Natural Phenolic Compounds Targeting The AMPK Activation For Metabolic Health

Khoa D.A Nguyen¹ and Khanh V. Doan^{2,*}

¹Department of Physiology, ²Department of Pharmacology, School of Medicine, Tan Tao University, Duc Hoa, Long An, 850000, Viet Nam., *Corresponding authors: E-mail: khanh.doan@ttu.edu.vn

ABSTRACT AMP-activated protein kinase (AMPK) is a cellular energy sensor which plays a crucial role in regulation of whole-body energy homeostasis. Activation of AMPK signaling results in favorable effects on mitochondrial function, autophagy, glucose/lipid metabolism, and insulin sensitivity, making it an important therapeutic target in treatment/prevention of metabolic disorders and cancer. Recently, pharmacological studies of natural phenolic compounds indicated that the benefits on metabolic health of these phytochemicals are not only related to their protogenic antioxidant property but also to their AMPK-activating potential. Due to their diverse structures, identification of phenolic compound molecules which have potential to target the AMPK activation for beneficial metabolic effects may be promising in order to develop novel therapeutics in the prevention and/or treatment of metabolic disorders. In this minireview, we summarize beneficial metabolic outcomes of AMPK activation and discuss the capability of natural polyphenols to activate the AMPK pathway focusing on the phenolic acids as potential lead compounds.

KEYWORDS

AMP-activated protein kinase (AMPK), Polyphenol, Phenolic acid, Metabolic benefits.

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INTRODUCTION

Obesity, insulin resistance, and dyslipidemia - typical features of "metabolic syndrome" - are strongly associated with globally increasing prevalence of diabetes and cardiovascular disease (CVD). Within the past two decades, our knowledge on molecular biochemical pathways modulating the pathogenesis of diabetes and metabolic syndrome has been expanded. Several novel therapeutic approaches based on the new molecular drug targets within these pathways have been considered, tested, and validated in animals and humans. [1]

AMP-activated protein kinase (AMPK) is a cellular energy sensor which plays a crucial role in regulation of whole-body energy homeostasis [2]. Activation of AMPK signaling results in favorable effects on mitochondrial function, autophagy, glucose/lipid metabolism, and insulin sensitivity, making it an important therapeutic target in treatment/prevention of metabolic disorders and cancer [3, 4]. Recently, pharmacological studies of phytochemicals indicated that benefits on metabolic health of natural polyphenols

are not only related to their protogenic antioxidant property but also to their AMPK-activating potential [5].

Polyphenols are a bulky group of small natural molecules which can be categorized into several classes including flavonoids, phenolic acids, stilbenes, and lignans based on the number and presence of phenol rings in their molecule [6]. They are found ubiquitously in plants and possess potent antioxidant activity as well as various bioactivities such as anti-inflammatory, antibacterial, antiproliferative, and anticarcinogenic effects [7]. While metabolic effects and molecular mechanistic action on AMPK pathway of flavonoids and resveratrol (stilbene) were extensively investigated and reviewed [8], the ability to activate AMPK signaling and metabolic actions of other phenolic compounds such as phenolic acids have been just recently explored. In this minireview, we summarize the benefits of AMPK activation on metabolism and discuss the potential for phenolic acids to target this activation and produce beneficial metabolic effects.

AMP-ACTIVATED PROTEIN KINASE (AMPK) AND ITS ACTIVATION

AMPK, a serine/threonine protein kinase, is a sensor of cellular energy status and plays a central role in the regulation of cellular metabolism [9]. It presents in all tissues as a heterotrimeric complex composed of a catalytic α subunit and regulatory β and γ subunits. Activation of AMPK requires phosphorylation at threonine residue 172 (Thr172) within the activation loop of the α subunit [9]. AMPK is activated by various types of metabolic stresses, drugs, and plant products through the mechanisms involved in the increase of cellular AMP, ADP, or calcium Ca^{2+} [10]. In mammalian cells, AMPK is activated directly by AMP/ADP or via phosphorylation at Thr172 by the upstream kinases such as iver kinase B 1 (LKB1) or Ca^{2+} /calmodium-dependent protein kinase kinases (CaMKKs) [10]. During energy stress, ratios of AMP/ATP and/or ADP/ATP rise markedly and AMP/ADP compete with ATP to bind to the AMPK γ subunit leading to the conformational changes in the kinase domain, which promotes phosphorylation of AMPK Thr172 meanwhile prevents its dephosphorylation [11]. While CaMKKs activate AMPK in response to elevated intracellular Ca^{2+} independent of changes in adenine nucleotides, LKB1 provides a high basal level of phosphorylation at Thr172 modulated by the binding of AMP to the subunit [11]. Once activated, AMPK in turn phosphorylates and inactivates acetyl-CoA carboxylase (ACC), an important enzyme in fatty acid synthesis. Hence, phosphorylations of AMPK at Thr172 site and the downstream ACC at the major phosphorylated sites are commonly used as indicators for AMPK activation [11].

AMPK ACTIVATION AND METABOLIC BENEFITS

AMPK activation and lipid metabolism

Generally, AMPK activation results in the inhibition of fatty acid synthesis while enhancing fatty acid oxidation [12]. By phosphorylating and inhibiting both isoforms of ACC (ACC1 and 2), AMPK activation lowers the concentration of malonyl-CoA, the product of the reaction catalyzed by ACC [13]. Malonyl-CoA serves as an intermediate in the fatty acid synthesis and an inhibitor of carnitine palmitoyl transferase-1, which is involved in fatty acid entry into mitochondria for beta oxidation. In addition, AMPK activation may also enhance fatty acid uptake by inducing the translocation of fatty acid translocase to the plasma membrane by undefined mechanisms [3]. Together with direct and acute effect on ACC, AMPK further inhibits fatty acid synthesis in the longer term by phosphorylating the sterol regulatory element-binding protein 1c (SREBP-1c, an important transcription factor of lipogenic enzymes) and inhibits cholesterol synthesis by phosphorylating and inactivating the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) as well as the phospholipid/triacylglycerol synthesis by inactivating the glycerol phosphate acyl transferase [12].

AMPK activation and mitochondrial biogenesis

Activated AMPK is known to enhance mitochondrial biogenesis by directly phosphorylating and activating the transcription of peroxisome proliferator-activated receptor gamma co-activator 1 α (PGC1 α), a master transcriptional regulator of genes involved in mitochondrial oxidative metabolism [3, 14]. PGC-1 α promotes expression of the peroxisome proliferator-activated receptors (PPARs) regulating the genes required for fatty acid oxidation and expression of nuclear respiratory factors (NRFs). NRFs stimulate expression of transcriptional factor A of mitochondria (TFAM), a mitochondrial matrix protein required for mitochondrial DNA

replication [15]. Through the direct regulation of PGC-1 α level and transcriptional activity, AMPK activation therefore enhances mitochondrial biogenesis by increasing new mitochondria and expression of nuclear-encoded mitochondrial genes [16]. In addition, PGC1 α can also be up-regulated indirectly through the effect of AMPK activation on sirtuin 1 (Sirt1) which is a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase enzyme that can deacetylate and subsequently increase activity of transcriptional factors [17, 18]. AMPK activation of Sirt1 might be mediated by the up-regulation of NAM-phosphoribosyl transferase (NAMPT), an enzyme that catalyzes the conversion of nicotinamide (NAM) to NAD^+ , or by altering NAD^+/NADH ratio independent of NAMPT activity, which ultimately leads to the upregulation of PGC1 α transcriptional activity [19, 20].

AMPK activation and autophagy

Together with increasing mitochondrial biogenesis, AMPK is involved in the disposal of damaged mitochondria and recycling of their contents for reuse as catabolic fuels through a cellular process called autophagy [16]. Autophagy is a “self-eating” process that is induced by nutrient starvation as a mechanism for cell survival [21]. The autophagy-related (Atg) proteins play a crucial role in the core machinery required for autophagosome formation and autophagy induction [22]. AMPK activation stimulates autophagy directly via phosphorylation of autophagy-related proteins in the mTORC1, ULK1 (Unc-51-like autophagy-activating kinase), and lipid kinase complex PIK3C3 (phosphatidylinositol 3-kinase, catalytic subunit type 3) [23, 24]. By recycling components of damaged organelles, AMPK-induced autophagy may play an important role in maintaining overall cellular ATP-generating capacity as the production of new mitochondria during energy starvation demands fuel resources [10]. Moreover, autophagy also regulates the lipid metabolism by preventing accumulation of lipid droplets [25].

AMPK activation, glucose metabolism and insulin sensitivity

AMPK increases glucose uptake in muscle and adipose tissues via a mechanism that enhances the translocation of glucose transporter 4 (GLUT4) from the cytoplasmic storage vesicles to the plasma membrane [26]. AMPK is also involved in the activation of the plasma membrane-located GLUT1 to promote glucose uptake into other cells [27] and enhances glucose metabolism by increasing mitochondrial biogenesis. In addition, AMPK can further increase insulin sensitivity by enhancing fatty acid oxidation and reducing triglyceride accumulation that is frequently associated with insulin resistance [28]. Consequently, AMPK activation improves insulin sensitivity and serves as a potential therapeutic intervention for the treatment of obesity and type 2 diabetes by regulating glucose and fatty acid metabolism, enhancing mitochondrial function, and clearing the damaged mitochondria through autophagy [3, 4, 11, 29].

PHARMACOLOGICAL AMPK ACTIVATORS

Physiological conditions of metabolic stresses such as exercise, calorie restriction, and starvation increase the AMP:ATP ratio and hence can physiologically activate the cellular AMPK [30]. In addition to metabolic stresses, it has been shown that AMPK can be activated in response to several pharmacological agents and a number of natural compounds.

Therapeutic agents as AMPK activators (Biguanide/ Metformin)

The biguanides have been used for the treatment of type 2 diabetes since the 1950s due to their abilities to inhibit hepatic glucose production and enhance peripheral insulin sensitivity [11]. Although phenformin was subsequently withdrawn due to the life-threatening side effect of lactic acidosis, metformin is still the most widely prescribed biguanide drug for the treatment of type 2 diabetes [31]. Despite their widespread use in clinics, the molecular mechanism of biguanides was first reported in 2000 when metformin was shown to inhibit complex I of the mitochondrial respiratory chain [32, 33], which consequently inhibited the mitochondrial ATP synthesis and caused a drop in intracellular ATP levels [11]. Indeed, activation of AMPK by biguanides/ metformin was subsequently demonstrated and the increase in AMP and/or ADP was shown to be required for the AMPK activation induced by biguanides [34, 35]. Although experimental studies revealed that other AMPK-independent mechanisms also contributed to the effect of metformin on hepatic gluconeogenesis suppression, metformin activation of AMPK was demonstrated to play an important role in this major therapeutic mode of action of metformin to lower the blood glucose in diabetes [36, 37]. Furthermore, activation of AMPK accounted for the beneficial effect of metformin on insulin sensitivity by stimulating fatty acid oxidation, inhibiting fatty acid synthesis, and reducing triglyceride storage [11].

Thiazolidinediones (TZDs)

TZDs, another major class of anti-diabetic drugs which include rosiglitazone, pioglitazone, and troglitazone, are known as insulin-sensitizing agents including. Although the primary target of TZDs is the nuclear hormone receptor peroxisome proliferator-activated receptor- (PPAR-), TZDs have also been shown to activate AMPK [38]. Similar to metformin, TZDs indirectly activate AMPK by inhibiting complex 1 of the mitochondrial respiratory chain [39]. In addition increase in adiponectin secretion triggered by PPAR- activation might also involve in AMPK activation induced by TZDs [40, 41].

Phytochemicals as AMPK activators

Various natural products and phytochemicals have been reported to activate AMPK and elicit metabolic benefits in type 2 diabetes and metabolic syndromes. These include resveratrol from red grapes, quercetin from fruits and vegetables, ginsenoside from *Panax ginseng*, curcumin from *Curcuma longa*, berberine from *Coptis chinensis*, epigallocatechin gallate and catechins from green tea, theaflavin from black tea, and hispidulin from snow lotus [11]. Although the structures of these compounds are quite variable, most of them can be classified as polyphenols. Except for the case with salicylate, it seems likely that most of the phytochemicals activate AMPK indirectly by inhibiting mitochondrial ATP production in a similar manner to the biguanides [11]. For example, berberine was shown to inhibit the mitochondrial respiratory complex I whereas resveratrol and quercetin were suggested to inhibit the F1 ATP synthase [42, 43]. This mechanism could be explained for the observation that AMPK activation by resveratrol, berberine, and quercetin was attenuated or abolished in cells expressing the AMP/ADP-insensitive AMPK mutant [11, 35]. It is suggested that mitochondrial poisonous effect at low doses of these phytochemicals probably leads to a mild inhibition of mitochondria and thus may be useful to activate AMPK without cellular toxicity [11] (Fig. 1).

Figure 1 Depicted diagram proposes the mechanistic and beneficial actions of phenolic compounds on AMPK activation

Figure 2 Structures of major polyphenols activating the AMPK pathway

Resveratrol and flavonoids

Resveratrol (3,4',5-trihydroxystilbene, Fig. 2) is found in grapes, red wine, and some berries and has been suggested to give beneficial effect on a variety of illnesses, including obesity/diabetes, cardiovascular disease, and cancer. It has also been suggested to enhance stress endurance and extend lifespan [8, 44] probably by mediating some crucial regulators of energy metabolism including AMPK, SIRT1 and PGC1α [18, 45, 46]. AMPK activation by resveratrol treatment has been observed in many cell types, including muscle cells, hepatocytes and neurons [46, 47]. Resveratrol enhances glucose uptake and mitochondrial biogenesis in muscle, increases mitochondrial biogenesis in the liver, and inhibits hepatic lipid accumulation resulting in improvement of lipid profiles and glucose homeostasis [46, 48, 47]. Resveratrol treatment showed consistent anti-obesity effects by improving lipid and glucose metabolism in diet-induced and genetic obese animal models [8]. However, clinical trials examining the effects of resveratrol on obesity-related energy expenditure, fat oxidation, plasma lipid profiles, and glucose hemostasis produced controversial results [49, 50]. The lack of resveratrol's effect may be due to the relative short-term intervention and low levels of plasma resveratrol achieved (1–2 μmol/L) compared to the concentrations usually used to activate AMPK in vitro [35]. Thus, although evidence from in vitro, in vivo studies, and limited clinical trials suggests potential anti-obesity effects of resveratrol, the optimal

Figure 3 Structures of phenolic acids reported to activate AMPK pathway

doses and study period of dietary supplementation of resveratrol for clinical benefits remain to be determined.

A number of flavonoid molecules have been reported to have potential to activate the AMPK pathway, among which epigallocatechin gallate (EGCG), curcumin and quercetin were extensively studied and reviewed elsewhere [8, 51, 52] (Fig. 2). The metabolic benefits and anti-obesity effects of EGCG, curcumin, and quercetin shown in numerous cell culture and animal studies were identically related to their significant effects on adipogenesis and fatty acid oxidation [8, 51, 52, 29]. Specifically, these flavonoid polyphenols showed inhibitory effects on pre-adipocyte differentiation and adipocyte proliferation leading to adipocyte apoptosis and lipogenesis suppression meanwhile promoting lipolysis and fatty acid beta-oxidation [8]. These effects on lipid metabolism could be mediated through AMPK activation which suppressed the expression of PPAR γ and CCAAT/enhancer binding protein α (C/EBP α), two key regulators of adipocyte differentiation [51, 52]. Remarkably, EGCG was shown to activate AMPK resulting in the suppression of hepatic gluconeogenesis similarly to the action of biguanide [53] while anti-obesity effect of curcumin might be further due to its action on hepatic production of fibroblast growth factor 21 (FGF21) [54]. Moreover, the autophagy induction resulted from AMPK activation leading to reduced lipid accumulation in the liver might also contribute to beneficial effects of these flavonoids on lipid metabolism [55, 56].

AMPK activation by phenolic acids

Phenolic acids are non-flavonoid polyphenolic compounds which can be classified into two sub-groups: hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acid group includes salicylic, gallic, vanillic, and protocatechuic acids while caffeic, ferulic, p-coumaric, and sinapic acids are hydroxycinnamic acids (Fig. 3). They are found in variety of food plants, often in the bound form. Similar to the flavonoids, phenolic acids possess potent antioxidant activity and have been shown to have various bioactivities including benefits on metabolism [57]. However, their abilities to activate AMPK signaling and regulate metabolic action of phenolic acids have been just recently explored. Except salicylate (salt or ester of salicylic acid) [58], there is a very limited number of studies investigating the potential effects of phenolic acids on AMPK activation.

Salicylic acid and salicylate (its salt or ester) are some of the oldest and most widely used medications which are well-known therapeutic agents in treatment of fever, pain and inflammation. Salicylic acid and salicylate are regarded as plants' cytokine hormones produced to combat against pathogens in response to the infection [15]. The well-defined mechanisms of action of salicylates

to inhibit cyclooxygenases (COXs) [59], enzymes catalyzing the prostaglandin synthesis, and NF- κ B signaling [60, 61] are known for their therapeutic treatment as non-steroidal anti-inflammatory drugs (NSAIDs) in clinical settings. Interestingly, it has been recently shown that salicylate can directly activate the AMPK pathway [62]. The binding of salicylate to AMPK protein was quite similar to AMP molecule, yet its binding site is distinct, causing an allosteric activation of AMPK while inhibiting its Thr172 dephosphorylation. This AMPK activation were demonstrated to account for many metabolic effects of salicylate on glucose/lipid metabolism and cancers [62, 63] which cannot be explained by their ability to inhibit COXs and NF- κ B signaling. In addition, it has been suggested that the well-known protective effects of aspirin (the acetyl ester of salicylic acid) in various types of cancers could be mediated, at least in part, by AMPK activation [58].

AMPK activation can also be induced by other hydroxybenzoic acids such as gallic, vanillic, and protocatechuic acids. While there was only one in vitro study reporting AMPK activation by protocatechuic acid [64], gallic and vanillic acids were demonstrated to activate AMPK pathway both in vitro and in vivo [65, 66]. These studies also implicated that AMPK activation plays an important role in mediation of the metabolic beneficial effects of these hydroxybenzoic acids similarly to the case of salicylate [65, 67]. Thus, these findings together suggest that AMPK activation could be the primary molecular target underlying metabolic benefits of these simple phenolic molecules.

There are increasing evidence showing that other hydroxycinnamic acids such as caffeic, ferulic, and p-coumaric acids can also activate AMPK pathway to exert metabolic actions. For example, caffeic acid and its esters, in addition to target the AMPK pathway in several cancer cell lines [68, 69], were shown to activate AMPK in skeletal muscle cells to stimulate glucose uptake and exert potential antidiabetic effects on glucose and lipid metabolism [70, 71]. Similarly, ferulic and p-coumaric acids displayed ability to activate AMPK pathway to regulate metabolic actions in the muscle and adipose cell lines [72, 73]. However, these metabolic effects of hydroxycinnamic acids on AMPK activation were only observed in in vitro models and thus further in vivo validating study is needed.

CONCLUDING REMARK

Numerous phenolic compounds activating AMPK may be used as useful functional foods or adjuvant therapy designed to improve metabolic health. However, since polyphenol is a broad class of plant secondary metabolites with variable structures, characterization of phenolic molecules targeting the AMPK pathway to improve metabolic homeostasis is an important step to study the relationship between polyphenolic structures and AMPK activation. In this regard, simple phenolic acids emerge as potential lead compounds targeting the AMPK activation to develop new effective therapeutics in prevention/treatment of metabolic diseases.

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