

AMPK activation: a therapeutic target for type 2 diabetes?

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Abstract: Type 2 diabetes (T2D) is a metabolic disease characterized by insulin resistance, β -cell dysfunction, and elevated hepatic glucose output. Over 350 million people worldwide have T2D, and the International Diabetes Federation projects that this number will increase to nearly 600 million by 2035. There is a great need for more effective treatments for maintaining glucose homeostasis and improving insulin sensitivity. AMP-activated protein kinase (AMPK) is an evolutionarily conserved serine/threonine kinase whose activation elicits insulin-sensitizing effects, making it an ideal therapeutic target for T2D. AMPK is an energy-sensing enzyme that is activated when cellular energy levels are low, and it signals to stimulate glucose uptake in skeletal muscles, fatty acid oxidation in adipose (and other) tissues, and reduces hepatic glucose production. There is substantial evidence suggesting that AMPK is dysregulated in animals and humans with metabolic syndrome or T2D, and that AMPK activation (physiological or pharmacological) can improve insulin sensitivity and metabolic health. Numerous pharmacological agents, natural compounds, and hormones are known to activate AMPK, either directly or indirectly – some of which (for example, metformin and thiazolidinediones) are currently used to treat T2D. This paper will review the regulation of the AMPK pathway and its role in T2D, some of the known AMPK activators and their mechanisms of action, and the potential for future improvements in targeting AMPK for the treatment of T2D.

Keywords: adenosine monophosphate-activated protein kinase, type 2 diabetes, insulin resistance, drug therapy

Introduction

Obesity, type 2 diabetes (T2D), and metabolic syndrome have reached epidemic proportions worldwide and the prevalence of these conditions continues to grow.¹ Although there are several medications currently available to help manage T2D, there is an increasing need for more effective treatments than those currently available. T2D is associated with many comorbidities, such as cardiovascular disease and certain cancers, and it was estimated that diabetes caused 5.1 million deaths in 2013.¹

The biological pathways involved in maintaining energy homeostasis have been targeted for pharmacological manipulation to combat the insulin resistance (IR) and metabolic dysfunction caused by chronic nutrient excess.² One such pathway is that of AMP-activated protein kinase (AMPK), an enzyme that has come to be known as a master regulator of metabolism.² This nutrient-sensing serine/threonine kinase is activated when cellular energy levels are low (ie, the intracellular AMP:adenosine triphosphate [ATP] ratio is high). Upon activation, AMPK signals through its downstream substrates to restore normal energy levels by stimulating processes that generate

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ATP (such as fatty acid [FA] oxidation) and inhibiting those that use ATP (such as triglyceride and protein synthesis).³ Overall, AMPK activation improves insulin sensitivity and glucose homeostasis, making it an attractive target for T2D and metabolic syndrome.

Interestingly, several drugs that have long been used for the treatment of diabetes, such as metformin and thiazolidinediones (TZDs), were later found to exert some of their beneficial effects through the indirect activation of AMPK.³ In addition to pharmaceutical agents, numerous natural compounds and hormones can also activate AMPK. Despite being a seemingly promising target for drug development, no direct AMPK activators have reached clinical use for the treatment of metabolic disease. Perhaps further research on AMPK's regulation will lead to new activation strategies or to the development of compounds with isoform specificity or better pharmacokinetic profiles, finally unlocking the potential for a clinically efficacious AMPK activator. This paper reviews AMPK's role and dysregulation in T2D, the currently known activators of AMPK, and the potential for a direct AMPK activator reaching the clinic.

Type 2 diabetes

T2D is a metabolic disease characterized by elevated blood glucose levels in the presence of peripheral IR.¹ According to the International Diabetes Federation, more than 350 million people worldwide had diabetes in 2013.¹ It is projected that this number will rise to nearly 600 million by 2035.¹ T2D is associated with a number of complications and comorbidities, including cardiovascular disease, blindness, kidney failure, and lower limb amputation.¹ The number one risk factor for T2D is obesity, in which the chronic overconsumption of food leads to hyperglycemia, IR, and impaired metabolic function.¹

Excess exposure to glucose, free FAs (FFA), or amino acids can be toxic to cells. To protect themselves from this toxicity, cells use a mechanism of IR to avoid taking up too many nutrients in environments of over-nutrition.⁴ However, this protective mechanism leads to pathological changes in the setting of prolonged exposure to nutrient overload. In this state of chronic IR, reduced glucose uptake in the muscles, liver, and adipose tissue, impaired suppression of hepatic gluconeogenesis, and impaired suppression of lipolysis lead to hyperglycemia, hyperinsulinemia, and hyperlipidemia.⁴ At first, the pancreatic β -cells can compensate by secreting even more insulin, but eventually these cells become dysfunctional, causing the patient to become dependent on injections of exogenous insulin. In normally insulin-responsive tissues (for

example, muscle, liver, and adipose tissue), the combination of impaired metabolism in the presence of excess glucose, insulin, and FFA causes pathological changes in gene and protein expression and activity.⁴ While there are many proteins and biological pathways involved in metabolic homeostasis that are dysregulated in IR and T2D, the remainder of this paper will focus on the AMPK pathway.

AMPK

AMPK is a phylogenetically conserved serine/threonine kinase that functions as a master metabolic regulator. It exists as a heterotrimer, consisting of a catalytic α -subunit and regulatory β - and γ -subunits. Each subunit has multiple isoforms ($\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, $\gamma 1$, $\gamma 2$, $\gamma 3$), making a total of 12 possible heterotrimer combinations. Whether there are functional differences between the different isoforms remains unclear; however, some isoforms are tissue-specific. For example, heterotrimers containing the $\alpha 1$ isoform predominate in the liver and adipose tissue, whereas those containing $\alpha 2$ predominate in the brain, heart, and skeletal muscles.^{5,6}

The activation of AMPK requires both an increase in the intracellular AMP:ATP ratio and phosphorylation of Thr172 on the "activation loop"⁷ of the α -subunit by one of its three upstream kinases: the tumor-suppressor liver kinase B1 (LKB1);^{8,9} the calcium-dependent calcium/calmodulin-dependent protein kinase kinase β (CaMKK β);¹⁰ or transforming growth factor- β activated protein kinase-1 (TAK1).¹¹ An inhibitory site at Ser485 of the $\alpha 1$ subunit also exists and has been shown to be phosphorylated by Akt,^{12,13} protein kinase A (PKA),^{14,15} or autophosphorylation¹⁴ in various cell types and tissues, such as the heart,¹² adipocytes,^{15,16} and vascular smooth muscle cells.¹³ Similarly, Ser491 of the $\alpha 2$ subunit can be phosphorylated by PKA,^{14,15} p70S6K,¹⁷ or autophosphorylation¹⁸ in tissues such as the adipocytes,¹⁵ hypothalamus,¹⁷ heart,¹² and HEK293 cells,¹⁸ resulting in reduced AMPK activity. Although previous studies suggested that Ser491 is also an Akt phosphorylation site,^{12,13} a recent study by Hawley et al¹⁸ showed that Akt does not phosphorylate Ser491 in a cell-free assay. The role and regulation of this site in insulin-responsive tissues and in T2D is not yet understood. Several other phosphorylation sites on the α subunit also exist, though their functional importance is not yet known.

The γ subunit contains four cystathionine-beta-synthase (CBS) domains (each pair is referred to as a Bateman domain) to which adenine nucleotides bind.¹⁹ Three of the four CBS domains bind adenine nucleotides; site three primarily has

AMP bound, however this can be replaced by ATP under specific conditions.²⁰ The other two binding domains can bind AMP, adenosine diphosphate (ADP), or ATP, depending on their relative concentrations.¹⁹ Under normal conditions, ATP is bound to these domains; however, when the AMP:ATP ratio is increased, AMP replaces ATP at the Bateman domains, causing an allosteric change that contributes to AMPK activation.¹⁹ This allosteric change makes AMPK a better substrate for its upstream kinases to phosphorylate it at α Thr172 and inhibits dephosphorylation of this site by the protein phosphatases, PP2A and PP2C.^{21,22} The combination of allosteric activation and phosphorylation at α Thr172 leads to a greater than 1,000-fold increase in kinase activity in cell-free assays,²³ although the changes under physiological conditions are likely much smaller.²⁴ Recently, it has been proposed that ADP, as well as AMP, may be able to activate AMPK by binding to the Bateman domains,^{25,26} although whether this occurs under normal physiological conditions remains under debate, as AMP is a much more potent allosteric activator.²⁴

Upon activation, AMPK phosphorylates its downstream targets, a main one being acetyl-CoA carboxylase (ACC).²⁷ AMPK phosphorylates ACC at Ser79 (an inhibitory site), preventing the conversion of acetyl-CoA to malonyl CoA, which allows long-chain FAs to enter the mitochondria for oxidation. Other downstream targets of AMPK include TSC2, which inhibits mammalian target of rapamycin complex 1 (mTORC1) and protein synthesis;²⁸ HMG-CoA reductase, which leads to the inhibition of cholesterol synthesis;²⁹ peroxisome proliferator-activated receptor-gamma coactivator (PPAR α) 1 α , which stimulates mitochondrial biogenesis,^{30,31} and many others. A more comprehensive list of AMPK's actions can be found in a recent review by Ruderman et al.³

AMPK activation has effects on a multitude of tissues (Figure 1). In skeletal muscles, its activation stimulates glucose uptake, FA oxidation, glucose transporter type (GLUT)4 translocation, and mitochondrial biogenesis, while inhibiting protein and glycogen synthesis.⁶ Similarly, in cardiac muscle, AMPK activation stimulates glucose uptake, FA oxidation, and glycolysis.³² AMPK stimulates glucose uptake and FA oxidation in liver, while inhibiting gluconeogenesis, as well as cholesterol, FA, and protein synthesis.³ In adipose tissue, it stimulates FA oxidation and reduces FA synthesis and lipolysis.⁶ AMPK inhibits insulin secretion from pancreatic β -cells,⁶ and it signals to increase food intake in the hypothalamus.³³ Nearly all of the physiological effects of peripheral AMPK activation

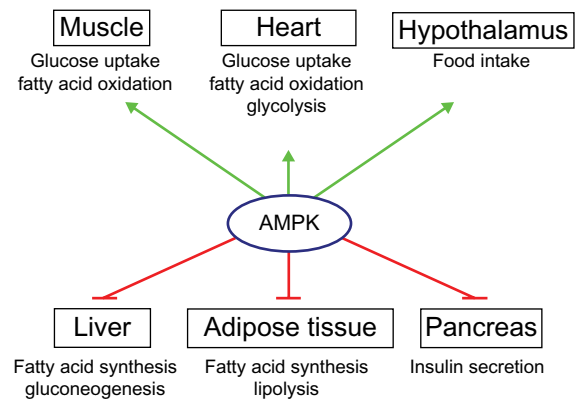


Figure 1 Roles of AMPK in the control of whole-body energy metabolism.

Notes: Activation of AMPK (green lines) stimulates the energy-generating pathways in several tissues while inhibiting the energy-consuming pathways (red lines). In skeletal muscle and heart, activation of AMPK increases glucose uptake and fatty acid oxidation. In the liver, AMPK activity inhibits fatty acid and cholesterol synthesis. Lipolysis and lipogenesis in adipose tissue are also reduced by AMPK activation. Activation of AMPK in pancreatic β -cells is associated with decreased insulin secretion. In the hypothalamus, activation of AMPK increases food intake.

Abbreviation: AMPK, adenosine monophosphate protein kinase.

would be beneficial for a patient with T2D. For this reason, the pharmacological activation of AMPK has been a seemingly promising target for drug discovery and development during the past 2 decades.

AMPK and T2D

The regulation of AMPK is of great interest in the study of T2D and metabolic syndrome due to accumulating evidence suggesting that the dysregulation of AMPK plays an important role in the development of IR and T2D, and that AMPK activation (either physiological or pharmacological) can prevent and/or ameliorate some of the pathologies of IR and T2D.³ Multiple animal models with a metabolic syndrome phenotype have exhibited decreased AMPK activity in muscle,² and evidence exists that AMPK activity is diminished in the skeletal muscle³⁴ or adipose³⁵ of humans with T2D or obesity.

AMPK activators

Numerous physiological, pharmacological, natural, and hormone activators of AMPK are known. Some of these are currently used clinically for the treatment of T2D. The following is a noncomprehensive list of some of the most established and newly identified AMPK activators (and their mechanisms of action, if known) that may have positive effects in patients with T2D (Figure 2). A more thorough list of AMPK activators can be found in other recent reviews by Steinberg and Kemp⁶ and Fogarty and Hardie.³⁶

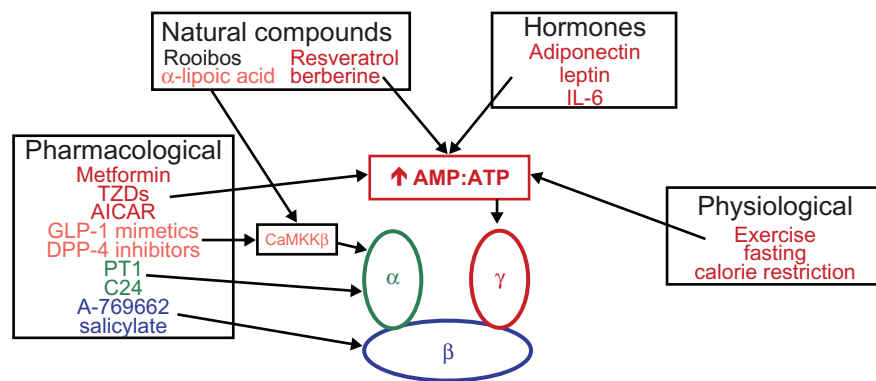


Figure 2 Physiological, pharmacological, natural, and hormonal activators of AMPK.

Notes: As discussed in the text, there are many known activators of AMPK. This noncomprehensive list highlights many of the well-established and newly discovered AMPK activators that have positive effects on T2D. Many activators activate AMPK via an increased AMP:ATP ratio (shown in red), causing AMP to bind to the γ -subunit. However, a subset of compounds stimulate AMPK activation via other mechanisms, such as binding directly to the α -subunit (shown in green), stimulating phosphorylation of α Thr172 through CaMKK β (orange), or binding directly to the β -subunit (blue). The mechanism by which rooibos activates AMPK is not known. AICAR is phosphorylated to ZMP, an analog of AMP that can activate AMPK via the γ -subunit.

Abbreviations: IL, interleukin; TZDs, thiazolidinediones; AICAR, 5-aminoimidazole-4-carboxamide riboside; GLP, glucagon-like peptide-1; DPP, dipeptidyl peptidase-4; CaMKK β , calcium/calmodulin-dependent protein kinase kinase β ; AMP, adenosine monophosphate; ATP, adenosine triphosphate; AMPK, adenosine monophosphate protein kinase; T2D, type 2 diabetes.

Physiological

Exercise and calorie restriction

Exercise and calorie restriction exert beneficial effects on metabolic health and decrease one's risk for a variety of diseases, including T2D and cardiovascular disease.³⁷ Both exercise and caloric restrictions are metabolic stresses that increase the AMP:ATP ratio in an organism's cells, and thus can activate AMPK.³⁷ Studies conducted in the past 2 decades have revealed that AMPK is stimulated by muscle contractions in both rodents^{38–42} and humans,^{43–45} and that it is a crucial enzyme through which exercise imparts many of its positive effects. ATP turnover in skeletal muscle is elevated over 100-fold during exercise, causing a rapid rise in AMP and ADP levels in an intensity-dependent manner.⁴⁶ High-intensity muscle contractions preferentially activate α 1-containing heterotrimers; while α 2 activity is stimulated by low-intensity exercise and increases progressively with intensity.⁵

Although AMPK activation has not been proven to be the mechanism by which exercise exerts its positive metabolic effects, several studies have shown that pharmacological AMPK activation mimics the effects of endurance training (for example, through increased FA oxidation or mitochondrial biogenesis) in rodents,³⁷ suggesting that AMPK may mediate the effects of exercise. A recent study showed that AMPK β 1/ β 2 skeletal muscle knockout mice not only have a reduced exercise capacity, but they also have reduced contraction-stimulated glucose uptake and skeletal muscle mitochondrial content.⁴⁷ Similarly, AMPK α 1/ α 2 skeletal muscle knockout mice have reduced exercise tolerance,

maximal force production, and fatigue resistance. However, in contrast to the β 1/ β 2 knockout mice, these mice have reduced their oxidative capacity, but not their mitochondrial number.⁴⁸ These findings suggest that AMPK is, at least in part, responsible for the exercise-induced stimulation of glucose uptake and mitochondrial biogenesis.

Pharmacological

5-Aminoimidazole-4-carboxamide riboside

5-Aminoimidazole-4-carboxamide riboside (AICAR) was the first compound identified to activate AMPK.^{49,50} It is structurally similar to adenosine and, upon entering the cells, is phosphorylated by adenosine kinase to become ZMP.⁴⁹ ZMP is an AMP analog that can bind to the CBS domains on AMPK's γ -subunit to cause allosteric activation and allow for increased phosphorylation of Thr172.⁴⁹ AICAR treatment has been shown to prevent and/or reverse some aspects of metabolic syndrome in animal models, such as ob/ob mice,⁵¹ fa/fa rats,^{52,53} and rats fed a high-fat diet.⁵⁴ For example, AICAR treatment improves glucose tolerance and whole-body glucose disposal, and reduces hepatic glucose output, as well as plasma triglyceride and FFA levels.^{51–54} AICAR also induces the expression of genes involved in oxidative metabolism and improves running endurance.⁵⁵ For these reasons, the World Anti-Doping Code banned the use of AICAR by athletes in 2011.⁵⁶

Despite these promising effects in animal models, AICAR is unlikely to be used in the treatment of human T2D or metabolic syndrome due to poor bioavailability and a short half-life.³⁶ Additionally, AICAR can mimic other actions of AMP

to exhibit AMPK-independent effects, such as the inhibition of the enzyme fructose-1,6-bisphosphatase,⁵⁷ and the stimulation of muscle glycogen phosphorylase.⁵⁸ However, AICAR may be useful in treating humans with acute lymphoblastic leukemia^{59,60} and cardiac ischemic injury.^{61,62} Interestingly, another recently described AMPK activator, compound 13, is taken up into the cells and converted to the AMP analog, compound 2, which is a much more potent and specific activator of AMPK when compared to ZMP.⁶³ Compounds of this series are being optimized for oral bioavailability and pharmacokinetics; however, their clinical utility remains to be seen.⁶³

Biguanides

Metformin, which belongs to the biguanide family of insulin-sensitizing drugs, is currently the first-line oral therapy for T2D according to national and international guidelines.^{64–67} The biguanides also include phenformin, buformin, and the antimalarial agent, proguanil. This class of drugs originates from the French lilac plant, which has been used in folk medicine to treat diabetes for centuries due to its glucose-lowering properties.^{68,69} Although phenformin and buformin are more potent insulin sensitizers than metformin, they also have a higher risk for unwanted side effects – namely, lactic acidosis.⁶⁴ For this reason, they were withdrawn from the market, leaving metformin as the only biguanide available for the treatment of T2D.⁶⁴ Within 12 weeks of receiving United States Food and Drug Administration approval in 1994, metformin became the most frequently prescribed oral antidiabetic drug in the US,⁷⁰ and it is currently prescribed to over 100 million patients worldwide.⁶⁴ Metformin reduces hemoglobin HbA_{1c} by 1%–2% in patients with T2D, and it reduces mortality when compared to diet modifications alone.⁷¹ In addition to its hypoglycemic effects, metformin has very few side effects, is weight neutral, and recent epidemiological studies suggest that patients taking metformin may have lower risks of cardiovascular disease⁷² and certain types of cancer.^{73,74}

Since metformin was discovered before the use of targeted drug discovery techniques, its mechanism of action was not known for a long time, and it is still not fully understood.⁶⁴ Zhou et al reported that metformin activates AMPK, and its insulin-sensitizing actions have been attributed to AMPK.⁷⁵ Metformin does not activate AMPK directly; instead, it has been shown to inhibit complex 1 of the mitochondrial respiratory chain,⁷⁷ which promotes a switch from aerobic to anaerobic glycolysis, thus increasing the AMP:ATP ratio and promoting AMPK activation. This

indirect mechanism is supported by the fact that metformin fails to activate AMPK in cell-free assays⁷⁸ and in cells expressing AMP-insensitive (R531G) gamma 2 variants.⁷⁹ Inhibition of hepatic gluconeogenesis is thought to be the primary means by which metformin exerts its effects on glucose homeostasis.⁷⁷ It can also stimulate glucose uptake in adipose and skeletal muscle, although the times and concentrations needed to stimulate AMPK and glucose uptake were much larger than would be found in vivo.^{76,80} A recent study using liver-specific AMPK $\alpha 1/\alpha 2$ or liver kinase B1 knockout mice brought into question the dependence of metformin's effects on AMPK, since metformin treatment lowered blood glucose levels in both of these mouse models.⁸¹ An even more recent study reported that metformin exerts its effects on the liver by antagonizing glucagon signaling through cyclic AMP and PKA, independent of AMPK.⁸² In contrast, mice with mutations in ACC1/2 that prevent phosphorylation and inactivation by AMPK are refractory to the lipid-lowering and insulin-sensitizing effects of metformin when made obese by high-fat feeding, suggesting that the inhibitory phosphorylation of ACC by AMPK is essential for metformin-induced improvements in insulin sensitivity.⁸³ Despite these conflicting results, AMPK is likely an effector of some of metformin's insulin-sensitizing effects, though further studies are needed to distinguish the AMPK-dependent from AMPK-independent effects.

Thiazolidinediones

TZDs are a class of insulin-sensitizing drugs and include rosiglitazone, pioglitazone, and troglitazone. Although their primary target is the nuclear hormone receptor peroxisome proliferator-activated receptor- γ , (PPAR γ) they are thought to exert some of their antidiabetic effects through AMPK activation.⁸⁴ TZDs have been shown to rapidly stimulate AMPK and ACC phosphorylation in a variety of tissues, including the skeletal muscle^{84,85} and liver.⁸⁶ Like metformin, they do so indirectly by inhibiting complex 1 of the mitochondrial respiratory chain to increase the cellular AMP:ATP ratio.^{79,84,87} Additionally, TZDs may indirectly activate AMPK through the effects of peroxisome proliferator-activated receptor- γ to stimulate adiponectin secretion (which will be described in more detail).^{88,89}

In patients with T2D, TZDs improve insulin sensitivity in the muscles, liver, and adipose tissues, improve glycaemic control (reduce HbA_{1c}), enhance endothelial function, and reduce inflammation.⁹⁰ However, the main drawbacks of TZDs are that they cause weight gain (particularly subcutaneous adiposity), they may increase one's risk of

bladder cancer,⁹¹ and they may worsen congestive heart failure, though they are not associated with increased mortality.^{90,92}

Glucagon-like peptide-1 receptor agonists

Glucagon-like peptide-1 (GLP-1) is an incretin that is secreted from intestinal L-cells following the ingestion of food.⁹³ GLP-1 stimulates insulin secretion in a glucose-dependent manner, decreases pancreatic glucagon secretion, increases β -cell mass and insulin gene expression, stimulates satiety in the brain, and increases peripheral insulin sensitivity.⁹³ Based on these antidiabetic actions, GLP-1 mimetics, such as exenatide and liraglutide, have been developed for the treatment of T2D.⁹⁴ An alternative strategy that has been undertaken to increase GLP-1 levels is the development of dipeptidyl peptidase-4 inhibitors, which prevent the inactivation of GLP-1.⁹⁵ Recent studies have shown that these compounds, as well as endogenous GLP-1, can activate the AMPK pathway.^{96,97} For example, exenatide treatment was shown to increase AMPK phosphorylation at Thr172 in hepatocytes,⁹⁶ decrease body weight, serum FFA, and triglyceride levels, and reverse the hepatic accumulation of lipids and inflammation in high-fat-fed mice, while increasing AMPK messenger ribonucleic acid (mRNA) and protein expression.⁹⁷

A-769662

The first compound to be identified as a direct activator of AMPK was A-769662.⁶ This thienopyridone, identified by Abbott Laboratories (Abbott Park, IL, USA), activates AMPK in a similar manner to AMP; it causes allosteric activation and prevents dephosphorylation of Thr172.^{98–100} Unlike AMP, however, A-769662 binds in a cleft between the kinase domain of the α -subunit and the carbohydrate-binding domain of the β -subunit.¹⁰¹ It is specific for the β 1-isoform and requires β Ser108 phosphorylation.^{100,102} Treatment of ob/ob mice with this compound caused improvements in glucose homeostasis and lipid levels.⁹⁸ Despite these benefits on the metabolic parameters, A-769662 is unlikely to be used to treat human metabolic syndrome due to its poor oral absorption and its reported AMPK-independent effects, whereby it can inhibit 26S proteasome activity and arrest cell cycle progression.¹⁰³ However, A-769662 has utility as a research tool to further study the effects of AMPK activation. Recently, another compound referred to as 991, which is a cyclic benzimidazole derivative that binds to the same site as A-769662, was shown to be a much more potent AMPK activator.¹⁰¹ Studies regarding the efficacy of this compound are in their infancy.

Salicylate

Salicylates are natural substances produced by many plants to defend themselves against infections.¹⁰⁴ The medicinal use of salicylate was first described thousands of years ago, when it was extracted from willow bark,¹⁰⁵ making it one of the oldest medicines used by humans. It is often taken in the form of acetyl salicylate (trade name, aspirin; Bayer AG, Leverkusen, Germany) or the diester salsalate, both of which are rapidly converted to salicylate in vivo.^{106,107} It was recently reported that salicylate, but not aspirin, activates AMPK in HEK-293 cells at concentrations found in the plasma of patients treated with high doses of aspirin.¹⁰⁸ Salicylate was determined to bind to the same site as A-769662 on the β 1-subunit based on findings that the ability of both compounds to activate AMPK is greatly diminished in complexes where the β 2 subunit, rather than β 1 subunit, is expressed, and that the effects of both compounds are nearly abolished by an S108A mutation in β 1.¹⁰⁸ Further confirmation of a role for salicylate-induced AMPK activation in vivo was found when mice treated with salicylate had lower respiratory exchange ratios following food withdrawal, indicating a switch to fat oxidation.¹⁰⁸ However, these effects were not seen in β 1 knockout mice.¹⁰⁸

These findings suggest that although salicylate is a less potent activator than A-769662, it may have some utility in improving metabolic parameters in patients with T2D. Indeed, two randomized controlled trials showed that oral salsalate treatment decreased plasma glucose levels and insulin C-peptide, and increased plasma adiponectin levels in obese young adults¹⁰⁹ and in patients with impaired fasting glucose and/or impaired glucose tolerance.¹¹⁰ Although these findings seem promising for the use of salicylate as an AMPK-mediated antidiabetic treatment, further research is needed to fully define the role of AMPK in these outcomes. High-fat-fed wild type and AMPK β 1-knockout mice treated with salicylate for 2 weeks both showed improved glucose tolerance, as well as reduced fasting glucose and insulin levels, suggesting that some of salicylate's insulin-sensitizing effects are AMPK-independent.¹⁰⁸

PTI and C24

PT1 is another small molecule compound that has recently been identified as a direct activator of AMPK.¹¹¹ Its mechanism of action is thought to be antagonism of the autoinhibitory (residues 313–335) domain of the α -subunit.¹¹¹ Treatment with PT1 dose-dependently increased AMPK activity and ACC phosphorylation in L6 myotubes and HepG2 cells, with no significant changes in the AMP:ATP ratios.¹¹¹ PT1 is not

effective *in vivo* due to a poor pharmacokinetic profile, but its structural optimization led to the discovery of the similar, but orally bioavailable compound, C24.^{112,113} C24 was shown to reduce glucose production and decrease triglyceride and cholesterol contents in hepatocytes.¹¹² Chronic oral treatment with C24 lowered blood glucose and lipid levels and improved glucose tolerance in db/db mice.¹¹² Whether C24 or a similar compound will make it to the clinic remains to be seen.

Natural compounds

Numerous naturally occurring compounds and phytochemicals have been shown to activate AMPK *in vitro* and *in vivo*,³⁶ and they elicit metabolic benefits that are dependent on AMPK activation.

Resveratrol

Resveratrol is a polyphenol found in red wine that has been suggested to mimic some of the effects of calorie restriction to increase one's lifespan.¹¹⁴ Treatment of high-fat-fed animals with resveratrol causes improvements in insulin sensitivity and decreases markers associated with aging.¹¹⁵ Resveratrol has been shown to stimulate AMPK activity in multiple cell types, including hepatocytes,^{116–118} muscle cells,^{119,120} and neurons.¹²¹ The mechanism by which resveratrol activates AMPK is thought to be an increase in AMP levels due to inhibition of the mitochondrial F1 ATPase.^{79,122} Resveratrol treatment stimulates glucose uptake¹²⁰ and mitochondrial biogenesis¹¹⁹ in muscle cells, and it stimulates mitochondrial biogenesis¹¹⁶ and reduces lipid accumulation in the liver.¹¹⁸ The latter effect is blocked by a dominant negative AMPK, suggesting that it is AMPK-mediated.¹¹⁸ Further studies are required to determine how much of resveratrol's effects are due to AMPK activation as opposed to the activation of sirtuin 1 (SIRT1), a redox-sensitive deacetylase whose activation has been shown to increase longevity.¹²³ Of note, however, Ruderman et al¹²⁴ have shown that AMPK and sirtuin 1 can both regulate each other and share many common target molecules.

Rooibos

Rooibos (*Aspalathus linearis*) is a plant grown in South Africa that is popularly used in tea and has been shown to activate AMPK.¹²⁵ Treatment of C2C12 myotubes with rooibos extract increases glucose uptake, mitochondrial activity, GLUT4 expression, and ATP production, and it reverses palmitate-induced IR.¹²⁵ *In vivo*, the rooibos extract was reported to reduce serum cholesterol, triglyceride, and FFA concentrations in mice fed a high-fat diet.¹²⁶ Adipocyte

size and triglyceride content were also reduced and hepatic steatosis was prevented. These metabolic improvements were attributed to AMPK activation in the liver and adipose.¹²⁶ Similarly, ob/ob mice fed a diet containing 0.1% rooibos extract had improved fasting blood glucose levels and improved glucose tolerance compared to mice fed a control diet.¹²⁷ Furthermore, rooibos treatment decreased the expression of gluconeogenic and lipogenic hepatic genes in these animals.¹²⁷

Berberine

Berberine is an isoquinoline alkaloid found in certain plants, and it has traditionally been used in Chinese and Korean cultures to treat fungal and bacterial infections, as well as T2D. Berberine has been shown to improve glucose tolerance,¹²⁸ reduce body weight,¹²⁸ increase the expression of the insulin receptor (IR) and low-density lipoprotein (LDL) receptor,¹²⁹ lower total and LDL cholesterol levels,¹²⁹ and reduce triglyceride levels^{128,129} in several rodent models. Berberine has also been shown to lower blood glucose, triglyceride, and cholesterol levels to nearly the same degree as metformin.¹³⁰ It has been shown to potently activate AMPK in skeletal muscle,¹³¹ hepatocytes,¹³² and adipose tissue,¹³³ although some of its antidiabetic effects are likely mediated through AMPK-independent mechanisms, such as dipeptidyl peptidase-4 inhibition¹³⁴ and the enhancement of superoxide dismutase activity.¹³⁵ Like metformin and TZDs, berberine is thought to activate AMPK by inhibiting complex 1 of the mitochondrial respiratory chain, thus increasing the AMP:ATP ratio.^{79,136,137} It has also been shown to increase adiponectin expression, which may contribute to both AMPK-dependent and independent effects.¹³⁸

α -lipoic acid

The short-chain FA α -lipoic acid is an essential cofactor for mitochondrial respiration and a powerful antioxidant and has been shown to activate AMPK in the skeletal muscle,^{139,140} heart,¹⁴¹ and endothelium.¹⁴² It also inhibits AMPK signaling in the hypothalamus,¹⁴³ thus reducing food intake. It has been shown to improve insulin sensitivity in obese rodents¹⁴⁴ and to reduce insulin secretion and β -cell growth.¹⁴⁵ Furthermore, *ex vivo* incubation of rat skeletal muscle with α -lipoic acid prevents high glucose- or leucine-induced impairments in insulin signaling,¹⁴⁰ skeletal muscle lipid accumulation, and hepatic steatosis in obesity.¹⁴⁴ Shen et al¹⁴⁶ showed that the mechanism by which α -lipoic acid activates AMPK is through the CaMKK β -mediated phosphorylation of Thr172. The authors reported that the selective inhibitor

of CaMKK β STO-609 prevented α -lipoic acid-stimulated AMPK activation and subsequent ACC phosphorylation. In addition, α -lipoic acid has also been reported to have beneficial effects on diabetic neuropathy, although whether AMPK is also involved in mediating these effects is unknown.^{147,148}

Hormones

In addition to the exogenous pharmacological and natural compounds that can activate AMPK, endogenous hormones also exist that can activate AMPK and elicit many of the same antidiabetic effects.

Leptin

Leptin is a hormone made and secreted by adipocytes that acts on the brain to regulate food intake and body weight. It can also act directly and indirectly on peripheral tissues, as almost all tissues express the leptin receptor.¹⁴⁹ Leptin increases the AMP:ATP ratio in skeletal muscle, thus activating AMPK and stimulating FA oxidation.¹⁵⁰ This activation occurs only in α 2-containing heterotrimers,¹⁵¹ although the reason for this isoform specificity is not known. In addition to directly acting on skeletal muscle to activate AMPK, leptin can also indirectly stimulate AMPK in muscles via α -adrenergic signaling from the central nervous system.¹⁵² This activation is more delayed and requires the melanocortin 4 receptor, since the intracerebroventricular administration of a melanocortin 4 receptor antagonist prevents central nervous system-mediated activation of skeletal muscle AMPK by leptin.¹⁵² In contrast to its effects in skeletal muscle, leptin inhibits AMPK in the hypothalamus to inhibit food intake.^{33,153} Yang et al¹⁵⁴ reported that it does so indirectly via a mechanism involving release of an opioid from a cell that is different from that in which AMPK is located.¹⁵⁴ However, it was recently reported that AMPK inhibition via phosphorylation of S491 on its α 2-subunit by p70S6 kinase is required to mediate leptin's anorectic effects.¹⁷

Adiponectin

Adiponectin is a protein secreted from adipose tissue, which circulates at high concentrations in the plasma in the form of low and high molecular weight multimers.¹⁵⁵ This circulating hormone acts through its two receptors (adipoR1 and adipoR2), which are expressed in tissues such as adipose and skeletal muscle to regulate glucose levels and stimulate FA oxidation.¹⁵⁶ Adiponectin levels are reduced in obese humans and animals.¹⁵⁷ AMPK activation by adiponectin is dependent on signaling through adipoR1¹⁵⁸ and requires the adaptor protein, phosphotyrosine interaction, PH domain

and leucine zipper containing 1.¹⁵⁹ Purified adiponectin from human plasma potently activates AMPK activity in C2C12 myotubes,¹⁶⁰ and adiponectin's ability to suppress hepatic glucose output has been shown to be AMPK-dependent.¹⁶¹ Adiponectin overexpression has been shown to reduce body weight, improve insulin sensitivity, and increase FA oxidation in various rodent models of genetic and diet-induced obesity.^{162–165} Interestingly, adiponectin trimers and hexamers, but not high molecular weight forms, stimulate food intake through AMPK activation in the hypothalamus.¹⁶⁶

Interleukin-6

Interleukin-6 (IL-6) is a proinflammatory cytokine that is elevated in obesity.¹⁶⁷ It is also produced and released from muscle during exercise to increase circulating levels almost 100-fold.¹⁶⁸ Interestingly, in obesity, IL-6 is associated with IR, whereas during exercise, it may enhance glucose uptake via AMPK activation. IL-6 increases muscle glucose uptake through an AMPK-dependent mechanism in cultured cells,¹⁶⁹ rodents,^{169,170} and humans.¹⁷¹ These effects of IL-6 are additive to those of insulin in stimulating glucose uptake.¹⁷² However, some studies have shown that these effects are only seen at super-physiological concentrations.¹⁷² AMPK activity is diminished in the muscle and adipose tissue of IL-6 knockout mice, and exercise-stimulated AMPK activity is diminished in these mice.^{167,170} Kelly et al¹⁶⁹ showed that IL-6 activates AMPK by increasing the concentration of cyclic AMP and, secondarily, the AMP:ATP ratio.

Conclusion

As the T2D epidemic continues to grow, the need for safe and efficacious antidiabetic medications also increases. Current therapies leave much room for improvement, as most patients require multiple medications to get their blood glucose levels under control, and T2D is the seventh leading cause of death in the US.¹⁷³ AMPK is an attractive target for T2D therapies because of its role as a master metabolic regulator. Since it is activated during calorie restriction and exercise (both of which promote insulin sensitivity), it is thought that pharmacological activation of this target could elicit many of their positive benefits.² Although several currently used T2D medications, such as metformin and TZDs, indirectly activate AMPK, no direct AMPK activators have made it to the clinic due to poor pharmacokinetic profiles or off-target effects. Furthermore, AMPK is an attractive target because its activity is decreased in tissues such as in the muscle and adipose tissue of obese or insulin-resistant animals and humans. For a more comprehensive review of

AMPK inhibition in response to over-nutrition, refer to the recent review by Coughlan et al.⁴

Despite accumulating evidence, both in vitro and in vivo, that AMPK activation positively affects numerous physiological processes that are dysregulated in metabolic diseases, whether the continued pursuit of direct AMPK activators is a worthwhile or promising strategy for drug development remains to be seen. An important consideration in pursuing AMPK activation as a treatment for metabolic disease is that although most of its effects are beneficial (for example, via the stimulation of glucose uptake and FA oxidation), excess activation can have unwanted consequences. For example, AMPK inhibits protein synthesis, which could be harmful, particularly to elderly patients in whom muscle wasting may be a concern.¹⁷⁴ However, calorie restriction has been shown to delay age-related loss of muscle mass and function, and to induce a younger muscle transcription profile.^{175,176} Perhaps isoform-specific activators will not have the same weaknesses as currently available nonspecific activators, as they may preferentially target particular cell types or tissues. Further insights into the regulation of AMPK's less-studied post-translational modifications may bring to light new strategies for more controlled pharmacological modulation. In summary, as a cellular energy sensor and master regulator of metabolism whose activity is diminished in states of IR, AMPK seems to be an attractive and promising target for the pharmacological treatment of T2D.

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Disclosure

The authors report no conflicts of interest in this work.

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