

Exercise activates AMPK signaling: Impact on glucose uptake in the skeletal muscle in aging

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Abstract

Aging is characterized by a decline in metabolism and functional capacities. On the other hand, physical exercise is a well-known strategy that provides metabolic and functional benefits. Since elderly subjects have insulin resistance and issues with carbohydrate metabolism, physical exercise can contribute to glucose homeostasis through its participation in skeletal muscle glucose uptake via insulin-dependent/insulin-independent mechanisms. Therefore, it is important to understand the effects of physical exercise on 5' AMP-activated protein kinase (AMPK) signaling in skeletal muscle and on positive metabolic adaptations and the prevention of age-related diseases. In this mini-review, we will discuss the participation of AMPK in controlling skeletal muscle glucose uptake in response to physical exercise and aging.

Introduction

The aging process has been widely studied from different perspectives in the scientific community. Aging is associated with the appearance of innumerable diseases and co-morbidities, especially in those with a lifestyle involving poor quality food and physical inactivity. One of the common causes of some of these age-related comorbidities is impaired insulin sensitivity and the downregulation of skeletal muscle glucose uptake, which contributes to impairments in whole-body glucose homeostasis¹. Since skeletal muscle is responsible for up to 70–90% of blood glucose uptake in the post-prandial state, the effects of aging and insulin resistance in this tissue may affect its functionality and metabolic capacity, leading to negative effects on glucose homeostasis^{2,3}.

Physical exercise is very well described for its role in the upregulation of skeletal muscle insulin sensitivity and glucose uptake, through mechanisms that are dependent and independent of insulin⁴. Regarding the insulin-independent glucose uptake mechanisms, AMPK has shown important participation in the skeletal muscle glucose uptake independently of insulin, being widely studied because due to its varied benefic effects on metabolism. Previous studies have shown the role of AMPK in the stimulation of skeletal muscle glucose uptake in the absence of insulin, and also in promoting fatty acid oxidation and mitochondrial biogenesis⁵⁻⁷. AMPK activity is also associated with autophagy activation and inhibition of mechanistic target of rapamycin (mTOR), a protein that when upregulated is involved with age-related comorbidities⁸. The benefic effects of exercise-induced AMPK activation in the skeletal muscle in the condition of advanced age will be reviewed in this mini-review⁹.

AMPK actions in metabolism

AMPK is regulated by the levels of adenosine monophosphate (AMP) and adenosine triphosphate (ATP) inside the cell, acting as an energy sensor. The heterotrimeric structure of this protein is composed of the α catalytic subunit, and the regulatory β and γ subunits. Although some studies suggest that the $\alpha 2$ catalytic subunit is essential to skeletal muscle glucose uptake, some other studies suggest that the regulatory subunits ($\beta 2$ and $\gamma 3$) are necessary to stimulate glucose uptake in response to AMPK activators¹⁰. Since AMPK acts as a crucial cellular energy sensor, the activation of catabolic mechanisms (glucose uptake/glycolysis, lipolysis, autophagy, mitophagy) and inactivation of anabolic mechanisms (lipogenesis, glycogenolysis, gluconeogenesis, protein synthesis) are involved^{11,12}.

In skeletal muscle, the glucose uptake in response to AMPK activation occurs independently of insulin to stimulate the glucose transporter 4 (GLUT4) vesicle trafficking to the cellular membrane. The mechanism involved in this process is tre-2/USP6, BUB2, cdc16 domain family member 1 (TBC1D1) and Akt substrate of 160 kDa (AS160, also known as TBC1D4) phosphorylation by AMPK, which activate Rab GTPases and stimulate the translocation of GLUT4 vesicles to the membrane^{13,14}. However, a recent study showed the role of AMPK in the regulation of insulin-stimulated glucose uptake¹⁵. The authors deleted the protein kinase B (Akt2) in the skeletal muscle of mice and observed no changes in the lean mass and skeletal muscle insulin sensitivity. The deletion of Akt1/2 led to decreased lean mass, but normal insulin-stimulated glucose uptake in the skeletal muscle. Moreover, the chronic Akt ablation induced AMPK activity in response to energetic stress, regulating the skeletal muscle insulin sensitivity. This model was confirmed with an experiment where insulin stimulated the *in vivo* and *ex vivo* glucose uptake in Akt1/2 knockout mice; however, the additional treatment with compound C (an AMPK inhibitor) blunted the insulin-stimulated glucose uptake¹⁵. Thus, the connection between AMPK and the insulin signaling pathway in skeletal muscle should be investigated in future studies to answer some of the remaining questions.

Besides the role of AMPK in GLUT4 vesicle translocation to the cellular membrane, AMPK induces mRNA expression for GLUT4 and hexokinase 2 (HK2), an essential enzyme that mediates the glycolysis flux¹⁶. In addition, AMPK inhibits glycogen synthesis through glycogen synthase (GS) inhibition¹⁷. Moreover, treatment with activator 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) (a pharmacological AMPK activator) increases glycogen synthase kinase (GSK) phosphorylation, facilitating glycogenolysis in the skeletal muscle¹⁸.

Fatty acid oxidation is one of the pathways regulated by AMPK activity. When activated, AMPK phosphorylates acetyl-CoA carboxylase (ACC), a protein that controls the conversion of acetyl-CoA to malonyl-CoA¹⁹. This conversion is involved in fatty acid synthesis; however, this shifting mediated by ACC phosphorylation favors fatty acid oxidation to provide substrate availability to myocytes²⁰. Therefore, it is essential to highlight the importance of AMPK in this pathway. The effect of AMPK in increasing the oxidative capacity of skeletal muscle appears to occur through transcriptional regulation. The increase in the content of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α) and other transcription factors are involved in the process of fat oxidation through AMPK in muscle tissue²¹.

The chronic activation of mammalian target of rapamycin (mTOR) is related to insulin resistance, tissue degeneration, and tumor development²². AMPK plays an important role in mTOR inhibition in response to energy depletion, through its association with liver kinase B1 (LKB1) and the consequent phosphorylation of tuberous sclerosis complex 2 (TSC2), a protein that inhibits mTOR activity²². At the same time, AMPK mediates important steps of autophagy activation through unc-51 like autophagy activating kinase 1 (ULK1) phosphorylation. In a fed state, mTOR complex phosphorylates ULK1 in inhibitory sites, blocking autophagy. On the other hand, under starvation conditions, these inhibitory sites in ULK1 are dephosphorylated by mTOR complex. ULK1 is also a target of AMPK, showing positive regulation of autophagy by ULK1 phosphorylation. Thus, AMPK indirectly activates autophagy by regulating mTOR inhibition, but also by direct phosphorylation of ULK1 in mammals²³. In this regard, activation of AMPK in skeletal muscle through exercise can be an efficient strategy for achieving metabolic health and preventing disease.

Role of physical exercise in AMPK activity

The importance of physical exercise throughout life to provide a healthier elderly life is very well described²⁴. One of the most well-known metabolic responses to physical exercise is the upregulation of skeletal muscle glucose uptake through direct mechanisms that improve insulin sensitivity and insulin-independent mechanisms that also contribute to glucose homeostasis²⁵. In addition, the enhanced energy expenditure promoted by physical exercise reduces intracellular ATP levels and increases AMP levels, inducing AMPK phosphorylation/activation and stimulating skeletal muscle glucose uptake²⁶.

As mentioned before, AMPK is composed of trimeric complexes (α , β , γ) that are differently influenced by exercise duration and intensity⁹. The $\alpha 1\beta 2\gamma 1$ and $\alpha 2\beta 2\gamma 1$ complexes are activated under moderate intensity exercise

with 1 hour of duration²⁷. On the other hand, the $\alpha 2\beta 2\gamma 3$ complex is activated by intense exercise with 20 minutes of duration⁹. The $\alpha 2$ subunit is also phosphorylated during prolonged exercise, but is not sufficient to maintain ACC phosphorylation in the skeletal muscle of humans²⁸. Well-trained individuals showed increased AMPK phosphorylation after physical exercise; however, the stimulus was different from that of the prior training modality²⁹. In this study, Coffey et al. investigated strength- and endurance-trained individuals and showed that AMPK phosphorylation increased after cycling in the strength-trained group, but not in the endurance-trained group. When the endurance-trained individuals were subjected to resistance exercise, AMPK phosphorylation was increased, something that did not happen in the resistance-trained group²⁹. Finally, the AMPK activity in response to physical exercise depends of the AMP levels independently of the exercise duration and intensity; however, increases in AMP levels are probably higher when the physical exercise is performed until exhaustion³⁰.

Besides the acute effects of physical exercise, chronic physical exercise also induces AMPK activity and PGC-1 α , contributing to mitochondrial biogenesis, energy homeostasis, thermoregulation, and glucose metabolism³¹. In addition, AMPK stimulates the peroxisome proliferator-activated receptor α PPAR α mRNA expression, leading to molecular modulations that favor fatty acid oxidation in skeletal muscle³¹. Therefore, it is of great importance for the elderly to engage in a regular exercise program in order to maintain health and prevent disease. In addition, at older ages, there is usually a decrease in insulin secretion, muscle atrophy, and hemodynamic changes, all of which have implications for the glucose uptake process through insulin signaling³². Therefore, AMPK activation and exercise-mediated glucose uptake is of great importance in aging. This suggests that the elderly should exercise more frequently, in order to promote the activation of AMPK and obtain the benefits associated with this signaling pathway and glucose uptake independent of insulin.

The role of physical exercise in promoting AMPK activation also results in autophagy stimulation. A family of proteins called sestrins are responsive to stress, and may be one mechanism that mediates the effects of physical exercise on AMPK phosphorylation, and consequently mTOR inhibition and autophagy stimulation³³. The adequate coordination of these mechanisms by AMPK should slow down some aspects of aging such as tissue degeneration and tumor growth in advanced ages³⁴. Another mechanism involved in AMPK activation by physical exercise is the increase in intracellular calcium (Ca²⁺) content through membrane depolarization, inducing the calcium/calmodulin-dependent protein kinase kinase (CaMKK) phosphorylation, which can phosphorylate and

activate AMPK^{35,36}. Additional information regarding the role of Ca²⁺ in skeletal muscle glucose uptake was reviewed by Jensen and co-workers³⁷. Moreover, endoplasmic reticulum stress in response to skeletal muscle contraction increases reactive oxygen species (ROS), which leads to CaMKK activation^{30,38,39}. The increase in muscle temperature may also play a role in AMPK activation during physical exercise⁴⁰. In summary, there is different evidence regarding the effects of physical exercise on AMPK activation through different mechanisms.

Effects of physical exercise and AMPK during aging

It has been shown that the activation of AMPK through exercise can promote metabolic health with advancing age. Previous animal studies have shown that physical exercise stimulates AMPK, as well as skeletal muscle glucose uptake. Wang and colleagues (2016) observed that older (30-month old) rats showed increased skeletal muscle AMPK phosphorylation in response to acute swimming exercise combined with caloric restriction when compared to control animals that performed only caloric restriction or at ad libitum⁴¹. Wang et al, also observed increased skeletal muscle glucose uptake in the groups that performed caloric restriction and physical exercise combined with caloric restriction compared to control groups⁴¹. These findings suggest that physical exercise can potentiate the effects of caloric restriction on AMPK phosphorylation; however, the same pattern of skeletal muscle glucose uptake observed in the sedentary and exercised groups with caloric restriction could be addressed by the single session of exercise, and not a chronic intervention. The study of Coqueiro et al., observed the effects of preventive and therapeutic aerobic exercise on the soleus and gastrocnemius muscles and cardiometabolic outcomes. It was observed that increases in AMPK gene levels in the soleus muscle, but not in gastrocnemius, of the preventive exercise group was associated with improved cardiometabolic health and glucose homeostasis. This study highlight the importance of long-term exercise in blood glucose homeostasis and longevity⁴². On the other hand, the therapeutic exercise improved glucose tolerance; however, it was not able to modulate AMPK and improve insulin sensitivity in the case of obesity and aging⁴². Moreover, the researchers found higher AMPK levels in the soleus muscle oxidative fibers than other skeletal muscle tissue⁴².

In the study of Mortensen et al, it was evaluated the mRNA and protein levels of AMPK in the skeletal muscle of 100 young and 82 older mono- and dizygotic nondiabetic twins⁴³. Firstly, the authors observed similar mRNA and proteins levels of AMPK between the twin samples, indicating minor genetic influence on skeletal muscle AMPK levels. There was a positive correlation AMPK $\gamma 3$ mRNA and protein expression, and between AMPK $\gamma 3$ protein and AMPK $\gamma 3$ activity. The AMPK $\gamma 3$ protein levels showed a

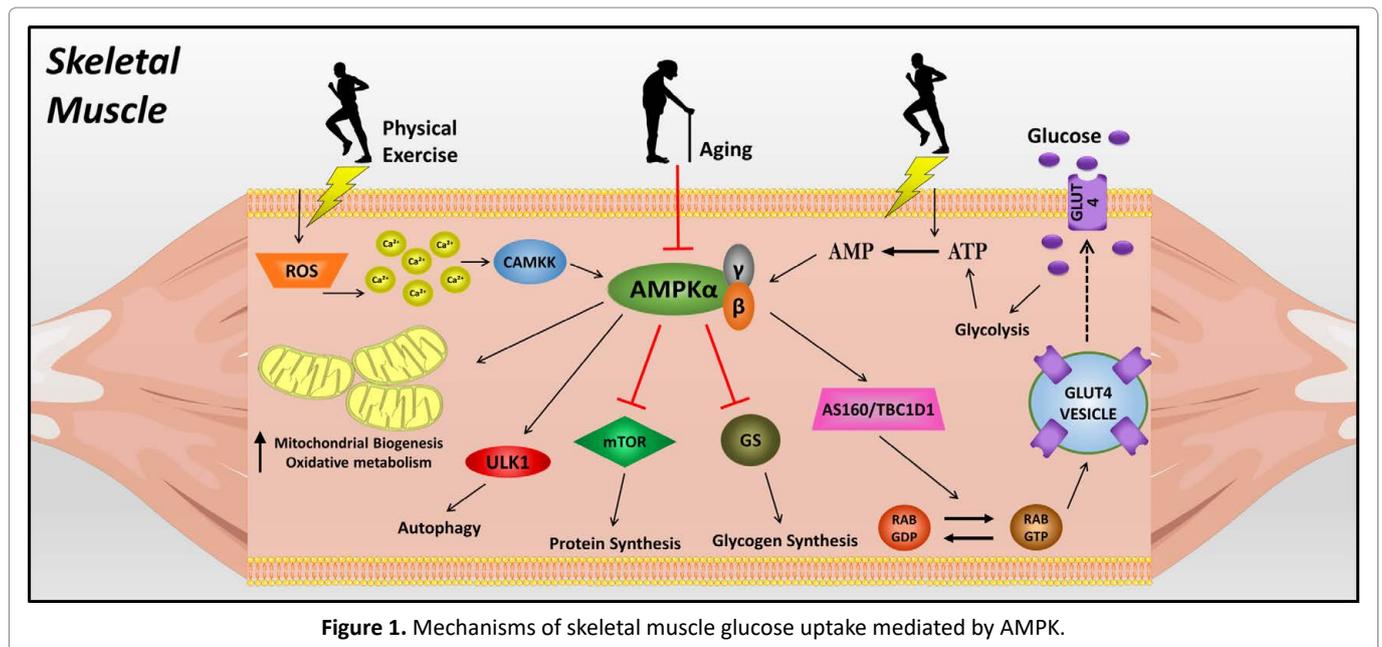


Figure 1. Mechanisms of skeletal muscle glucose uptake mediated by AMPK.

negative correlation with MHC type 1 mRNA levels, and a positive correlation with type 2 MHC type 2a and 2x mRNA levels. In the older twin, there was lower levels of AMPK γ 3 mRNA, protein and activity compared to young individuals, but not in the other AMPK subunits (α 1 and α 2). It was also observed negative correlation between AMPK γ 3 and glycogen content⁴³. This study highlights the importance of environmental factors on AMPK activity, such as age, sex and aerobic capacity.

Aging-related sarcopenia is characterized by a loss of muscle mass and its functionality. There is strong evidence that muscle mass in elderly subjects has a negative correlation with mortality^{44,45}. In addition, is important to highlight the association between lower muscle mass and impaired glucose metabolism in humans⁴⁶. Thus, physical exercise is the most effective way to prevent this muscle loss⁴⁴. A study using 19-month old mice showed that AMPK activation in response to physical exercise (4 weeks of treadmill exercise) reduced phenotypes of cellular senescence, by decreasing senescence markers (p16 and p21) compared to sedentary mice⁴⁷. Moreover, an interesting finding of this study was that physical exercise increased AMPK phosphorylation in the skeletal muscle of young mice; however, the old exercised group showed only a minor increase in AMPK phosphorylation compared to the old sedentary group⁴⁷. This result may suggest an impaired AMPK response to physical exercise in aged mice.

Although the studies discussed here focus on the effects of physical exercise and AMPK activity in the control of skeletal muscle glucose uptake, there is a correlation with AMPK and positive outcomes in other metabolic tissues such as liver, adipose tissue, endothelium, and

hippocampus⁴⁸⁻⁵⁰. In addition, AMPK is also responsive to physical exercise interventions in these tissues⁴⁸⁻⁵⁰.

Therefore, the AMPK pathway is an important mediator of glucose homeostasis through its positive role in skeletal muscle glucose uptake, affecting insulin-dependent/insulin-independent mechanisms (Figure 1). Moreover, AMPK contributes to the benefic effects of physical exercise on whole-body glucose homeostasis. In the aging process, this relationship between exercise and AMPK is extremely positive for metabolic health and the prevention of age-related diseases. Finally, the known effects of physical exercise as a therapeutic strategy to combat obesity, type 2 diabetes, metabolic syndrome, and cardiovascular diseases, may be due to some of the molecular effects of AMPK described here. The current knowledge indicates that moderate and high intensity exercises can activate AMPK in relation to rest and low intensity exercise conditions; however, further studies are needed to investigate which type of exercise is capable of being more efficient in activating AMPK in the elderly. Future studies investigating older population groups and different types and characteristics of exercise will provide more information about AMPK activity in skeletal muscle, and its contribution to whole-body glucose homeostasis.

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