Review

Can Food Factors Provide Us with the Similar Beneficial Effects of Physical Exercise?

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Abstract Metabolic diseases have got global health issues. Physical exercise as well as diet therapy is a potent strategy for fighting against the diseases. However, it is often difficult to continue to keep exercise regularly enough to take sufficient effect. Thus, good substitutes for the therapeutic exercise would be greatly beneficial. Recent studies have suggested that 5'AMP-activated protein kinase (AMPK) play important roles in the metabolic alterations by muscle contraction. The notion that AMPK mediates broad effects of physical exercise has been widely accepted, though it has been challenged by observations in some genetically AMPK-disrupted animals. We have demonstrated metabolome-wide significance of AMPK activation in contracting muscles. Thus, pharmacological activation of AMPK can be a promising way to obtain similar effects of the exercise. The relevance of AMPK will be introduced, and possible strategies for obtaining similar effects to the exercise from food factors will be discussed in the current review.

Keywords: phytochemicals, physical exercise, 5'AMP-activated protein kinase (AMPK), metabolome, muscle contraction

Introduction

Widespread metabolic diseases including diabetes, hyperlipidemia, obesity, and so on, have been a global issue. As such disorders are often called lifestyle-related diseases, modern sedentary life style seems to exacerbate the spread. On the other hand, physical exercise has been well known for being potent stimulator of whole body metabolism. Thus, regular physical exercise as well as diet therapy lies in the first-line therapy against most of the metabolic disorders. As for the treatment of type2 diabetes for instance, the official guidelines from diabetes associations in the United States (ADA), Europe (EASD), Canada (CDA), Japan (JSD), including IDF (International Diabetes Federation), and so on, recommend starting with the therapeutic exercise and diet (1-3). Furthermore, physical exercise might help to prevent cancer, mental diseases, among others such as the metabolic derangements.

However, it is often difficult to keep regular exercise, as you know, and it might be hard for the inpatients. Therefore, it would be of great benefit if we could gain the desirable effects of physical exercise without doing real exercise. In this review article, current overviews of molecular mechanisms mediating beneficial effects of physical exercise including our recent results will be introduced briefly, as well as possible strategies for obtaining the metabolic effects of physical

exercise from food factors will be discussed.

AMPK activation plays a key role in metabolome-wide alterations by skeletal muscle contraction

Physical exercise primarily evokes catabolic responses in contracting skeletal muscles (4-6). As for blood sugar control, glucose uptake rate is acutely facilitated in the contracting skeletal muscles, which is presumably mediated by translocation of Glut4. In addition, chronic exercise promotes insulin sensitivity as well as upregulation of Glut4. Though a series of pathways have been proposed as molecular mechanisms underlying the muscle contraction-caused Glut4 control, 5'AMP-activated protein kinase (AMPK) has been widely accepted as a key metabolic regulator in contracting skeletal muscles which mediates metabolic alterations by physical exercise; a concept known as the AMPK hypothesis. AMPK was originally isolated from rat liver as an upstream kinase of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase and acetyl-coenzyme A carboxylase (ACC) (7). Stimulation by 5-aminoimidazole-4-carboxamide 1- β -D-ribofuranoside (AICAR), AMP-analogue activating AMPK was demonstrated to promote glucose uptake in skeletal muscles in a similar manner to muscle contraction, which induces striking AMPK activation (8-10) (Fig. 1). A

Fig. 1. A scheme representing the concept of conventional AMPK hypothesis. Muscle contraction facilitates glucose uptake with Glut4 translocation as well as upregulation of Glut4. AMPK is strikingly activated in contracting skeletal muscles. AICAR, a pharmacological activator of AMPK, also evokes glucose uptake and Glut4 expression in quite similar manners to the muscle contraction. AMPK is originally hypothesized to play a key role in the glucose regulation during physical exercise according to these observations.

lot of investigations have provided with evidences suggesting that AMPK activation plays widespread roles beyond glucose uptake promotion by skeletal muscle contraction (10-12). However, several studies using genetic disruption of AMPK did not support the theory that AMPK is necessary to get the metabolic responses related to skeletal muscle contraction, though all the observations are not consistent (13-16). The relevance of AMPK remained obscure.

Consequently, we conducted a comparative study adopting a novel powerful tool, metabolomic analyses to clarify the significance of AMPK more deeply (17,18). Surprisingly enough, whole picture of changes in the metabolites determined by time-of-flight mass spectrometry combined with capillary electrophoresis (CE-TOFMS) was highly similar throughout the metabolome between AICAR stimulation and muscle contraction, which obviously demonstrates high relevance of AMPK-related metabolic effects in the muscle contraction (Fig. 2A). Sub-analyses revealed that most pathways related to glucose, nucleic and ureic acids metabolisms should be dependent on the AMPK activation while some amino acids appear to be regulated independently of AMPK (17,18). Results of principal component analysis (PCA), a popular statistical method, support the predominant significance of AMPK. The primary component (PC1) representing 63.8% of the information clearly showed the high similarity between AICAR stimulation and muscle contraction compared with the control, while the AICAR stimulation and muscle contraction were different almost only in the minor component (PC2) (Fig. 2B). In brief, the AMPK activation is demonstrated to play vast roles in metabolomic regulation in contracting skeletal muscles. Furthermore, AMPK activators such as AICAR could work as ideal mimetics of muscle contraction in terms of metabolome. It is probable that we can obtain some of the similar merits of physical exercise by taking AMPK activators.

(A) Alterations of metabolites

(B) Principal Component Analysis

Fig. 2. Metabolomic comparison between pharmacological AMPK activation and muscle contraction. Metabolomic alterations by AICAR stimulation (x-axis) and electrical muscle contraction (y-axis) in isolated rat epitrochlearis muscles of all the metabolites detected by CE-TOFMS metabolome analysis (A). Characteristics of the metabolome evaluated by principal component analysis (PCA) (B).

Molecular bases of AMPK activity control

AMPK is a ubiquitous serine/threonine kinase which consists of catalytic alpha, scaffolding beta and regulatory gamma subunits. As its name designates, it is activated in response to an increased intracellular AMP level, in other words, AMPK is an energy sensing molecule. When AMPK is activated, ATP-consuming anabolic processes are suppressed, while catabolic ones promoting ATP synthesis are facilitated, generally. Therefore, AMPK is a central metabolic regulator as well as an energy sensor.

AMP seems to bind to the gamma subunit, which is believed to stabilize the conformation of the alpha subunit which contains threonine 172 residue subject to be phosphorylated by upstream kinases such as liver kinase B1 (LKB1) and calmodulin-dependent kinase kinase beta (CaMKK β) by avoiding dephosphorylation by phosphatases like protein phosphatase 2c (PP2c) (Fig. 3) (19-26). Some other kinases including transforming growth factor-beta-activated kinase1 (Tak1) have also been suggested to phosphorylate the threonine residue, though their relevance in skeletal muscles has not been well established (27,28). The conformational alteration induces higher catalytic activity of the alpha subunit. AMPK is sensitively activated in contracting skeletal muscles predominantly due to increase in the AMP concentrations. The contraction-induced

Molecular regulation of AMPK activity

Fig. 3. Proposed molecular mechanisms regulating AMPK activity. AMPK is a heterotrimeric kinase which consists of alpha, beta and gamma subunits. AMP predominantly interacts with the gamma subunit allosterically allowing phosphorylation of threonine 172 residue of the catalytic alpha subunit, of which enzymatic activity is high.

calcium ion may contribute the activation through CaMKK β . Most of the known AMPK activators are analogues of AMP like AICAR or deplete ATP by inhibiting ATP synthesis as described below.

Can we obtain exercise-related benefits just by eating foods?

As aforementioned, AMPK activators could be ideal metabolomic exercise mimetics (17,18). Fortunately, many food factors have been known to potentially activate AMPK. We also have reported that hot water extract from *Morus alba*, namely, condensed mulberry tea evokes AMPK activation with facilitation of glucose uptake in isolated skeletal muscles, for instance (29). Furthermore, lots of molecules have been identified as potential AMPK-activating compounds (Table 1). 1-Deoxynojirimycin, a constituent rich in the mulberry leaf, was shown to activate AMPK in cultured 3T3-L1 adipocytes, while we did not find the activation in isolated skeletal muscles or cultured myocytes in our preliminary experiments (30).

Coffee is well known to be beneficial to our health. Though controversial results had been reported whether caffeine, a multipotent bioactive compound dominant in the coffee, could stimulate AMPK activation or not, caffeine is accepted as an α 1-isoform preferable AMPK activator, which is presumably involved in the metabolic action (31-33). Berberine is a typical ingredient of Chinese traditional medicine such as Huang Bo (mainly from *Phellodendron amurense*) and Huang Bai (mainly from *Coptis japonica*). Berberine has been reported to activate AMPK by inhibiting complex 1 of mitochondrial respiratory chain (34-36).

Polyphenols as well as alkaloids are popular AMPK-regulating phytochemicals as most of polyphenols are nonspecific inhibitors of mitochondrial FOF1-ATP synthase (37,38). Catechins are characteristic polyphenols of green tea. Epigallocatechin-3-gallate among the catechins harbors AMPK-activating property, and the AMPK activation by epigallocatechin-3-gallate was demonstrated in various cultured cells and tissues *in vivo* (39-42). Interestingly enough, galloyl moiety

Table 1. Typical phytochemicals reported as potential AMPK activators

Potential AMPK activating food factors
a) Alkaloids
Deoxynojirimycin
Caffeine
Berberine
b) Phenolic compounds
Flavonoids
Catechins
Quercetin
Tiliroside
Diarylheptanoids
Curcumin
Stilbens
Resveratrol
Phenol carboxylic acids
Salicylic acid
Chlorogenic acid
c) Saponins
Gypenosides

seems important for the action though diverse polyphenols are active on AMPK. Quercetin, a prevalent flavonoid with multiple healthy effects, is also known to activate AMPK possibly by down-regulation of PP2C and ATP depletion (43-45). Some pharmacological competence of quercetin including anti-obesity, diabetes, cancer, neurodegeneration, and so on, has been claimed to be at least partially due to the AMPK activation (38,43,45,46). In addition, quercetin was shown to activate another energy sensing metabolic regulator, sirt1. Resveratrol, a red wine polyphenol, as well as quercetin was reported as a dual activator of AMPK and sirt1, of which actual molecular mechanisms remain obscure and under discussion (45,47-53).

There are plenty of food factors harboring AMPK-stimulating potency in addition to the above mentioned chemicals. We believe that we can gain some of the beneficial metabolic effects of physical exercise at least partly by taking such AMPK-activating compounds without performing exercise as described before. However, some problems remain unsolved. ADME (absorption, distribution, metabolism, and excretion, which characterizes the property of chemicals *in vivo*) should be an important issue. It remains unknown whether these dietary chemicals could be properly distributed to skeletal muscles in order to activate AMPK, although AMPK activation will improve our health in most tissues generally. Many phytochemicals, especially, polyphenols are too hydrophobic to be absorbed into the body enough. We have been developing a novel technology for improving water-solubility of the hydrophobic molecules by conjugating it with branched glycerol oligomers (BGLs) (11,54-57). Pharmacological, pharmacokinetic, and physical properties of such highly hydrophobic compound as fenofibrate were strikingly ameliorated when conjugated with the BGL trimer without any adverse effects. Hydrophilic prodrug like BGL should endow such hydrophobic phytochemicals like polyphenols with more hydro-

philicity, and this will enhance higher absorption.

This kind of food factors might be used as lead compounds in case of drug discovery. Superior compounds have been developed based on resveratrol in terms of sirt1 activators for instance, although the genuineness seems to remain under debate.

In conclusion, our recent metabolomic analysis clearly demonstrated that pharmacological activation of AMPK can evoke vast metabolic beneficial effects similar to physical exercise. There are lots of food factors that had been demonstrated to activate AMPK, and we should be able to obtain some of the benefits just by taking meals containing such compounds. However, further studies will be required to reveal what kind of foods or food factors can provide us with more beneficial effects like physical exercise, which must enrich our quality of lives.

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