylidene-D-xylose-diethyl-dithioacetal) stimulates the rate of hexose transport in skeletal muscle cells by increasing the abundance of glucose transporter-4 (GLUT-4) in the plasma membrane through activation of AMPK. This compound reduces blood glucose levels in diabetic mice and therefore offers a novel strategy of therapeutic intervention strategy in type 2 diabetes. The present review describes various classes of chemically-related compounds that activate AMPK by direct or indirect interactions and discusses their potential for candidate antihyperglycemic drug development.

Keywords: diabetes · hyperglycemia · AMP · antihyperglycemic · drug target · energy metabolism · glucose transport · skeletal muscle · D-xylose

The structure of the AMPK complex

he enzyme AMP-activated protein kinase (AMPK) is expressed in all eukaryotic cells as a heterotrimeric complex. The heteromeric structure and functions of this enzyme complex have been widely investigated. In this section, we focus on structural elements in this complex relevant for its interaction with synthetic pharmaceuticals.

The AMPK complex combines two regulatory subunits (β , 30 kDa; γ , 38-63 kDa) and a catalytic subunit (α , 63 kDa). These subunits are encoded by different genes and several isoforms of each have been discovered: $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, $\gamma 1$, $\gamma 2$ and $\gamma 3$ [1]. Theoretically, there are 12 possible heterotrimeric combinations of AMPK. Moreover, variable gene splicing of these gene products also contributes to the diversity of the AMPK complex composition. It appears that different

types of cells and tissues express distinct combinations of the complex. For instance, human skeletal muscles display three complexes in which the $\beta 2$ subunit is conserved: $\alpha 2\beta 2\gamma 1$, $\alpha 1\beta 2\gamma 1$ and $\alpha 2\beta 2\gamma 3$ [2]. Among these three complexes, the latter is predominantly associated with exercise-induced AMPK activation in human skeletal muscles, while the function of the other is less defined [3]. The $\beta 1$ subunit is more ubiquitously expressed and is found in other tissues such as the liver [4].

The first crystal structure of a heterotrimeric AMPK complex was obtained from *Saccharomyces cerevisiae* [5]. However, a full length crystal structure of mammalian AMPK has not yet been obtained. Therefore, structural analyses of the human AMPK subunits are largely based on partial domain structure identification and analysis of interactions among proteolytic truncated fragments of these subunits [6]. Detailed structural analysis of these complexes will enhance the effort to design, synthesize and test novel molecules that interact with distinct AMPK complexes expressed in different cells and organs.

The α subunit of the AMPK complex

This catalytic subunit contains a classical serine/threonine protein kinase domain close to the N-terminal [7]. Free catalytic α subunits are usually inactive due to the presence of an autoinhibitory domain that is located in the center of this subunit. When the α subunit forms a functional complex with the β and γ subunits, this autoinhibitory function no longer blocks the catalytic function. Interestingly, Pang *et al.* have recently suggested that small molecules can directly relieve this inherent autoinhibition in the α subunit, rendering the complex constitutively active and possibly resistant to intracellular degradation [8]. Although $\alpha 1$ and $\alpha 2$ subunits share similar substrate specificity [9], $\alpha 2$ is typically localized in the nucleus whereas $\alpha 1$ is predominantly found in the cytoplasm [10]. It has been shown in HeLa cells that certain stressful conditions induce $\alpha 1$ isoform translocation to the nucleus [11]. This compartmentalization and subcellular redistribution may explain some diverse functions attributed to AMPK in cells.

The β subunit of the AMPK complex

The C-terminus of the β subunit contains recognition sequences that interact with the two other AMPK subunits and serves as the scaffold of the heterotrimeric complex. In addition, the central part of the β subunit contains a specific sequence that binds to gly-

cogen particles. It has been proposed that this interaction is closely associated with a tight regulation of glycogen metabolism [12]. A myristoylation site located in the N-terminus of this subunit functions as a molecular switch for reversible membrane binding. The functional significance of this membrane docking has been demonstrated when a point mutation in the myristoylation site resulted in a 4-fold increase in the activity of the AMPK complex [13]. A direct autoinhibitory domain located in the N-terminal part of the β subunit has a critical role in autoregulating the activity of the complex [14]. In addition, the nuclear localization of the AMPK complex has been associated with targeted serine-phosphorylations in the β subunit [13]. These recent findings indicate that in addition to its established scaffold function, the β subunit also has important autoregulatory functions in the AMPK complex.

The γ subunit of the AMPK complex

Four tandem repeats of cystathionine- β -synthase sequences (CBS) that are located in the N-terminus of this subunit form two Bateman domains, which selectively bind adenosine containing molecules, such as AMP or ATP [15, 16]. Upon binding, the phosphate group of AMP/ATP lies in a groove on the surface of the γ subunit and interacts with basic residues of amino acids there [6]. Mutations in these basic amino acids result in several human hereditary metabolic disorders, such as impaired cardiac glycogen storage [17]. The binding of AMP to these Bateman domains activates AMPK, whereas ATP binding antagonizes this process. The two Bateman domains bind two pairs of AMP molecules in a positive cooperative manner, suggesting that the second domain is inaccessible for binding unless two AMP molecules occupy the CBS binding sites in the first Bateman domain. This allosteric interaction increases the sensitivity of the AMPK complex to minute changes in intracellular levels of AMP. In contrast, the binding of ATP molecules to these domains antagonizes activation of the complex. These disparate allosteric modulations in the complex, induced by the binding AMP or ATP, form the molecular basis for the metabolic switch function of AMPK and a plausible target for synthetic activators [16].

Mutations in the hydrophobic patch in the γ subunit (Arg¹⁷¹-Phe¹⁷⁹), which interacts with the glycogen binding loop of the β subunits, substantially impede AMP-dependent activation of the AMPK complex [14]. It seems, therefore, that the activation process of the complex is also influenced by the γ subunit. Of in-

terest is the finding that the $\gamma 2$ subunit confers the complex with the greatest AMP dependency in comparison with the $\gamma 3$ subunit, which moderately affects the sensitivity to AMP [18].

Activation of the AMPK complex

Among stressful conditions, which deplete ATP stores and increase AMP content in cells, heat shock, hypoxia, hyperosmolarity, glucose deprivation, ischemia and prolonged contraction of skeletal muscles have been studied most intensively [19, 20]. It is, however, important to note that some conditions known to activate AMPK in cultured cells are physiologically extreme. For instance, exaggerated hyperosmolarity or severe glucose deprivation are not compatible with real life situations. AMPK is activated in an AMP-dependent manner by targeted phosphorylation of Thr¹⁷² in the α subunit [21, 22]. The binding of AMP to the CBS domain in the γ subunit induces conformational changes in the heterotrimeric complex that promote the phosphorylation of this Thr¹⁷² moiety by several upstream kinases. The dissociation of the AMP molecules is followed by the binding of ATP, which then increases the susceptibility of pThr¹⁷² to dephosphorylation by protein phosphatases [16].

The “energy charge hypothesis” assigns AMP regulatory metabolic functions in eukaryotic cells [23]. AMP is a very sensitive metabolic indicator of cellular energy metabolism due to the function of adenylate kinases in cells. These enzymes interconvert two molecules of ADP to AMP and ATP [21]. Because these kinases function at near equilibrium, the AMP:ATP ratio in cells varies as the square of the ADP:ATP ratio. When ATP is consumed or depleted and not adequately replenished, the ratio of ADP:ATP rises and drives the adenylate kinase reaction forward to generate ATP and AMP. The latter then further activates the AMPK pathway to conserve energy stores in cells. When cellular ATP levels are high enough and the generation of AMP is reduced, the former molecule preferentially binds to AMPK and blocks it [23]. Because the affinity of ATP binding to the Bateman domain in the γ subunit is lower than that of AMP, relatively higher concentrations of the former are required to attenuate the function of the complex [24]. The recent finding that only a small fraction of the total amount of cellular AMPK is amenable to AMP activation at any given time suggests that additional regulatory interactions, such as compartmentalization or covalent modifications (e.g., phosphorylation), are also involved in this binding process [6].

The tumor suppressor LKB1 is the major upstream serine/threonine kinase that phosphorylates Thr¹⁷² in the α subunit and activates the AMPK complex. Of importance is the finding that the LKB1 complex is not directly activated by AMP, but that the latter induces conformational changes in AMPK rendering it a preferable substrate to LKB1 [24]. The human LKB1 gene encodes a single 433-amino acid protein [25], which includes three structural domains: an N-terminal nuclear localization domain, a central catalytic domain and a C-terminal prenylation site that targets the enzyme to membranes [26]. This enzyme plays an important role during embryogenesis and is involved in epithelial, glial and neuronal cell polarity determination [27]. LKB1-knockout mouse embryos die in uterus due to multiple abnormalities, including neural tube and vascular defects [25]. LKB1 is most active in a complex with two accessory proteins: the STE20-related adaptor (STRAD) and the mouse embryo scaffolding protein (MO25) [24, 28, 29]. The pseudokinase STRAD, which binds ATP but lacks a phosphorylation function, enhances the catalytic function of LKB1. MO25, which interacts with the C-terminus of STRAD, stabilizes the entire LKB1 complex [24]. Interestingly, LKB1, like AMPK, shuttles between the nucleus and the cytoplasm [25, 30]. Ribosomal S6 kinase (RSK)- and protein kinase A (PKA)-induced phosphorylation of Ser⁴³¹ enhances the association of LKB1 with intracellular membranes [31]. It is assumed that this membrane targeting is important to maintain enzymatic activity.

Although AMPK activation is lost when LKB1 expression is silenced, the basal activity of AMPK remains intact [32]. The ability of the AMPK complex to maintain an intrinsic Thr¹⁷²-autophosphorylating capacity is independent of the fluctuation in AMP levels and seems to maintain a basal intrinsic activity of the complex that allows cells to respond immediately to energetic challenges [33].

Calmodulin-dependent protein kinase kinases (CaMKKs), the upstream kinases for calmodulin-dependent protein kinase I and IV, can also phosphorylate Thr¹⁷² and activate AMPK. CaMKK β , rather than CaMKK α , is the main isoform of the enzyme that activates AMPK [34-36]. Because in many cases intracellular Ca²⁺ signaling often precedes energy utilization and demand, it is conceivable that CaMKK β prepares cells for a significant increase of energy demand by activating AMPK [37].

Transforming growth factor- β -activated kinase-1 (TAK1) directly phosphorylates AMPK in yeast [38]. Recently, the mammalian TAK1 homolog (previously

known as AMPK-kinase), which also phosphorylates Thr¹⁷² in AMPK has been found in various mammalian tissues, including skeletal muscle [38-40]. It has been suggested that tumor necrosis factor- α (TNF- α) and transforming growth factor- β activate TAK1 [41]. In addition, 5-aminoimidazole-4-carboxamide-1 β -D-ribofuranoside (AICAR) and the biguanide metformin also activate TAK1. This effect of metformin was lost in mice cardiomyocytes following the disruption of TAK1 expression [42]. Further studies will ascertain the physiological function of TAK1 in cellular energy metabolism. Protein phosphatases 2A and 2C (PP2A and PP2C) inactivate AMPK by dephosphorylating pThr¹⁷² [33, 43]. The activity of these enzymes is negatively regulated by AMP and free fatty acids [44].

In summary, changes in energy and calcium metabolism in cells underlie the activation process of AMPK. Among the effectors that act in concert to induce Thr¹⁷² phosphorylation, LKB1, CaMKK β and TAK1 seem to be prominent.

Metabolic Functions of AMPK

AMPK phosphorylates serine moieties in target proteins. It mostly interacts with a serine moiety within a 9-amino acid motif. In human, this motif is Φ - Ψ -X-X-S/T-X-X-X- Φ , where Φ , Ψ and X denote hydrophobic, basic or any other amino acid, respectively [45-47]. Many of these target proteins regulate key metabolic functions, such as glucose uptake, glycolysis, fatty acid oxidation, cholesterol synthesis, glycogen synthesis, gluconeogenesis, protein synthesis or lipolysis [48]. These various functions inhibit anabolic processes and conserve ATP, on one hand, and stimulate catabolic pathways to produce ATP, on the other [46].

Skeletal muscles and heart

Skeletal muscle mass is the main target for insulin-stimulated glucose disposal, whereas insulin resistance is a major contributing factor to the development type 2 diabetes. AMPK-dependent stimulation of glucose transport in skeletal muscles is independent of insulin, phorbol ester or passive stretch [19, 46]. In contrast, muscle contractions, hypoxia, hyperosmolar shock, mitochondrial uncouplers and electron transport inhibitors activate AMPK due to relative energy depletion [49]. Both hypoxia and mitochondrial uncoupling increase the rate of glucose transport in mouse skeletal muscles but fail to produce a similar response in skeletal muscles bearing a dominant-negative AMPK α 2 subunit [50, 51].

The activation of muscle AMPK by exogenous compounds or by contraction recruits GLUT-4 to the plasma membrane and augments the rate of glucose transport in a non-insulin-dependent manner [52, 53]. AMPK-induced translocation of GLUT-4-containing vesicles to the plasma membrane is preceded by the phosphorylation of the protein AS-160 at Thr⁶⁴². This phosphorylated form of AS-160 releases the vesicle from intracellular storages and allows their recruitment to the plasma membrane [54, 55]. In addition, AMPK upregulates the expression of genes encoding GLUT-4 and hexokinase II and stimulates glycogen synthesis in muscles by allosteric activation of glucose-6-phosphatase-induced activity [56-58]. Various studies link the glucose transport stimulatory effect of AMPK in skeletal muscles to the activation of ERK1/2, p38-MAPK, Pyk2, PLD, α PKC and Grb2 [19]. In addition to the GLUT-4 translocation, AMPK also exerts its anabolic function in skeletal muscles by activating two major citric acid cycle enzymes: citrate synthase and succinate dehydrogenase [59-61]. AMPK also stimulates glycolysis in cardiomyocytes (and hepatocytes) by activating 6-phosphofructo-2-kinase (PFK2) [62, 63]. AMPK (predominantly complexes with the α 2 isoform) has a cardioprotective role in augmenting glucose transport and glycolysis in ischemic hearts [64, 65]. Increased myocardial ischemia injury due to enhanced post ischemic myocardial apoptosis, extended infarct size and worsened cardiac functional recovery were inflicted in mice bearing a dominant negative AMPK α 2 in their cardiomyocytes [66]. In non-insulin-sensitive cells that do not express GLUT-4, AMPK increases glucose uptake possibly by activating the ubiquitous GLUT-1 that resides in the plasma membrane [67].

Although the outcome of insulin action and AMPK activation of the glucose transport system in skeletal muscles is similar, the transduction mechanism employed by insulin to recruit GLUT-4 to the plasma membrane is entirely different and independent of that recruited by AMPK. Indeed, when costimulated, both the AMPK- and the insulin-dependent pathway increase the rate of glucose transport in an additive manner [68]. The interactions between these two main pathways that regulate glucose metabolism in skeletal muscles have been investigated [69]. Disruption of the AMPK α 2 function in skeletal muscles in mice resulted in glucose intolerance and insulin resistance, lending more support to the hypothesis that in addition to normal insulin function, the AMPK α 2 isoform also plays a role in maintaining normal insulin sensitivity and reactivity of muscles [19]. In cardiac muscle, how-

ever, insulin antagonizes the activation of AMPK by an Akt/PKB-dependent phosphorylation of Ser⁴⁸⁵ or Ser⁴⁹¹ in AMPK $\alpha 1$ or $\alpha 2$, respectively, which attenuates the phosphorylation of Thr¹⁷² by LKB1 [70-72].

The observation that physical activity induces translocation of AMPK $\alpha 2$ from the cytoplasm to the nucleus supports the idea that AMPK also regulates transcriptional functions, such as the increased expression of GLUT-4 in exercising muscles [73-75]. AMPK phosphorylates histone deacetylase-5 and releases it from a complex with myocyte enhancer factor-2 (MEF2), rendering the latter accessible to threonine residues phosphorylation by p38-MAPK and allowing it to form an active transcription complex of the GLUT-4 gene [76].

The blood glucose lowering effect of exercise in type 2 diabetic patients results from an insulin-independent increased rate of glucose transport and enhanced lipid oxidation in skeletal muscles [58]. Interestingly, exercise and AMPK activation also render skeletal muscles more sensitive to insulin action. Indeed, preincubation of isolated rat skeletal muscles with AICAR enhanced insulin-stimulated glucose transport [77]. However, such effects were not observed in cultured myotubes (C2C12) or primary human muscle cells, in which pretreatment with AICAR did not alter their sensitivity to insulin [78]. AMPK-dependent inactivation of IRS-1 by Ser⁷⁸⁹ phosphorylation in these cells may explain this phenomenon [79].

It is important to note that contraction-mediated glucose uptake is only partially increased in isolated muscles from AMPK $\alpha 2$ whole body knockout mice [80]. Moreover, the simultaneous silencing of the $\alpha 1$ and $\alpha 2$ subunits in mouse skeletal muscles led to a modest reduction in the rate of glucose uptake in contracting muscles [19]. Such findings support the claim that AMPK is not required for contraction-mediated augmentation of glucose transport in skeletal muscles. In contrast, others have found that inhibition of the AMPK complex with Compound C decreased contraction-stimulated glucose transport in rat skeletal muscles [81]. Therefore, further investigation of the specific role of AMPK in the regulation of the glucose transport system in resting and contracting skeletal muscles during acute exercise or endurance training is required. This may lead to the discovery of new cellular targets for the development of novel drugs that mimic effects of muscle contraction and augment glucose transport in skeletal muscle in non-insulin and non-AMPK-dependent mechanisms.

Reduced AMPK activity in insulin resistance and type 2 diabetes has recently been associated with mito-

chondrial dysfunction and impaired metabolism of lipids in skeletal muscles [82]. Glucose storage in skeletal muscles serves as an immediate source for ATP production, especially during exercise and short-term fasting. Free fatty acids provide an abundant and long-term stock of energy for peripheral energy demands. Fatty acid oxidation in working muscles produces energy 3-fold greater than comparable carbohydrate resources [83]. Inhibition of acetyl-CoA carboxylase (ACC) by AMPK in skeletal muscles improves β -oxidation of fatty acid in the same mechanism as described below for hepatocytes.

Peroxisome proliferator-activated receptor γ (PPAR γ) coactivators-1 α and -1 β (PGC-1 α/β) are critical stimulators of mitochondrial biogenesis in response to various stimuli and stressful conditions, such as different diets or physical activity [84]. Muscle PGC-1 α is activated by AMPK and by NAD⁺-dependent type III deacetylase (SIRT1). It has recently been shown that AMPK indirectly controls SIRT1 activity by increasing NAD⁺ content. SIRT1, in turn, deacetylates and activates PGC-1 α [85, 86]. This introduces a functional crosstalk between two important energy sensors that synchronize energy production in mitochondria.

Adipose tissue

The activation of AMPK in fat tissues leads to decreased lipogenic flux, massive fatty acid oxidation and decreased triglyceride synthesis. Fasting, physical exercise or treatment with β -adrenergic agonists activates AMPK via a cAMP-dependent mechanism. The $\alpha 1$ catalytic subunit is the predominant isoform expressed in adipocytes and is critical for the major effects of the AMPK complex [87]. Not only that AMPK activation in adipocytes just marginally increases glucose uptake, but active AMPK antagonizes the augmenting effects of insulin on GLUT-4-mediated glucose uptake. The mechanism of this phenomenon is not clear, but these findings agree with the view that unlike skeletal muscles, glucose in adipocytes is predominantly utilized anabolically for lipid storage [88].

AMPK also regulates lipolysis in adipocytes by inactivating hormone-sensitive lipase (HSL) by a targeted serine phosphorylation [87, 89]. Normally, receptor-coupled adenylate cyclase increases lipolysis via cAMP-dependent protein kinase-A1, which activates HSL. Interestingly, exposure of adipocytes to AICAR blocks lipolysis, which is induced by this mechanism [90]. Both basal and isoproterenol-stimulated lipolysis were elevated and the antilipolytic effect of AICAR was lost in adipocytes from AMPK $\alpha 1$ knockout mice [91]. It

appears that AMPK prevents recycling and release of fatty acids from triglycerides, a process which consumes ATP. AMPK α 2 has a direct or indirect role in adipose tissue function since its total deletion in mice resulted in an excessive weight gain upon a high-fat diet, but did not entail glucose intolerance [92].

Activation of AMPK in human adipose tissue leads to an increased expression of adiponectin, which is a potent insulin sensitizer in skeletal muscles [87]. The cAMP response element binding protein (CREB), which is over-activated in adipocytes of obese mice, triggers the expression of the ATF3. The latter is a transcriptional repressor that binds to and inhibits the transcription of the adiponectin and GLUT-4 genes. These interactions have recently been linked to the development of hyperglycemia in type 2 diabetic patients [93]. The peripheral effects of adiponectin may explain the significant contribution of AMPK activating drugs to the prevention and improvement of insulin resistance in obese diabetic patients.

Liver

The main function of AMPK in the liver is to augment fatty acid oxidation and to prevent cholesterol and triglycerides biosynthesis. Liver-specific AMPK α 2 deletion in mice enhances hepatic lipogenesis, increases plasma triglyceride levels and hepatic glucose production. Conversely, overexpression of AMPK α 2 in hepatocytes decreases plasma triglyceride level [94, 95]. AMPK also reduces mRNA content of the sterol regulatory element binding protein-1 (SREBP-1) [96]. Over-function of this factor has been associated with the increased prevalence of dyslipidemia in type 2 diabetes. Activation of AMPK also reduces the cellular content of the mRNA of the carbohydrate responsive element-binding protein (ChREBP). This factor, otherwise, upregulates lipogenesis and therefore plays a key role in inducing of hyperlipidemia in type 2 diabetes patients [97].

Adipose tissue-derived adiponectin also improves lipid metabolism in the liver of diabetic obese mice by decreasing fatty acid biosynthesis and increasing mitochondrial fatty acid oxidation [98]. The beneficial effects of AMPK in the liver are attenuated when adiponectin synthesis and secretion from adipose tissues is decreased in diabetes and obesity [93].

Type 2 diabetes is characterized by fasting hyperglycemia and impaired peripheral glucose utilization. A key contributing factor to these abnormalities is the failure of insulin to suppress gluconeogenesis and hepatic glucose production. Liver-specific AMPK α 2-knockout mice develop hyperglycemia and glucose in-

tolerance and this is associated with an increased hepatic glucose production. Conversely, the stimulation of AMPK in wild type mice dramatically reduces hepatic glucose output [99]. The suppression of gluconeogenesis by AMPK results from the inhibition of the transcription of phosphoenol pyruvate carboxy kinase (PEPCK), the key regulatory gluconeogenic enzyme [57]. In addition, AMPK attenuates the synthesis of cholesterol and glycogen in hepatocytes by deactivation of HMG-CoA reductase and glycogen synthase, respectively. In addition, AMPK downregulates the expression of enzymes that are centrally involved in fatty acid synthesis and gluconeogenesis by inhibiting the transcription factors SREBP-1c, ChREBP and HNF-4 α , and by attenuating the activity of transcriptional coactivators, like p300 and TORC2 [46]. For example, when the latter is phosphorylated it forms a cytoplasmic complex with 14-3-3 protein, which inhibits the transcription of gluconeogenic enzymes [100].

AMPK also phosphorylates and deactivates ACC [101], an enzyme that exists as two isoforms: ACC1 (cytoplasmic) and ACC2 (predominantly mitochondrial) [102]. The inhibition of the former reduces fatty acid synthesis in cells. Malonyl-CoA, the product of ACC2, is a potent blocker of carnitine palmitoyltransferase-1 (CPT1), which transports long chain fatty acids to mitochondria. When ACC2 is inhibited, the flux of these fatty acids to mitochondria and their oxidation is increased. In addition, AMPK directly stimulates free fatty acid uptake to cells by translocating the fatty acid translocase CD38 to the plasma membrane [46].

β -cells

Low glucose levels activate AMPK in β -cells. Overexpression of wild type AMPK or constitutively active AMPK, or its pharmacological activation attenuate glucose-induced insulin secretion, whereas the expression of a dominant-negative AMPK in cultured β -cells increases it [103]. It has been suggested that the biguanide metformin affects insulin secretion in β -cells by activating AMPK [104, 105].

Unlike peripheral tissues like skeletal muscle, which predominantly express the α 2 subunit of AMPK, the α 1 subunit is more abundant in rodent β -cells than the α 2 subunit. The former is found most frequently in the cytoplasm, while the latter is distributed between the cytoplasm and the nuclear compartment. It has been proposed that the AMPK α 1 complex participates in the electrochemical activity of the cells, whereas the nuclear AMPK α 2 complex is involved in regulating gene transcription. Interestingly, neutralization of the

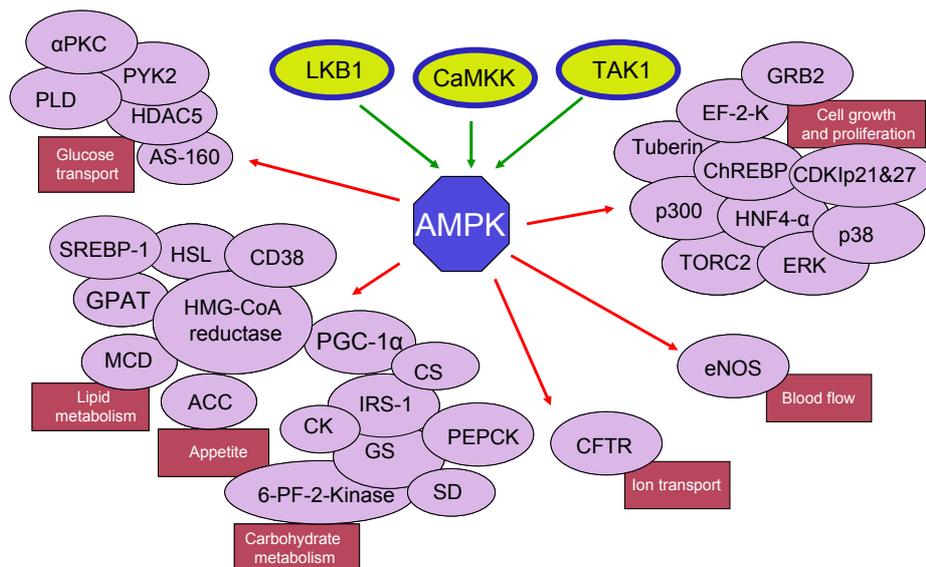


Figure 1. Metabolic pathways and functions regulated by AMPK. AMPK can be activated directly by three kinases, LKB1, TAK1 and CaMKK. When activated in various tissues and organs different AMPK complexes mediate a variety of cellular and physiological responses by activating cell-specific targets (e.g., enzymes, transcription factors and docking proteins). Major effects of AMPK activations are metabolic (carbohydrate and lipid metabolism), appetite regulation, cell growth and differentiation, vascular function (blood flow) and basic cellular functions (chloride ion transport). *Abbreviations:* ACC: acetyl-Co-A carboxylase. AS-160: Akt substrate of 160 kDa. CDKlp21&27: cyclin-dependent kinase inhibitors p21 and p27. CFTR: cystic fibrosis transmembrane conductance regulator. ChREBP: carbohydrate responsive element binding protein. CK: creatine kinase. CS: citrate synthase. EF-2-K: elongation factor 2 kinase. eNOS: endothelial nitric oxide synthase. ERK: extracellular signal-regulated kinases. GPAT: glycerol-3-phosphate acyltransferase. GRB2: growth factor receptor-bound protein 2. GS: glycogen synthase. HDAC25: histone deacetylase 25. HSL: hormone sensitive lipase. HMG-CoA reductase: 3-hydroxy-3-methyl-glutaryl-CoA reductase. HNF4- α : hepatocyte nuclear factor 4 α . IRS-1: insulin receptor substrate 1. MCD: malonyl-CoA decarboxylase. p38: p38 mitogen-activated protein kinase. PEPCK: phosphoenolpyruvate carboxy kinase. 6-PF-2-Kinase: 6-phosphofructo-2-kinase. PGC-1 α : peroxisome proliferator-activated receptor- γ -coactivator-1 α . PYK2: proline-rich tyrosine kinase 2. SD: succinate dehydrogenase. SREBP-1: sterol regulatory element binding protein. TORC2: target of rapamycin complex 2.

Non-metabolic functions of AMPK

AMPK is involved in numerous cellular and physiological interactions not directly related to metabolic homeostasis or diabetes. It is clear that non-specific and non-tissue/cell targeted AMPK activators for the treatment of diabetes and metabolic disorders may also affect these processes and produce diverse side effects. The following is a short summary of such critical functions of AMPK.

An important cross-talk between AMPK and endothelial NO synthase (eNOS) regulates vascular endothelial cell dilatory functions. The AMPK activity in vascular endothelial cells is controlled directly by AMP and indirectly by nitric oxide, the product of eNOS. Following the activation of soluble guanylate cyclase by NO, the former activates CaMKK, a positive upstream activator

$\alpha 2$ subunit in β -cells enhances the transcription of the preproinsulin gene [106]. Therefore, it seems that the transcriptional regulation of AMPK $\alpha 2$ in β -cells greatly differs from the transcriptional regulation it mediates in skeletal muscles [76].

Recent studies on the role of AMPK in the regulation of insulin secretion in β -cells have associated it with the mTOR pathway, energy availability, protein synthesis, cell growth and apoptosis [69]. Collectively, these results demonstrate the complexity of AMPK function in regulating insulin secretion in β -cells and the need for thorough investigations of the complexity of such direct and/or indirect interactions.

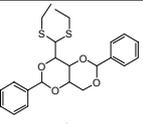
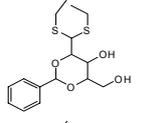
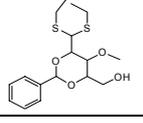
of AMPK [107]. In a positive feedback loop, AMPK $\alpha 1$ complex phosphorylates eNOS at Ser¹¹⁷⁷ in an Akt/PKB-dependent manner and increases NO production [108]. This reciprocal and positive feedback mechanism integrates stressful stimuli (e.g., hypoxia) and metabolic (e.g., hypoglycemia) signals to maintain an adequate circulation in critical organs.

AMPK inhibits DNA replication in various cell lines by causing a G1/S-phase cell cycle arrest. This phenomenon is accompanied with the accumulation of tumor suppressor factor p53 and the cyclin-dependent kinase inhibitors p21 and p27 [46]. Another mechanism that may explain some antiproliferative effects of

AMPK is the targeting of the RNA-binding protein HuR to the nucleus, where it interacts with and decreases the half-life of mRNAs encoding p21, cyclin A, and cyclin B1 [109]. AMPK has also been implicated in downregulating protein synthesis in cells by inhibiting mammalian target of rapamycin (mTOR) [79], and by phosphorylating the elongation factor-2 kinase, which than inhibits the elongation step [110]. A rare familial deactivating mutation in LKB1 has been linked to the development of hamartomas in humans (Cowden syndrome, Peutz-Jeghers syndrome and tuberose sclerosis). Despite the abnormal growth, cells in these hamartomas retain a normal differentiated state. Interestingly, some reports claim that diabetic patients treated with metformin have a decreased risk of cancer-related mortality than patients treated with other antidiabetic drugs or insulin. These beneficial effects of metformin have been attributed to the activation of the AMPK pathway [111]. These and other findings have initiated a great interest in the potential antimetastatic activity of AMPK-targeted drugs [112].

Critical functions of primary immune cells, such as chemotaxis and cytokine secretion, are also regulated by AMPK [113]. In fact, the stimulation of AMPK in macrophages from diabetic mice was reduced in comparison with normal macrophages. This perhaps underlies the impeded functional capacity (i.e., reduced macrophagocytosis) of the former cells in diabetes [114].

Table 1. Structure and function of the D-xylose derivative compounds 19, 21 and 24

Test compound	Structure	<i>In vitro</i> MEC	<i>In vivo</i> MEC
Compound 19		5 μ M	3 μ M
Compound 21		100 μ M	N/D
Compound 24		50 μ M	N/D

Legend: The table shows the structures of three compounds and their minimal effective concentrations (MEC) required to augment the rate of hexose uptake in cultured L6 myotubes and to reduce the blood glucose levels towards normoglycemia in STZ-diabetic C57/black male mice. Mice were injected subcutaneously twice daily, for 5 days, with 50 mg/kg of each compound dispersed in sesame oil. N/D: not determined.

It has recently been shown that AMPK also participates in the regulation of appetite and food intake. Constitutive activation of AMPK in the mouse hypothalamus increases significantly food uptake and body weight, whereas its inhibition has the opposite effects [115, 116]. Noteworthy are the observations that some drugs and factors have opposite effects on hypothalamic and peripheral AMPK complexes. For example, leptin and metformin activate AMPK in skeletal muscles while inhibiting it in the hypothalamus. In contrast, ghrelin and cannabionoids stimulate AMPK in the hypothalamus, but inhibit it in the liver and adipose tissues [117]. These disparate functions are not well-investigated, but seem to be related to diverse heteromeric structures of AMPK complexes in various regions of the central nervous system and in peripheral tissues.

An interesting relationship has been found between AMPK and the cystic fibrosis transmembrane conductance regulator (CFTR). Both proteins are colocalized in the apical membrane of lung secretory epithelial cells. AMPK phosphorylates CFTR at two serine moieties that maintain the chloride channel closed. Therefore, systemic activation of AMPK may have detrimental effects whereas site-specific pulmonary inhibition of AMPK might be considered therapeutically relevant for the treatment of cystic fibrosis. The potential of such treatments with AMPK disruptors or inhibitors in the lung remains to be elucidated [118].

Figure 1 depicts the three main kinases that activate AMPK and its multiple downstream targets, classified according to metabolic, cellular and physiological functions.

In summary, the heterotrimeric structure of the AMPK complex does not only differ among tissues and organs, its activation results in an array of cellular and metabolic responses. Therefore, global activators of AMPK aimed at producing favorable therapeutic effects may exert undesirable side effects due to peripheral and central interactions. This leads to the conclusion that more specific drugs need to be developed. Drugs targeted chemically or by way of administration to specific organs expressing distinct AMPK heterotrimeric complexes present a potential solution to a rational AMPK-targeted therapy.

Chemical activators of AMPK

The search for novel AMPK activators by rational drug design, screening of vast chemical libraries and testing of various plant extracts has produced numerous reports on new compounds. In the following sec-

tion, we discuss and classify a collection of such drugs and compounds that activate AMPK. We make the classification according to chemical structures and function.

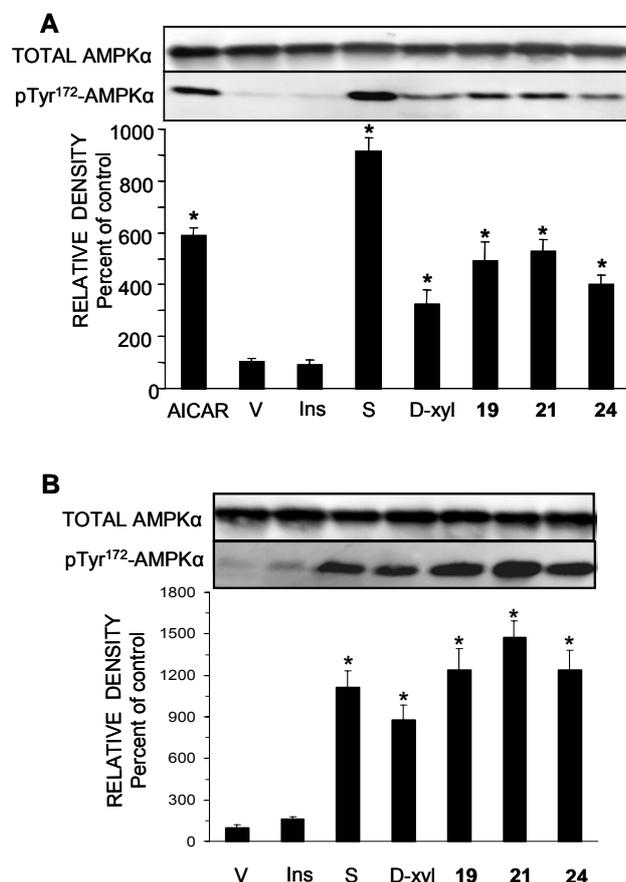


Figure 2. D-Xylose and Compounds 19, 21 and 24 activate AMPK. **A:** L6 rat myotube cultures were washed and received fresh medium supplemented with 2% (v/v) FCS, 23.0 mM D-glucose supplemented with 20 mM of D-xylose (D-xylose), 5 μ M of Compound 19, 150 μ M of Compound 21 or 50 μ M of Compound 24. These compounds were present in the medium for 40 min, 12 h, 30 min and 2 h, respectively. Control myotubes received the vehicle (V) only. AICAR (4 mM), 100 nM of insulin (Ins) and 0.25 M of D-sorbitol (S) were present for 1h, 20 min and 30 min, respectively. Whole cell lysates were prepared and Western blot analyses were performed with antibodies against AMPK α and pTyr¹⁷²-AMPK α . **B:** Human myotubes were treated as described above and taken for Western blot analysis of AMPK α and pTyr¹⁷²-AMPK α . Representative blot and a summary of $n = 3$ ($p < 0.05$) in comparison with the respective controls. Reproduced with permission from [135].

Metformin and thiazolidinediones

Two groups of common antidiabetic drugs, biguanides and thiazolidinediones (TZD), are believed to mediate part of their effects through AMPK activation. Biguanides, and specifically metformin, inhibit hepatic gluconeogenesis and augment the rate of glucose uptake in skeletal muscles [119]. Some of these effects have been attributed to the activation of AMPK α 2 in skeletal muscles. However, the pharmacological significance of this mechanism in diabetic patients is questionable because the required effective concentration of metformin *in vitro* was as high as 0.5 mM, whereas the plasma effective concentration of the drug in man is at least one order of magnitude lower [96]. Since metformin is excreted in the urine unmetabolized, no active metabolites of this drug can be implicated in its action in skeletal muscles and other tissues [120]. Metformin does not induce LKB1 phosphorylation and activity in skeletal muscles, it fails to increase calcium influx and it does not alter protein phosphatase activity [121]. The main antidiabetic effect of metformin is attributed to hepatic activation of AMPK followed by the inhibition of gluconeogenesis.

Pharmacokinetic data on metformin also supports the hypothesis that the liver is the main target for this drug. Orally administered metformin is effectively absorbed from the gastrointestinal tract to the portal vein. Thus, due to this first-pass effect, the liver is exposed to high concentration of the drug. Therefore, in contrast to skeletal muscle, the effect of metformin in the liver is mediated by LKB1 as the specific hepatic knockout of LKB1 in diabetic mice completely rendered them insensitive to metformin [116]. Of concern is a recent report that links metformin-induced activation of AMPK to an increased biogenesis of Alzheimer's amyloid in mice brains [122]. Therefore, novel AMPK-activating drugs devoid of such serious side effects are needed.

The second class of antidiabetic drugs that stimulate AMPK is thiazolidinediones (TZD). Members of this group that bind to and activate PPAR γ improve insulin sensitivity in various peripheral tissues. TZD, such as rosiglitazone and pioglitazone, downregulate lipolysis and reduce the content of fatty acids in adipocytes [123]. These drugs also inhibit the release of several adipokines from adipose cells, such as tumor necrosis factor α (TNF- α), interleukin-6 and resistin, which induce muscle insulin resistance. Concomitantly, TZD augment the secretion of the insulin-sensitizing factor adiponectin from adipose tissue in man and rodents [124]. Adiponectin stimulates glucose uptake and fatty acid oxidation in skeletal muscles and inhibits

gluconeogenesis in the liver by activating AMPK [98]. Expression of a dominant negative AMPK α 1 mutant in adipocytes or treatments with synthetic inhibitors of AMPK abolished these effects of adiponectin [125]. These findings indicate that AMPK also plays a key role in mediating hepatic metabolic effects of adiponectin.

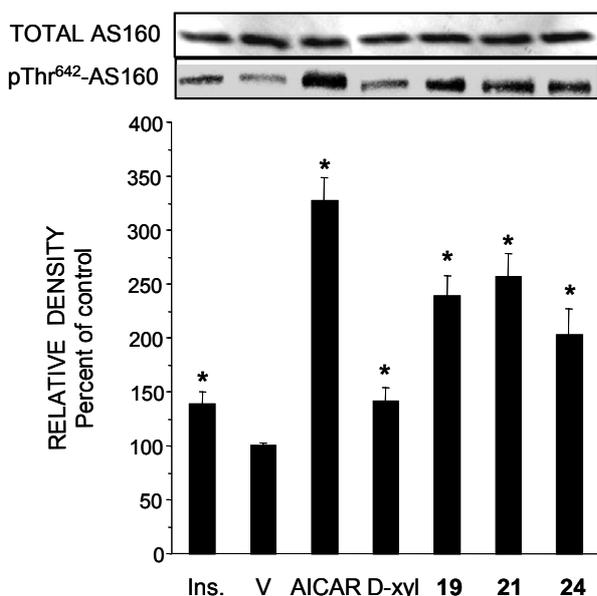


Figure 3. D-Xylose and Compounds 19, 21 and 24 activate AS160. Whole cell content of AS160 and pThr⁶⁴²-AS160 was determined by Western blot analysis in samples that were prepared from L6 myotubes, as described in the legend to Figure 1. Representative blot and a summary of $n = 3$ ($p < 0.05$) in comparison to the respective controls. Reproduced with permission from [135].

It has also been reported that the skeletal muscle levels of several oxidative phosphorylation enzymes and of PGC-1 were increased in diabetic patients treated with TZD over 6 months [126]. PGC-1 regulates the expression of several genes that are involved in mitochondrial bioenergetics [127]. Moreover, TZD also increase the expression of super oxide dismutase-2 (SOD2) and quinone oxidoreductase-1 (NQO1), providing an efficient antioxidant defense against elevated levels of reactive oxygen species [128]. Importantly, *in vitro* experiments suggest that TZD-dependent activation of AMPK-dependent pathways in insulin sensitive tissue is not exclusively mediated by adiponectin. Finally, TZD effects were recorded in adipocytes in

which PPAR γ expression was blocked [129]. It has recently been reported that the TZD derivative BLX-1002, which does not bind to PPAR γ , stimulates insulin secretion from isolated normal and diabetic pancreatic islets or dispersed β -cells only upon incubation under high glucose. Part of this effect has been attributed to the activation of AMPK in β -cells [130].

D-xylose and lipophilic D-xylose derivatives

A decade ago Winder *et al.*, introduced AMPK as a target for developing novel drugs for the treatment of type 2 diabetes [131]. Dozens of molecules that activate AMPK, directly or indirectly, were synthesized or extracted from plants. In the course of our work, we found that the pentose D-xylose augmented the rate of glucose transport in L6 and human myotubes under high glucose conditions by activating AMPK [132]. Because this effect of D-xylose was obtained at very high concentrations (10-20 mM), we synthesized more potent lipophilic derivatives of D-xylose. Among these, three highly lipophilic compounds 2,4;3,5-dibenzylidene-D-xylose-diethyl-dithioacetal (Compound 19), 2,4-benzylidene-D-xylose-diethyl-dithioacetal (Compound 21) and 2,4-benzylidene-D-xylose-3-O-methyl-diethyl-dithioacetal (Compound 24) exerted significant glucose transport stimulatory effects at low concentrations (5-100 μ M) in rat and human cultured myotubes (Table 1). These effects were decreased in the presence of the AMPK inhibitor Compound C. All three derivatives and the parent compound, D-xylose, induced Thr¹⁷² phosphorylation of AMPK α and of Thr⁶⁴² in the downstream substrate AS160 (Figure 2 and Figure 3). Our study also shows that the inhibition of the insulin transduction pathway by wortmannin and an AKT inhibitor did not interfere with the glucose transport stimulatory function of these derivatives. The exact mechanism of action of these compounds is still under investigation. Of major importance is the finding that Compound 19 significantly reduced blood glucose levels towards the normoglycemic range in streptozotocin-diabetic mice and in the genetically diabetic KKAY mice. Table 1 depicts the structures of these compounds and their minimal effective concentrations in augmenting the rate of glucose transport in L6 myotubes and in lowering blood glucose levels in streptozotocin-diabetic C57/Black mice. These findings point to the potential of these compounds in the development of novel and long-acting antihyperglycemic compounds.

These three benzylidene derivatives share some structural similarities to other known activators of AMPK. Figure 4 shows compounds that contain three

to five aromatic and non-aromatic rings, connected to each other directly or with short linkers. Some of these rings contain heteroatoms, such as oxygen and nitrogen. For example, berberine, an isoquinoline alkaloid derived from *Hydrastic Canadensis*, and its synthetic derivative dihydroberberine are claimed to be effective antilipogenic and hypoglycemic agents in rodents [133, 134]. Some studies have shown that both are indirect activators of AMPK, probably by inhibiting complex 1 in mitochondria [134, 135]. The furancarboxylic acid derivative compound D942 has also been found to activate AMPK indirectly, due to its ability to bind to NAD(P)H dehydrogenase and attenuate the function of complex 1 [136].

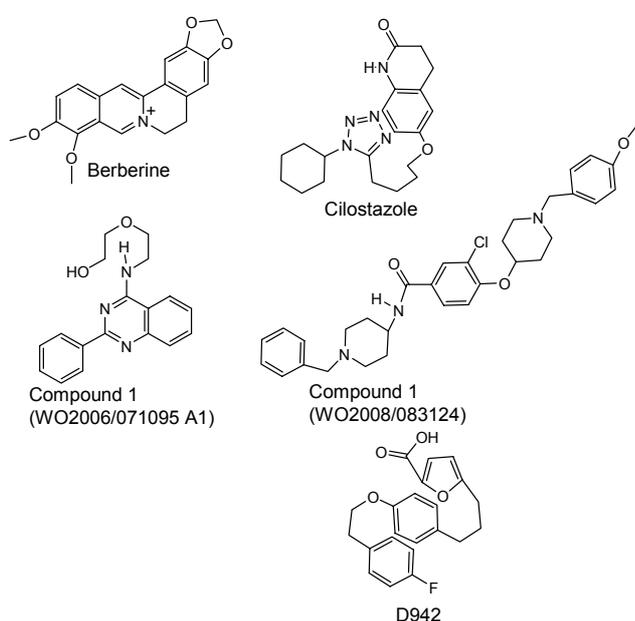


Figure 4. Molecular structures of AMPK activators sharing structural similarities to the D-xylose derivatives, Compounds 19, 21 and 24.

Cilostazol

Cilostazol is a selective inhibitor of phosphodiesterase-3 (PDE3) that shares structural similarities with the compounds shown in Table 1 and Figure 4. One of the striking effects of this compound is the enhancement of Thr¹⁷² phosphorylation and activity of AMPK in human umbilical vein endothelial cells. This activation is followed by downstream phosphorylations of ACC and eNOS. Cilostazol restores vascular

endothelial cell function in diabetic rats by PDE3 inhibition [137]. In a study on the effects of cilostazol on thrombospondin-1 expression in hearts of streptozotocin-diabetic rats, the investigators reported that a 4-week treatment reduced blood glucose levels significantly from 22.1 to 16.7 mM [138]. Others have shown that cilostazol ameliorates metabolic abnormalities in diabetic mice or rats via activation of PPAR γ and the suppression of inflammatory markers [139]. This drug also improved arterial compliance in patients with peripheral arterial disease while also improving their lipid profile [140]. Further clinical observations and trials will determine the efficacy of such auxiliary antidiabetic effects this drug may possess.

Recently, Zhou *et al.* reported the structure of two patented polycyclic activators of AMPK that may belong to the group presented in Figure 4 [141]. Compound 1 (patent WO2006/071095) increased AMPK activity 6-fold via an unknown mechanism [142]. Compound 1 (patent WO2008/083124) activated AMPK indirectly. It has been suggested that this effect is mediated via adiponectin receptors. It remains to be investigated whether these compounds also affect the mitochondrial complex 1 [143].

Phytoestrogens

It has been assumed that phytoestrogens could reduce diabetic complications by improving glucose homeostasis and insulin resistance. For example, antilipogenic effects of the phytoestrogen genistein (Figure 5) and its capacity to decrease adiposity were related to the activation of AMPK [144]. Genistein, quercetin, isoginkgetin and epigallocatechin-3-gallate (Figure 5), contain isoflavone and isoflavone-like moieties in their structures. They activate AMPK in 3T3-L1 preadipocytes and adipocytes, adiposarcoma cells and primary mouse hepatocytes, respectively [145]. The activation of AMPK by epigallocatechin-3-gallate seems to be mediated by CaMKK [146]. Interestingly, the antioxidant properties of the gallic acid polyphenol moiety in epigallocatechin-3-gallate may also contribute to the antidiabetic effects of this compound [147]. The mechanism of AMPK activation of the other compounds is not yet clear.

Momordicosides

Momordicosides, such as maslinic acid, cucurbitane and ginsenosides represent another class of natural compounds that activate AMPK and may also possess antidiabetic properties (Figure 6). The main natural source of maslinic acid is olive's skin; the rest are ex-

tracted from *Momordica charantia* (bitter melon) [148, 149]. In addition to the four ring triterpenoidic structure, the majority of momordicosides contain one or more sugar moieties bound to the ring structure via glycoside bonds. The most abundant sugar moieties are β -D-glucopyranoside, β -D-allopyranoside and β -D-xylopyranoside. Several studies indicate that these compounds increased the rate of glucose transport and induce GLUT-4 translocation to the plasma membrane in L6 or C2C12 myotubes and in 3T3-L1 adipocytes by activating AMPK. When tested *in vivo*, some momordicosides enhanced fatty acid oxidation and glucose disposal during glucose tolerance tests in insulin-sensitive or insulin-resistant mice or augmented glucose-stimulated insulin secretion in mice [149, 150]. *In vitro* studies suggest that ginsenosides increase AMPK phosphorylation and activity in 3T3-L1 cells; however, the relevant molecular interactions have not yet been ascertained [151].

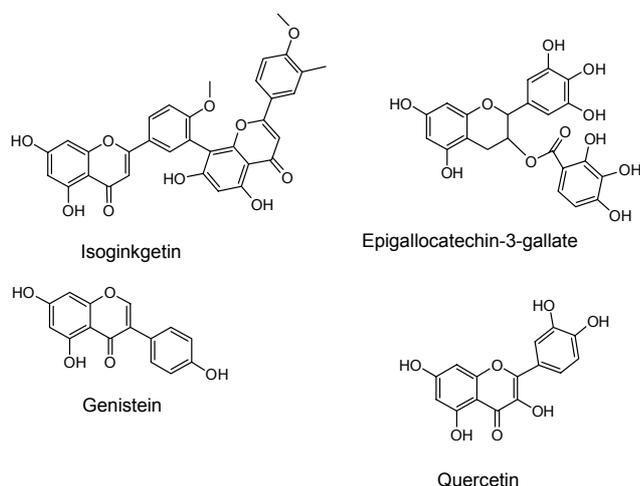


Figure 5. Molecular structures of phytoestrogens that interact with AMPK.

Capsaicinoids

Another large group of AMPK activators have one or two phenol, polyphenol or phenolmethyl ether moieties in their structures (Figure 7). The majority of these compounds have been extracted from plants. For example, capsaicinoids, a group of the natural compounds extracted from hot peppers, have been reported to promote fatty acid oxidation and decrease body fat accumulation in diabetic mice. The synthetic

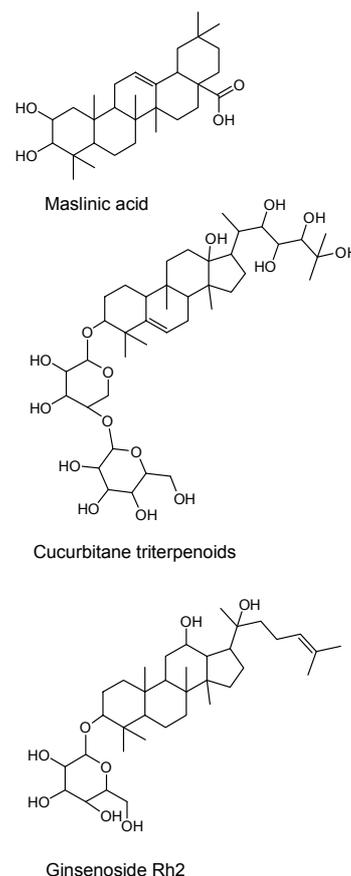


Figure 6. Molecular structures of momordicosides representing a class of natural compounds that activate AMPK.

structural ester isomer isodihydrocapsiate activates LKB1 both *in vitro* and *in vivo* [152]. This substance also increases glucose uptake in L6 myotubes and when administered orally to diabetic mice it substantially reduces blood glucose. A recent report indicated that salidroside, the active ingredient purified from *Rhodiola Rosea*, stimulated glucose transport and enhanced insulin sensitivity in L6 myotubes and 3T3-L1 adipocytes. The inhibition of AMPK activity by compound C completely abolished these effects of salidroside in L6 myotubes. This suggests that this compound exerts its effects by activating AMPK [153]. Another member of this group is the antioxidant resveratrol, which increases insulin sensitivity and lowers lipids in blood of diabetic and obese mice, most likely by activating SIRT1 [154]. Several natural analogues of resveratrol also activate AMPK [155]. One derivative, combretastatin A-4, a natural *cis*-stilbene is isolated from the

plant *Combretum caffrum*. It is the most effective and potent member of this group of compounds in down-regulating the expression of gluconeogenic enzymes in the liver and reducing the fasting blood glucose level in diabetic mice. The effect of combretastatin A-4 on AMPK is indirect and seems to result from the inhibition of the mitochondrial respiratory chain and a subsequent reduction in ATP levels [156].

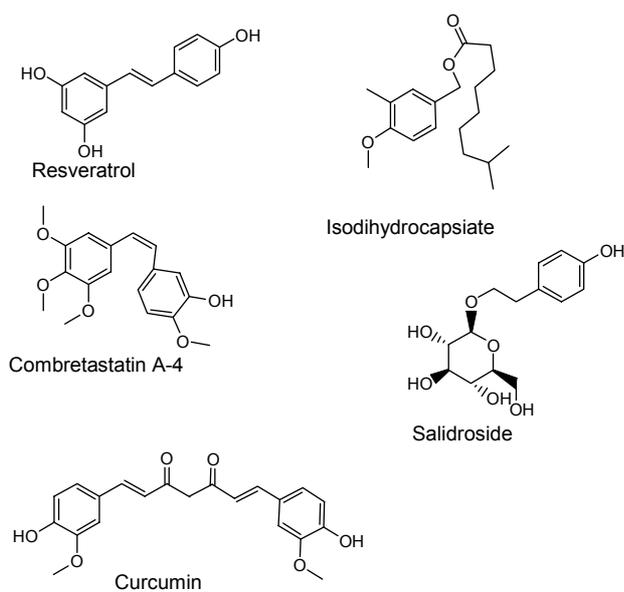


Figure 7. Molecular structures of compounds that have one or two phenol, polyphenol or phenolmethyl ether moieties.

Curcumin (diferuloylmethane), a polyphenol natural product of the plant *Curcuma longa*, has attracted significant attention and is undergoing early clinical trials as a novel anticancer agent [157]. It has been shown that curcumin-induced death of ovarian cancer cells is mediated by activating AMPK [158]. In other studies, curcumin rescued isolated mouse pancreatic islets from cytokine-induced death *in vitro* and prevented streptozotocin-induced diabetes *in vivo* [159]. Of particular interest is the finding that curcumin-induced activation of AMPK underlies the reduction in hepatic glucose production [160].

The thienopyridone derivative A769662 possesses antidiabetic effects by direct activation of AMPK [161] (Figure 8). It has been proposed that this compound activates AMPK in an AMP-independent manner, by

binding to an alternative allosteric site in the AMPK complex [162]. Others have shown that A769662 binds to the carbohydrate-binding moiety in the γ subunit of the complex and to several amino acid moieties of the $\beta 1$ subunit, none of which participates in AMP-binding [14]. Noteworthy is the finding that A769662 activates AMPK complexes that exclusively contain the $\beta 1$ subunit. This property limits the potential therapeutic use of this compound to tissues expressing the ubiquitous $\beta 1$ subunit, but not to tissue like skeletal muscle that assemble AMPK $\beta 2$ complexes. Therefore, the main target of A769662 is most likely the liver in which an activation of $\beta 1$ -containing AMPK complex reduces the expression of gluconeogenic enzymes and hepatic liver production [161]. Another patented thienopyridone derivative recently reported by Zhou *et al.* is Compound 202 (patent EP1754483A1) [141, 163] (Figure 8). Like A769662, this molecule, which contains a thienopyridone moiety, activates AMPK most likely by interacting with the $\beta 1$ subunits.

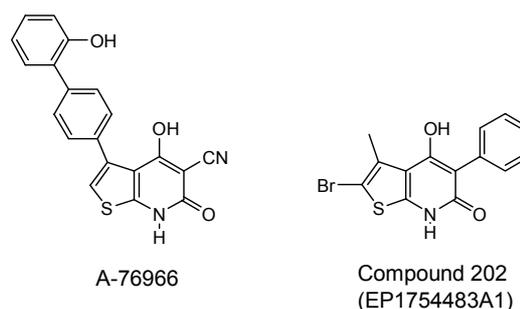


Figure 8. Molecular structure of the thienopyridone derivative A769662 and Compound 202.

Furanothiazolidine

While screening chemical libraries for direct activators of the α -subunit of AMPK Pang *et al.* [8] discovered a furanothiazolidine derivative, PT1 (Figure 9). When tested in hepatoma HepG2 cells it lowered their lipid content and activated AMPK in a dose-dependent manner. It interacted with the autoinhibitory domain in the $\alpha 1$ subunit and converted AMPK to a constitutively active complex [8]. Experiments with L6 myotubes showed that PT1 effectively increased the phosphorylation of AMPK with no apparent changes in the

ATP/ADP ratio in the cells. Moreover, this effect was not mediated via LKB1 because it also occurred in the LKB-deficient cells. PT1-induced phosphorylation of AMPK was lost in cells co-treated with STO-609, a specific CaMKK β inhibitor, suggesting a critical role for Ca²⁺-dependent CaMKK β activation in this process. *In vivo* tests with this compound are required to ascertain its potential as a prototype molecule for the development of novel antidiabetic drugs.

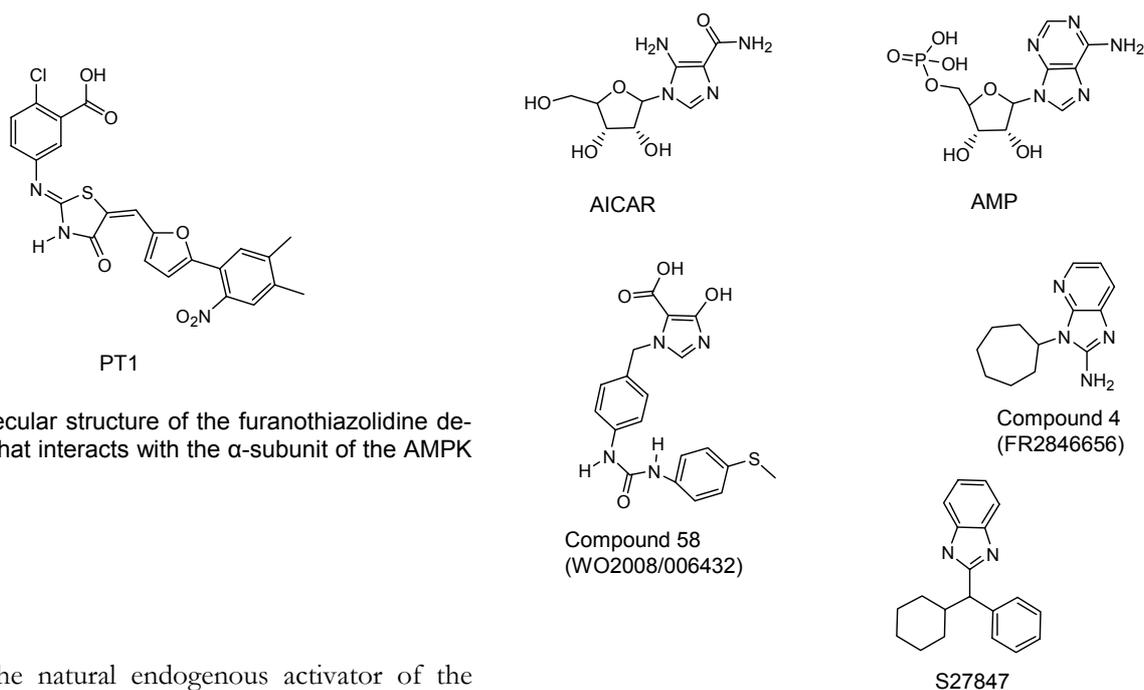


Figure 9. Molecular structure of the furanothiazolidine derivative, PT1, that interacts with the α -subunit of the AMPK complex.

AICAR

AMP is the natural endogenous activator of the AMPK complex. As explained above, two pairs of AMP molecules interact with two Bateman domains in the γ subunit and allosterically activate the entire AMPK complex. Figure 10 shows AMP and structurally related compounds that induce similar effects. AICAR is metabolized intracellularly to ZMP (5-aminoimidazole-4-carboxamide-1- β -D-ribofuranotide) by adenosine kinase. ZMP is an AMP analog that interacts with Bateman domains in the γ subunit of AMPK and induces allosteric changes in AMPK conformation, allowing kinase activation [52]. Some researchers, however, have claimed that ZMP can also regulate glucose metabolism by direct inhibition of fructose-1,6-biphosphatase in hepatocytes, thereby blocking gluconeogenesis independently of the activation of AMPK [164]. AICAR was tested in animal models of diabetes and exhibited antidiabetic effects as many pathological metabolic parameters in these animals such as, blood glucose level, lipids profile, hepatic

glucose output and glucose disposal were improved [165]. AICAR infusion in both healthy and diabetic individuals increased skeletal muscle glucose uptake [166]. It also dramatically reduced the formation of reactive oxygen species in blood and preserved normal endothelial cell function in diabetic patients [167]. In another study, AICAR increased glycogen content in rat skeletal muscles, mostly due to an increased influx of glucose [73].

Figure 10. Molecular structures of AMP and structurally related compounds that induce allosterical activation of the AMPK complex.

AICAR also negatively regulates IL-6 and IL-8 gene expression and their secretion from rat adipocytes and skeletal muscle cells. These two proinflammatory mediators are secreted from adipose tissues and play an important role in the etiology of insulin resistance in obese patients. Thus, these effects of AICAR led to the idea that it may serve as a potent drug for increasing insulin sensitivity in peripheral tissues [168]. Nevertheless, AICAR suffers from unfavorable pharmacokinetic properties (i.e., high effective concentration, poor bioavailability and short half life) and severe metabolic complications (e.g., lactic acidosis and massive uric acid production) [169].

Another serious shortcoming of AICAR is the lack of a strict specificity for AMPK. In fact, AICAR interacts with other enzymes, such as S-adenosylhomocysteine hydrolase and glycogen phosphorylase [170]. To solve these problems many groups have tried to develop more potent derivatives of AICAR. One example is the imidazo[4,5-b]pyridine derivative S27847, which increased the activity of AMPK 7-fold in hepatocytes at micromolar concentrations (Figure 10) [171]. Other structurally related imidazol analogues of AICAR and S27847 are the patented Compound 4 and Compound 58 [141, 172, 173]. These derivatives are less effective than S27847; at 500 and 200 μM they increased AMPK activity 3- and 2-fold, respectively, in comparison with the control treatments.

Other structurally unrelated compounds

Chromium picolinate

Some other molecules that activate AMPK do not belong to the various classes of chemically and structurally related compounds described above. One such compound is chromium picolinate, which is used as an antidiabetic food supplement. Recent reports suggest that some antidiabetic effects of this compound result from AMPK activation and the inhibition of resistin secretion from adipocytes. Resistin is a 12.5 kDa cysteine-rich adipokine known to induce insulin resistance in rodents [174]. Such effects are not found in man due to the lack of resistin expression and secretion [175]. Therefore, this resistin-dependent mechanism is not involved in the antidiabetic effects of chromium picolinate in man.

α -lipoic acid

α -lipoic acid decreases lipid accumulation in rat skeletal muscle and steatosis in the obese rat liver by augmenting AMPK phosphorylation [176]. It has been recently shown that an incubation of C2C12 myotubes with α -lipoic acid increased the activity and Thr¹⁷² phosphorylation of the AMPK α 2 subunit, followed by Ser⁷⁹ phosphorylation in ACC. Furthermore, inhibition of CaMKK with the selective inhibitor STO-609 abolished α -lipoic acid-stimulated AMPK activation, with a concomitant reduction of Ser⁷⁹ phosphorylation in ACC. When short interfering-RNA against CaMKK was used to silence its expression, it abolished α -lipoic acid-induced AMPK activation. These data indicate that CaMKK is possibly the target for α -lipoic acid-induced AMPK activation in myotubes [177].

Kainic acid

Another interesting molecule that activates AMPK in the brain is kainic acid (extracted from various seaweeds) [178]. Of interest are the reports showing specific receptors for kainic acid in the brain. It remains to be investigated whether kainic acid affects energy metabolism in the brain and possibly some important metabolic functions, like appetite.

Cannabinoids

Cannabinoids interact with AMPK complexes in various tissues in opposing manners. They activate AMPK in the hypothalamus and the heart, and inhibit it in the liver and adipose tissues. It has been assumed that cardioprotective effects of cannabinoids result from activating AMPK-dependent metabolic pathways [179]. Nevertheless, no significant benefits of these compounds in terms of blood glucose normalization in diabetes have yet been reported.

Long chain-fatty acids

It has also been proposed that long chain-fatty acids activate AMPK [180]. However, these effects are short-lived and transient due to efficient β -oxidation. Notwithstanding, such effects of long chain fatty acid have been documented in rats and mice and isolated insulin-sensitive tissues. Substituted α,ω -dicarboxylic acids of 14-18C fatty acids (MEDICA analogs), which are not metabolized beyond their acyl-CoA thioesters, also activate recombinant AMPK in a free cell system, in cell lines such as HepG2 and 3T3-L1 and in diabetic db/db mice. Activation of AMPK in the latter animal model normalized blood glucose and suppressed hepatic glucose production [180]. Of interest is the recent finding that oral administration of the short chain fatty acid butyric acid also activated AMPK in skeletal muscles and brown adipocytes of dietary-obese C57 black mice. This treatment also prevented the development of insulin resistance and obesity, decreased blood glucose level and reduced body fat content in the treated animals [181]. Thus, both long and short chain fatty acid may activate AMPK.

Reactive oxygen species

Reactive oxygen species (ROS) such as peroxy-nitrite (ONOO⁻) have been associated with the activation of AMPK in cells, especially in tissues with a high oxygen demand [182]. Pathologically relevant concentrations of peroxy-nitrite are capable of activating AMPK independently of variations in the AMP/ATP

ratio. It has been suggested that this process is significantly enhanced following hypoxia and reoxygenation in the heart [183]. Similar AMPK stimulatory activity has also been assigned to the free radical hydrogen peroxide [182]. The idea that activated AMPK prevents oxidative stress associated with diabetes by upregulating mitochondrial uncoupling protein-2 (UCP-2) presents an attractive pathway for antidiabetic effects of such AMPK activators [167]. Similarly, the finding that nitric oxide (NO) *per se* activates AMPK via the Ca²⁺-dependent CaMKK pathway in vascular endothelial cells points to another mechanism for AMPK activation, especially in cells and tissues where this radical generation is increased due to an of induction of NOS isotypes.

Leptin

Leptin is a 16 kDa peptide that is secreted from adipocytes and regulates energy metabolism and satiety. Several reports show that it activates AMPK in skeletal muscles but inhibits its activation in the hypothalamus. These disparate tissue-specific effects are remarkably related to the overall metabolic regulation in the organism: activation of AMPK in skeletal muscles potentiates the rates of glucose transport and fatty acid oxidation, whereas in the hypothalamus, the inhibition of AMPK reduces the appetite and food intake. Leptin-overexpressing mice exhibit reduced tissue triacylglycerol content and an increased energy expenditure, due to the peripheral phosphorylation of AMPK α 2 isoform [184, 185]. This finding was confirmed when leptin infusion increased AMPK α 2 activity in skeletal muscles of control and STZ-diabetic mice [184]. The molecular mechanism of leptin-induced activation of AMPK is not yet clear.

Ghrelin

Ghrelin has the opposite effects of leptin on AMPK. It is secreted from the gastric mucosa and acts as a growth hormone-releasing- and an appetite stimulating factor. Several studies have found that ghrelin activates AMPK in the hypothalamus and in the heart and inhibits it in the liver and adipose tissues [186]. It seems to lack any effect on AMPK phosphorylation in skeletal muscles [187]. The upstream kinase by which ghrelin exerts its effects on AMPK has not yet been identified. However, ghrelin has been shown to induce CaMKK β -dependent signaling in hypothalamic neurons [188].

Interleukin-6

Interleukin-6 (IL-6) rapidly and markedly increases AMPK activity in skeletal muscles. It also increases fatty acid oxidation and basal and insulin-stimulated glucose uptake by translocation of glucose transporter-4 to the plasma membrane of L6 myotubes. These effects are lost in L6 myotubes overexpressing the dominant negative form of AMPK α 2 [189]. The relevance of these finding to diabetes treatment is still under investigation.

AMPK inhibitors

Iodotubercidin and arabinose-adenosine (AraA)

The inhibitor iodotubercidin is a synthetic adenosine derivative that has been used to inhibit AMPK *in vitro*. It should be noted that this compound also interacts with and inhibits other enzymes, such as glycogen synthase, phosphokinase A, phosphokinase C or casein kinase [190-193].

Arabinose-adenosine (AraA) was originally isolated from the Caribbean sponge *Tehya crypta*. This compound, which has some antiviral activity, also inhibits various kinases including AMPK [194]. Another shortcoming of these two inhibitors is their inability to inhibit contraction-induced AMPK activation in skeletal muscles [195]. Thus, results on AMPK inhibition that were obtained from experiments with these inhibitors should be reevaluated.

Compound C

The synthetic Compound C is a reversible and an AMP-competitive inhibitor of AMPK [196]. When tested in cells, it fails to completely block all AMPK-dependent stimuli. For instance, while AICAR-dependent activation of AMPK in skeletal muscles was blocked by Compound C, the dinitrophenol-induced activation of AMPK was not affected by it. Moreover, some reports claim that Compound C inhibits adenosine transport into the cells. Thus, when presented together with AICAR it can limit the influx of the latter and therefore prevent its potential to activate AMPK intracellularly [196]. An additional limitation of Compound C is its inconsistent level of inhibition of AMPK activity in different tissues: for example, it is very effective in hepatocytes but much less so in skeletal muscles [81]. This may indicate an AMPK-subunit specificity of this inhibitor. Recent studies show that Compound C also inhibits other protein kinases, such as, ERK1/2 and PHK. Therefore, cellular effects me-

diated by Compound C should be carefully studied and evaluated.

Conclusions

New compounds that activate AMPK in a complex- and tissue-specific manner may eventually become novel antidiabetic and antiobesity drugs. The ideal AMPK activator should have several properties. It should specifically activate AMPK at a much lower concentration than AICAR. The activation should be targeted to AMPK subunits specifically expressed in the liver, adipocytes or skeletal muscles, but not in the central nervous centers where AMPK activation increases appetite and food consumption. Obviously, oral formulation of drugs that produce minimal side effects is essential.

This review describes some classes of direct and indirect activators of AMPK. Further studies are re-

quired to understand the molecular interactions of these compounds and structural requirements for their specificity to the various subunits of AMPK or its upstream effectors. Such ongoing efforts may provide a novel class of drugs to treat diabetes and related metabolic abnormalities in the future.

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Conflict of interest statement: The authors declare that they have no competing conflict of interests with respect to financial or other issues.

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