



The role of 5'-adenosine monophosphate-activated protein kinase (AMPK) in skeletal muscle atrophy

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Abstract: As a key coordinator of metabolism, AMP-activated protein kinase (AMPK) is vitally involved in skeletal muscle maintenance. AMPK exerts its cellular effects through its function as a serine/threonine protein kinase by regulating many downstream targets and plays important roles in the development and growth of skeletal muscle. AMPK is activated by phosphorylation and exerts its function as a kinase in many processes, including synthesis and degradation of proteins, mitochondrial biogenesis, glucose uptake, and fatty acid and cholesterol metabolism. Skeletal muscle atrophy is a result of various diseases or disorders and is characterized by a decrease in muscle mass. The pathogenesis and therapeutic strategies of skeletal muscle atrophy are still under investigation. In this review, we discuss the role of AMPK in skeletal muscle metabolism and atrophy. We also discuss targeting AMPK for skeletal muscle treatment, including exercise, AMPK activators including 5-amino-4-imidazolecarboxamide ribonucleoside and metformin, and low-level lasers. These studies show the important roles of AMPK in regulating muscle metabolism and function; thus, the treatment of skeletal muscle atrophy needs to take into account the roles of AMPK.

Introduction

As a central coordinator of energy homeostasis, 5'-adenosine monophosphate (AMP)-activated protein kinase (AMPK) is the cellular sensor of energy and coordinates metabolism, especially of glucose and fatty acids. AMPK responds to conditions of low energy in cells, such as nutrient depletion, hypoxia, and the inhibition of mitochondrial respiratory chain complex, and regulates the synthesis and degradation of proteins (Herzig and Shaw, 2018). AMPK has been shown to play a key role in regulating muscle metabolism (Merrill *et al.*, 1997; Winder and Hardie, 1996). Skeletal muscle atrophy is a common muscle disease attributed to muscular inactivity, caused by immobilization, denervation, unloading, aging, and starvation (Rudrappa *et al.*, 2016). The main pathological characteristics of skeletal muscle atrophy are a decrease in protein contents, fiber, and force and providing resistance to fatigue. The molecular aspects of muscle atrophy have

been well studied for over 20 years, and AMPK has been shown to play a crucial role in skeletal muscle atrophy (Thomson, 2018).

In the present review, we discuss the role of AMPK in skeletal muscle and the involved cellular processes, including protein synthesis and degradation, and autophagy. Furthermore, we also discuss the treatment of skeletal muscle atrophy through the modulation of AMPK.

5'-Adenosine Monophosphate-Activated Protein Kinase

AMPK, a serine/threonine protein kinase, is a key enzyme in coordinating cellular energy metabolism. AMPK mainly activates the uptake and oxidation of glucose and fat in conditions of energy deficiency in cells. It is a member of a eukaryotic protein family that is highly conserved from yeast to humans, and the AMPK protein consists of three subunits required for enzyme function. AMPK is widely expressed in organs, including the brain, liver, and skeletal muscle. AMPK activation is initiated upon binding ADP and AMP and leads to the stimulation of glucose uptake and fatty acid oxidation, the inhibition of cholesterol synthesis, lipogenesis and lipolysis, and the regulation of insulin secretion (Herzig and Shaw, 2018).

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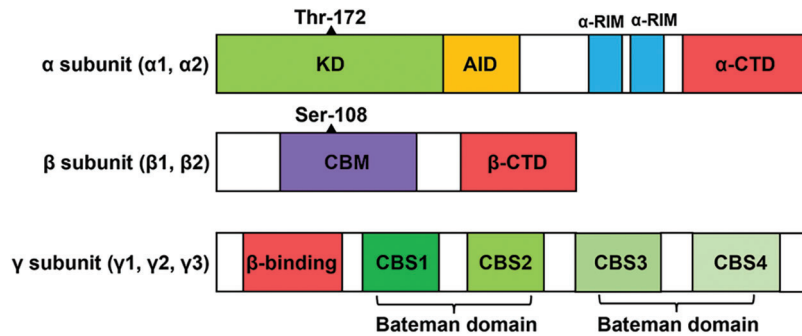


FIGURE 1. The structure of 5'-adenosine monophosphate-activated protein kinase (AMPK). AMPK consists of α , β , and γ subunits. The α subunit is the catalytic subunit. The kinase domain contains the predominant regulatory site, Thr172. The α subunit also has the autoinhibitory domain, two α -regulatory subunit-interacting motif-2 domains, and a C terminal domain (α -CTD). The β subunit is the scaffolding subunit; it contains an internal carbohydrate-binding motif (CBM) and a conserved C terminal domain (β -CTD). The Ser108 in the CBM is important for the effect of some AMPK activators. The γ subunit is the regulatory subunit, comprising four domains called cystathionine β -synthase domains, two of which are called Bateman domains. N terminal domains in the γ subunit are responsible for the binding of the β subunit.

The structure of 5'-adenosine monophosphate-activated protein kinase

AMPK is a heterotrimeric protein complex consisting of α , β , and γ subunits. Each subunit has a specific role in the stability and activity of AMPK (Kim *et al.*, 2016) (Fig. 1). The α subunit ($\alpha 1$ and $\alpha 2$ isoforms, encoded by *PRKAA1* and *PRKAA2* genes, respectively) is the catalytic subunit. Its kinase domain (KD) contains the Thr172 regulatory site, which is phosphorylated by AMPK kinase, which is required for the functioning of AMPK (Ross *et al.*, 2016). The α subunit also has the autoinhibitory domain (AID), two α -regulatory subunit-interacting motif-2 domains that trigger a conformational change when AMP binds to the AMPK γ subunit, and a C terminal domain (α -CTD) that binds to the β subunit.

The β subunit of AMPK ($\beta 1$ and $\beta 2$ isoforms, encoded by *PRKAB1* and *PRKAB2* genes, respectively) is the scaffolding subunit. It contains an internal carbohydrate-binding motif (CBM) and a conserved CTD (β -CTD). Ser108 in the CBM is necessary for the effect of some AMPK activators (Kim *et al.*, 2016). The α subunit-binding site and the γ subunit-binding site of the β subunit are both located in β -CTD.

The γ subunit ($\gamma 1$, $\gamma 2$, and $\gamma 3$, encoded by *PRKAG1*, *PRKAG2*, and *PRKAG3* genes, respectively) is the regulatory subunit and contains four domains called cystathionine β -synthase domains, which are four binding sites for ATP, ADP or AMP. Two of them are called Bateman domains, which are sites for AMP binding, making AMPK sensitive to the change in the AMP:ATP ratio (Ross *et al.*, 2016). The binding of AMP and one Bateman domain can increase the affinity of AMP to the other Bateman domain. When both Bateman domains are bound with AMP, the conformation of the γ subunit changes, thus exposing the catalytic domain in the α subunit. The N terminal domain in the γ subunit binds the β subunit.

Among the isoforms of α , β , and γ subunits, $\alpha 1$, $\beta 1$, and $\gamma 1$ are the most common in cells. Various combinations of the different isoforms of these three subunits result in twelve AMPK configurations. However, only $\alpha 2/\beta 2/\gamma 1$, $\alpha 2/\beta 2/\gamma 3$, and $\alpha 1/\beta 2/\gamma 1$ configurations are observed in human skeletal muscle (Birk and Wojtaszewski, 2006). In mouse muscle, only $\alpha 1/\beta 1/\gamma 1$ and $\alpha 2/\beta 1/\gamma 1$ are detected. The functional

implications of different configurations are not yet clear, and a more exhaustive understanding of the functions of these trimers in different tissues and muscles is needed.

The regulation of 5'-adenosine monophosphate-activated protein kinase

AMPK is regulated structurally and by post-translational modification (Jeon, 2016). Phosphorylation of Thr172 in the AMPK α subunit is the main mechanism of AMPK activation. Blockage of the phosphatase access to Thr172 can increase AMPK activation. AMPK can be allosterically regulated by the competitive binding of its γ subunit to ATP and AMP or ADP. ATP allows the access of phosphatase to Thr172, while AMP or ADP blocks this access. Thus, AMPK is a sensitive sensor of AMP/ATP or ADP/ATP ratios, considered indicators of cell energy level (Hardie *et al.*, 2012). The molecular mechanism of the allosteric activation of AMPK by AMP binding has been demonstrated earlier (Steinberg and Carling, 2019). The autoinhibitory domain in the AMPK α subunit interacts with the KD, leading to the inactive conformation of AMPK. Upon the binding of AMP to the AMPK γ subunit, the α -regulatory subunit-interacting motif-2 and α -CTD interact with the adenosine nucleotides, resulting in conformation changes that expose the KD to activate the AMPK subunit complex.

Many kinases and related proteins are involved in the phosphorylation and dephosphorylation of Thr172 in AMPK. The kinases that phosphorylate Thr172 include liver kinase B1 (LKB1), Ca^{2+} /calmodulin-dependent protein kinase kinase 2 (CAMKK2), and TGF- β -activated kinase 1 (TAK1) (Neumann, 2018). Protein phosphatase 2A, protein phosphatase 2C, and $\text{Mg}^{2+}/\text{Mn}^{2+}$ -dependent protein phosphatase 1E are the main phosphatases that dephosphorylate Thr172 (Yan *et al.*, 2021). LKB1-mediated AMPK phosphorylation at Thr172 can be enhanced by AMP binding to the AMPK γ subunit (Kim *et al.*, 2016). However, CAMKK2 responds to the increase in cellular Ca^{2+} and activates AMPK without influencing the ATP/ADP/AMP levels (Mairet-Coello *et al.*, 2013). Furthermore, myristoylation of the AMPK β subunit N terminal is also involved in the effect of AMP on Thr172 phosphorylation (Ali *et al.*, 2016).

AMPK is inhibited by insulin, leptin, and diacylglycerol (Jeon, 2016). AMPK also controls gene expression through metabolites of intermediary metabolisms, such as glucose, fatty acids, and ketogenic amino acids (Sukumaran *et al.*, 2020). The interaction of AMPK and glycogen alters the conformation and activity of AMPK (Janzen *et al.*, 2018). LKB1, CAMKK, and TAK1 are important kinases for AMPK activation in skeletal muscle, and LKB1 is regarded as the primary AMPK kinase (Thomson, 2018).

The physiological function of 5'-adenosine monophosphate-activated protein kinase

In response to metabolic stress, AMPK plays a pivotal role in maintaining anabolic and catabolic balance, contributing to glucose/lipid homeostasis (Trefts and Shaw, 2021). AMPK phosphorylates acetyl-CoA carboxylase 1 (ACC1) and sterol regulatory element-binding protein 1c (SREBP1c), thus inhibiting the synthesis of fatty acids and cholesterol and activating the uptake and oxidation of fatty acids (Jeon, 2016). AMPK also phosphorylates Rab-GTPase-activating proteins TBC1D1 and TBC1D4, thus inducing the fusion of glucose transporter 1 to the plasma membrane and stimulating glucose uptake (Sakamoto and Holman, 2008; Stockli *et al.*, 2008). AMPK stimulates glycolysis by activating the phosphorylation of 6-phosphofructo-2-kinase and glycogen phosphorylase. AMPK inhibits glycogen synthesis via phosphorylation of glycogen synthase (Jeon, 2016). AMPK also inhibits transcription factors such as hepatocyte nuclear factor 4 and cAMP response element-binding (CREB) regulated transcription coactivator 2 and also inhibits gluconeogenesis in the liver (Lee *et al.*, 2010). AMPK regulates the energy-related protein biosynthesis process through phosphorylating tuberous sclerosis complex 2 (TSC2). Upon TSC2 activation, TSC then inhibits the mechanistic target of rapamycin complex 1 (mTORC1), resulting in a decrease in protein synthesis. In energy-deficient conditions in cells, AMPK is activated; all energy-consuming pathways, such as protein synthesis are halted, and energy-producing pathways are activated (Hardie, 2011). AMPK activates autophagy by activating Unc-51 like autophagy activating kinase 1 (ULK1) directly and indirectly (Mao and Klionsky, 2011) and regulates mitochondrial biogenesis via peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1 alpha (PGC-1 α), which can promote gene transcription in mitochondria (Canto and Auwerx, 2009).

AMPK is a therapeutic target in the treatment of metabolic diseases such as diabetes (Day *et al.*, 2017) and also plays a role in tumorigenesis. The upstream regulatory kinase LKB is also a tumor suppressor (Chuang *et al.*, 2014), and AMPK controls mTORC1 and TIF-1A, which are involved in cell proliferation (Sukumaran *et al.*, 2020). Given its protective role in tumor cells in conditions of limited nutrients, hypoxia, and/or toxicity, AMPK activation should be considered for cancer treatment.

Skeletal Muscle Atrophy

Skeletal muscle atrophy is the loss of skeletal muscle mass, caused by muscle disuse, malnutrition, medications, aging,

injuries, or nervous system diseases, leading to muscle weakness and disability (Powers *et al.*, 2016). Muscle disuse can lead to muscle atrophy, which often occurs after motion limitation of limb or bed-rest during related illness. Skeletal muscle atrophy induced by disuse can be fully recovered with activity if the illness is cured. Malnutrition and cachexia caused by an underlying disease can also progress to skeletal muscle atrophy, which can be reversed either completely or incompletely by nutritional therapy. Age-related skeletal muscle atrophy can be slowed down by exercise but not completely reversed. Muscle diseases (such as muscular dystrophy) and nervous system diseases (such as stroke) can result in skeletal muscle atrophy, and recovery depends on the duration and severity of the underlying diseases along with the health of the patient. The predominant symptom of skeletal muscle atrophy is increasing weakness, leading to difficulty or inability in physical tasks. Skeletal muscle atrophy can be undetected until significant loss of the muscle occurs, and symptoms are different depending on the affected muscles.

Pathologically, skeletal muscle atrophy results from the imbalance between the synthesis and degradation of the protein. The mechanisms of muscle atrophy are not yet completely understood and vary depending on the underlying reason. Skeletal muscle is a storage center for amino acids that are used for producing energy when the energy supplies are low, or energy demands are high. When the metabolic level is greater than that of protein synthesis, the loss of muscle mass will occur (Atherton and Smith, 2012). Mitochondria are vital for skeletal muscle health, and harmful changes in mitochondria may lead to skeletal muscle atrophy. Decreased density and quality of mitochondria are commonly observed in skeletal muscle atrophy when skeletal muscle is disused (Harper *et al.*, 2021). ATP-dependent protein degradation pathway is also one of the main mechanisms for protein metabolism in muscles, and any change in this pathway might lead to muscle atrophy.

Treatment approaches for skeletal muscle atrophy include enhancing the pathways leading to muscle hypertrophy or reducing muscle breakdown. Physical exercise, especially resistance, is one of the crucial approaches to slow or reverse skeletal muscle atrophy (Lavin *et al.*, 2019). In individuals unable to exercise due to immobilization, such as spinal cord injury, electrical stimulation of muscle can be used (Ho *et al.*, 2014). Calories and protein supplements are also beneficial for skeletal muscles, especially for patients with malnutrition. Protein or amino acids, such as leucine, can stimulate protein synthesis in muscles and inhibit protein degradation and are, therefore, beneficial for some cases of skeletal muscle atrophy, such as cachexia (Argiles *et al.*, 2016). A metabolite of leucine, β -hydroxy β -methylbutyrate (HMB), is effective in preventing muscle loss in patients with sarcopenia and is used as a dietary supplement (Cavallucci and Pani, 2021). HMB is also used to prevent muscle loss in the elderly. However, the detailed effect of HMB needs more investigation. Furthermore, the administration of anabolic steroids and selective androgen receptor modulators to patients with skeletal muscle atrophy are also being

evaluated. Their effectiveness in promoting muscle growth and regeneration, as well as a few side effects, requires further investigation before clinical application.

The Role of 5'-Adenosine Monophosphate-Activated Protein Kinase in Skeletal Muscle Atrophy

Since skeletal muscle atrophy occurs due to the imbalance between protein synthesis and degradation and AMPK-involved pathways play important roles in protein synthesis and degradation, the role of AMPK-related mechanisms in the pathogenesis of skeletal muscle atrophy is important.

5'-adenosine monophosphate-activated protein kinase inhibits muscle protein synthesis

Skeletal muscles are the largest reservoir of proteins and amino acids in the body and are important for locomotion (Argiles et al., 2016). Muscle mass maintenance is crucial for life quality. Loss of muscle mass is the typical feature of skeletal muscle atrophy. Understanding the mechanisms of skeletal muscle atrophy, especially protein synthesis and degradation in muscle, is important for muscle rehabilitation.

The most well-recognized player in regulating muscle mass in skeletal muscle is mTOR (Yoon, 2017). Like AMPK, mTOR is also a serine/threonine kinase; it can sense intracellular and extracellular changes in nutrient and energy levels and regulates various processes in cells, such as cell growth and differentiation, autophagy, metabolism, and survival. mTORC1 and mTORC2 are two mTOR complexes with distinct functions (Grahammer et al., 2021). mTORC1 is composed of the regulatory-associated protein of mTOR (RAPTOR), the mammalian lethal with SEC13 protein 8 (mLST8), a 40 kDa proline-rich Akt substrate, and the DEP domain-containing mTOR-interacting protein (DEPTOR). mTORC2 is composed of mLST8, DEPTOR, the mammalian stress-activated map kinase-interacting protein 1, the rapamycin-insensitive companion of mTOR (RICTOR), and a protein with RICTOR 1 and 2 (PROTOR1/2). mTORC1 is sensitive to rapamycin and it is the key to regulating muscle mass in muscle contraction and loading. mTORC1 responds to cellular signals, including intracellular energy status, oxygen levels, growth factors, and amino acid availability. When activated, mTORC1 stimulates protein synthesis by phosphorylating its downstream targets, including S6 kinase 1, and inhibiting 4E-binding protein 1, promoting cell growth.

AMPK inhibits muscle protein synthesis by regulating the mTORC1 pathway. It can inhibit mTORC1 activity via the following mechanisms. a. AMPK phosphorylates mTOR at Thr2446, which can halt mTORC1 activity by preventing its phosphorylation at Ser2448; however, the impact of Ser2448 phosphorylation on mTORC1 activity has not been determined (Melick and Jewell, 2020). b. AMPK can also phosphorylate TSC2 to inhibit mTORC1 activity (Kim and Lee, 2015); TSCs can activate GTPase, which can convert GTP to GDP and can form a complex with TSC1 and TBC1D7, reducing the ability of a GTPase, Rheb, whose binding with GTP is needed for mTORC1 activity. c. AMPK

can phosphorylate RAPTOR, leading to impaired mTORC1 activity mediated by 14-3-3 proteins (Thomson, 2018).

In 2002, Bolster et al. (2002) reported that the injection of 5-amino-4-imidazolecarboxamide ribonucleoside (AICAR), an AMPK activating drug, can reduce the rate of protein synthesis in skeletal muscle; this finding was considered the first evidence of the role of AMPK in protein metabolism in skeletal muscle. Since then, the negative effect of AMPK on protein synthesis has been confirmed in many other cell types, including hepatocytes (Dubbelhuis and Meijer, 2002; Reiter et al., 2005), myocytes (Chan et al., 2004), and tumor cells (Fay et al., 2009; Xiang et al., 2004). In addition to the inhibitory effect on mTORC1, AMPK can also regulate protein synthesis by inhibiting the activity of eukaryotic elongation factor 2 (eEF2) (Yamada et al., 2019). AMPK can directly phosphorylate and activate eEF2 kinase (eEF2K), which can phosphorylate eEF2 at Thr56. The phosphorylation of eEF2 leads to eEF2 inactivation, indicated as the inhibition of its binding to the ribosome, slowing down the elongation rate and the subsequent protein synthesis. AMPK also prevents the inhibitory effect of S6k, which can phosphorylate and inhibit eEF2K and lead to eEF2 activation by inhibiting the mTOR pathway (Wang et al., 2014). Thus, while AMPK acts as a negative regulator of skeletal muscle mass, the regulation of eEF2 by AMPK in skeletal muscle has not been fully unveiled and needs more in-depth investigation.

5'-adenosine monophosphate-activated protein kinase stimulates protein degradation through autophagy/ubiquitin/FoxO3a-dependent ways

In addition to the inhibitory effect on protein synthesis, AMPK is also involved in protein degradation in muscles. AMPK activator was shown to increase protein degradation in cultured mouse myoblast C2C12 cells (Das et al., 2012) And stimulate protein degradation through autophagy/ubiquitin/FoxO3a-dependent ways (Fig. 2).

Autophagy is the process through which defective cellular components, such as organelles, are degraded and recycled in nutrition-deprived or low-energy supply conditions (Kitada and Koya, 2021). Autophagy involves the engulfment of the target into autophagosomes, followed by the fusion of the autophagosome with a lysosome and subsequent degradation of the autophagosome. Autophagy is one of the main pathways for protein degradation in skeletal muscle atrophy, especially in nutrient deprivation-induced conditions. AMPK can promote autophagy via regulating the ULK1 complex, which consists of ULK1, mTORC1, AMPK, the FAK family kinase-interacting protein of 200 kDa (FIP200), and autophagy-related 13 (ATG13) (Akers et al., 2012). ULK1, a serine/threonine kinase, plays a crucial role in the initiation of autophagy. Activation of AMPK can result in its association with the ULK1 complex, which facilitates the phosphorylation of ATG13 and FIP200, thus activating autophagy (Akers et al., 2012). AMPK also directly phosphorylates ULK1 to increase the activity of ULK1 kinase, taking part in autophagosome formation (Kim et al., 2011).

Ubiquitin-proteasome-mediated degradation is another protein degradation pathway that leads to skeletal muscle

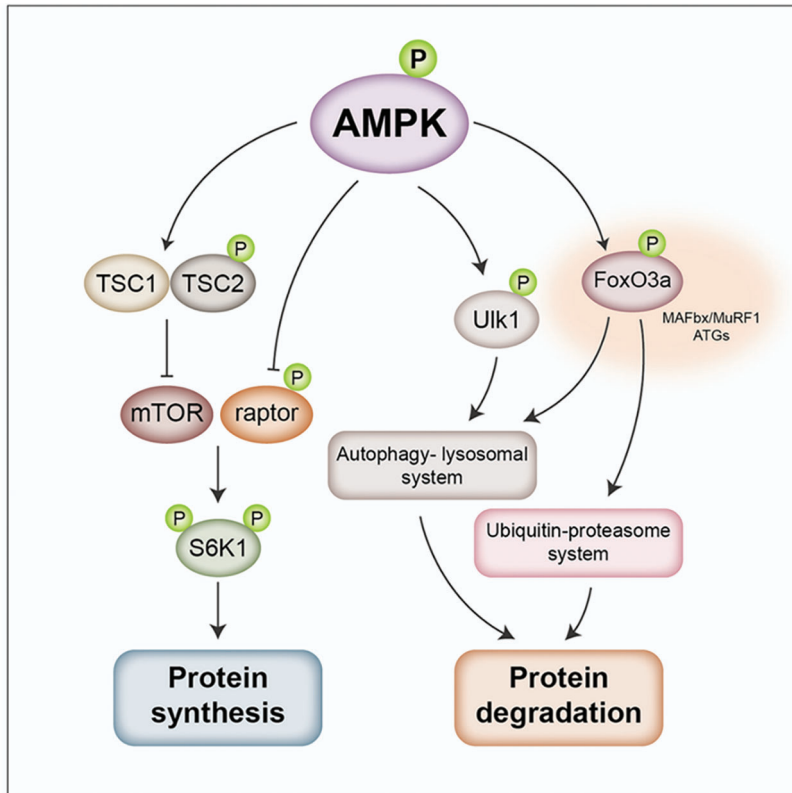


FIGURE 2. 5'-adenosine monophosphate-activated protein kinase (AMPK) regulates protein synthesis and degradation in skeletal muscle. AMPK inhibits muscle protein synthesis by regulating the mTORC1 pathway. It also stimulates protein degradation through autophagy/ubiquitin/FoxO3a-dependent ways. TSC1, tuberous sclerosis complex 1; TSC2, tuberous sclerosis complex 2; Mtor, mammalian target of rapamycin; S6K1, ribosomal protein S6 kinase 1; ULK1, Unc-51 like autophagy activating kinase 1; FoxO3a, Forkhead box O3a; ATGs, autophagy-related genes; MAFbx, muscle atrophy F-box; MuRF-1, muscle ring finger-1.

atrophy. In this process, target proteins are modified by ubiquitin in a mechanism involving three enzymes, E1, E2, and E3. The 26S proteasome then degrades the tagged proteins. In skeletal muscle atrophy, atrophy-related proteins, the muscle-specific ubiquitin ligases muscle atrophy F-box (MAFbx, also called atrogin-1) and muscle ring finger-1 (MuRF-1), are the main E3 ligases that regulate protein breakdown (Bodine and Baehr, 2014). AMPK activation can increase the mRNA content of MuRF-1 and MAFbx, leading to the initiation of the ubiquitin-proteasome-mediated degradation system (Baskin and Taegtmeier, 2011). No evidence has shown AMPK-mediated regulation of E1 and E2 in skeletal muscle, which requires more research.

The transcription of *MuRF-1* and *MAFbx* genes is also under the control of Forkhead box O3a (FoxO3a), which can upregulate the transcription of many genes in the autophagy pathway, such as *LC3B*, *beclin1*, *ULK2*, and *ATG4B*. FoxO proteins are evolutionarily conserved transcription factors and regulated by nuclear/cytoplasmic shuttling in a phosphorylation-dependent manner (Boccitto and Kalb, 2011). FoxO proteins play roles in the regulation of protein degradation as well as tumor suppression and development. In skeletal muscle, AMPK can activate FoxO3a via phosphorylation at Ser413/588 and regulate both autophagy and ubiquitin-proteasome degradation. The activation of FoxO3 leads to the increased expression of beclin 1 and LC3B, promoting autophagosome formation. There are debatable views on the effect of AMPK activation on FoxO3a shuttling between the nucleus and cytoplasm (Fasano et al., 2019). Nevertheless, studies have shown that AMPK can activate FoxO3a directly to increase its

transcriptional activity and thus has a role in the stability of proteins (Fig. 3). AMPK can regulate the activities of class IIA histone deacetylases, such as by histone deacetylase 5 (HDAC5) phosphorylation at Ser258 and Ser498, and affect myogenesis and muscular regeneration (Fu et al., 2013).

The role of 5'-adenosine monophosphate-activated protein kinase in mitochondrial dysfunction

Mitochondria are the main organelles responsible for the production of energy. The fusion and fission proteins regulate the morphology and subcellular location of mitochondria (Glancy et al., 2020). The inclination to fission machinery, which involves AMPK activation, can cause muscle dysfunction. The disruption of the network in mitochondria contributes to skeletal muscle atrophy. As observed in cases of muscle atrophy, such as in disuse, aging, and diabetic amyotrophy, mitochondria dysfunction has a pivotal role in skeletal muscle atrophy, characterized by loss, morphological change, and localization of the mitochondria.

AMPK can regulate mitochondrial biogenesis, as mentioned above, by phosphorylation of PGC-1 α (at Thr117 and Ser538), which is the primary controller for mitochondrial biogenesis and inhibits muscle atrophy under fasting and denervation conditions (Vainshtein et al., 2015). AMPK also directly phosphorylates FoxO3 on its regulatory sites and induces the mitochondrial fission cascade in skeletal muscle (Herzig and Shaw, 2018). The role of AMPK in regulating mitochondria function in muscle might depend on the specific conditions. By regulating ULK1, as discussed above, AMPK also modulates mitophagy, the autophagy-lysosome system in mitochondria (Fig. 4).

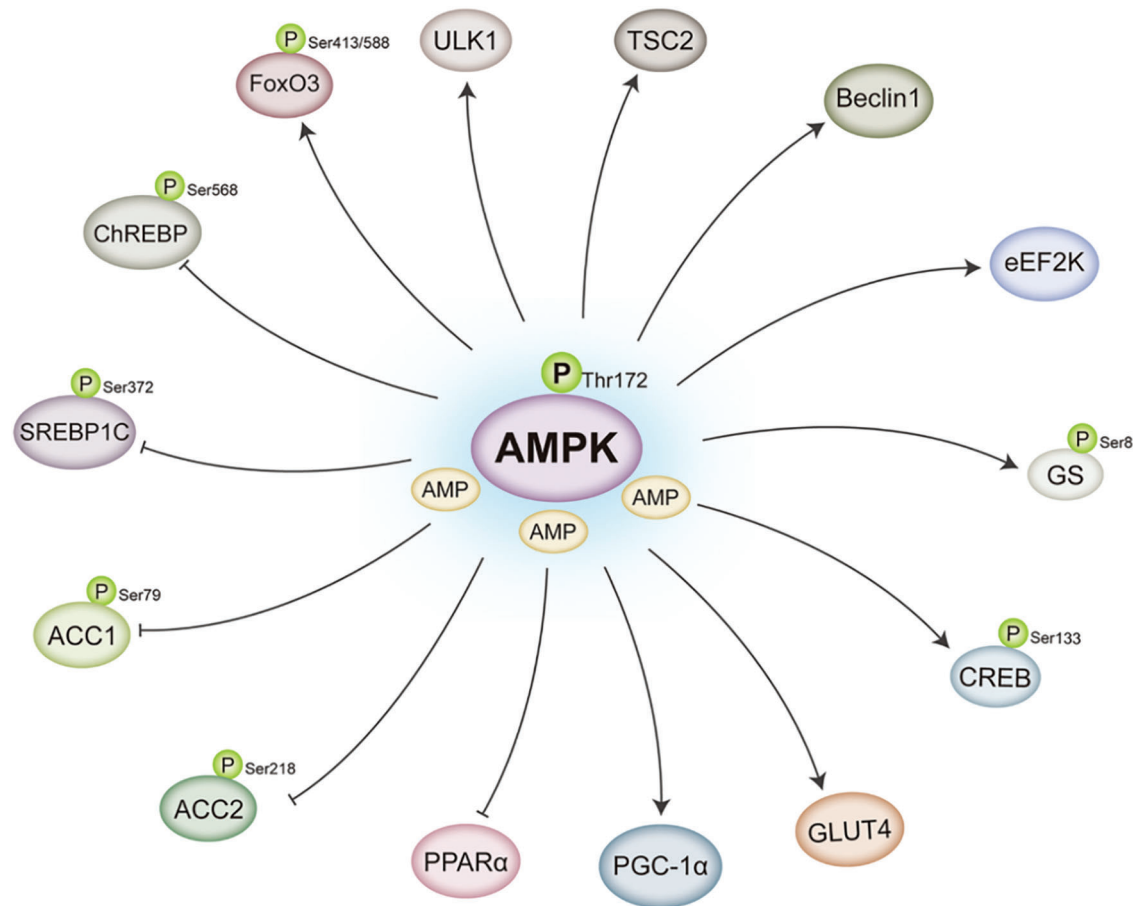


FIGURE 3. The regulatory role of 5'-adenosine monophosphate-activated protein kinase (AMPK) in skeletal muscle. AMPK can exert its biological functions by regulating the phosphorylation of many kinases, some of which are listed in this figure. AMP, 5'-adenosine monophosphate; ULK1, Unc-51 like autophagy activating kinase 1; TSC2, tuberous sclerosis complex 2; eEF2K, eukaryotic elongation factor 2 kinase; GS, glycogen synthase; CREB, cAMP response element-binding; GLUT-4, glucose transporter-4; PGC-1 α , peroxisome proliferator-activated receptor-gamma coactivator-1 α ; PPAR α , peroxisome proliferator-activated receptor α ; ACC2, acetyl-CoA carboxylase 2; ACC1, acetyl-CoA carboxylase 1; SREBP1c, sterol regulatory element-binding protein 1c; ChREBP, carbohydrate response element binding protein; FoxO3a, Forkhead box O3a.

Targeting mitochondria and improving mitochondrial function may provide a promising method for the treatment of skeletal muscle atrophy.

5'-adenosine monophosphate-activated protein kinase promotes glucose uptake and fatty acid and cholesterol metabolism

In the anaerobic and aerobic metabolism in skeletal muscle, insulin-dependent transport and metabolism of glucose are under the regulation of AMPK. The expression and translocation of glucose transporter-4 (GLUT-4) are induced by AMPK activation, followed by an increase in glucose uptake and oxidation (Webster *et al.*, 2010). The effect of AMPK on GLUT-4 is thought to be mediated by AMPK-mediated phosphorylation of TBC1D1 at Ser231 (Vichaiwong *et al.*, 2010). By phosphorylating CREB at Ser133, AMPK increases the transcription of hexokinase-2, which is responsible for the synthesis of glucose-6-phosphate (Feng *et al.*, 2020), promoting glycolysis. Moreover, AMPK can phosphorylate and thus inactivate glycogen synthase at Ser7, resulting in the inhibition of glycogenogenesis (Janzen *et al.*, 2018). This effect is dependent on the $\alpha 2$ subunit of AMPK. In skeletal muscle,

AMPK activation can promote more glycolysis and inhibit glycogenolysis, resulting in more glucose uptake.

AMPK can also regulate the metabolism of fatty acid and cholesterol in skeletal muscle by phosphorylating Ser79 of ACC1, Ser871 of the hydroxymethylglutaryl-coenzyme A reductase, and Ser565 of hormone-sensitive lipase (Srivastava *et al.*, 2012; Wang *et al.*, 2018). AMPK also decreases the transcription of lipogenic genes by stalling the activity of SREBP1c through Ser372 phosphorylation and the activity of carbohydrate response element binding protein (ChREBP) by phosphorylation at Ser568; both these transcription factors regulate lipogenic genes, repressing the synthesis of fatty acids (Li *et al.*, 2011; Liangpunsakul *et al.*, 2013). AMPK can phosphorylate the Ser212 of acetyl-CoA carboxylase and inhibit its activity, leading to reduced malonyl-CoA, which can allosterically inhibit carnitine palmitoyltransferase 1 (Lee *et al.*, 2018; Wakil and Abu-Elheiga, 2009), which is the rate-limiting enzyme in fatty acid oxidation, that transfers cytosolic long chain fatty acyl CoA into mitochondria. Thus, AMPK activation promotes the oxidation of fatty acids by downregulating malonyl-CoA in mitochondria. Furthermore, AMPK phosphorylates PGC-1 α at Ser538 and Thr177 and activates

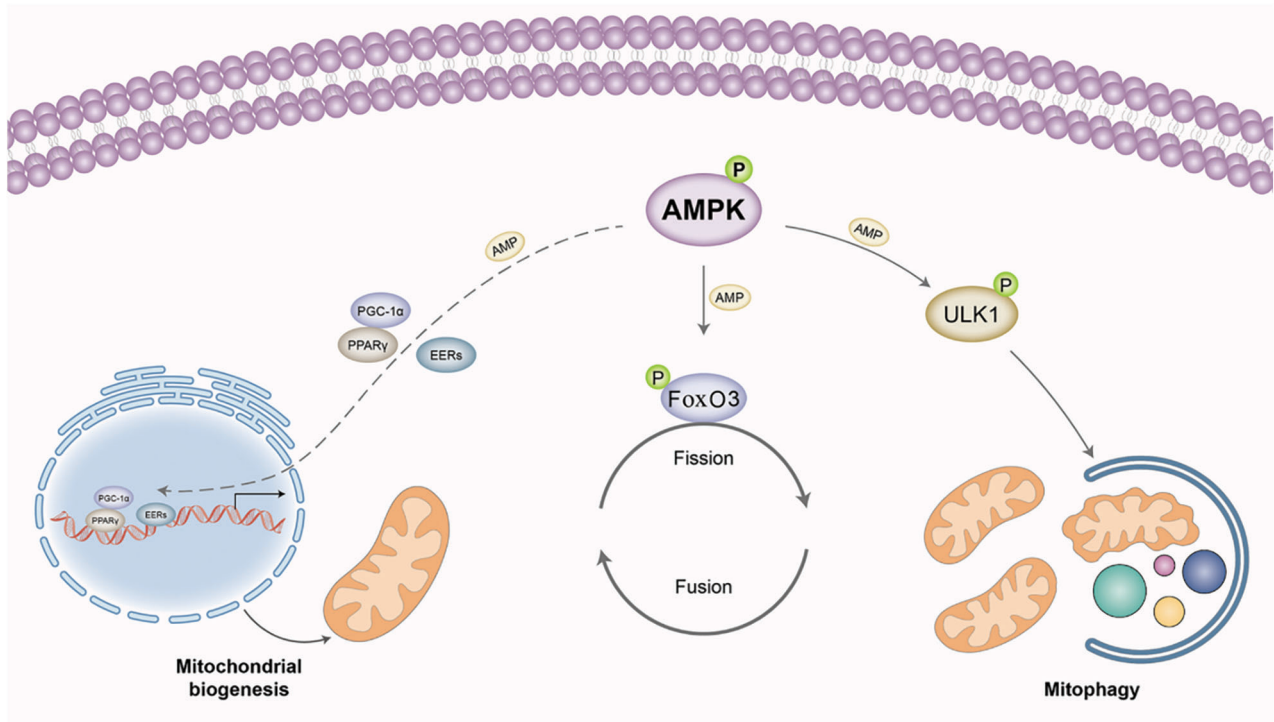


FIGURE 4. 5'-adenosine monophosphate-activated protein kinase (AMPK) regulates mitochondrial homeostasis in skeletal muscles. AMPK can enhance mitochondria biogenesis by phosphorylation of peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1 α , which is the main controller for mitochondrial biogenesis. AMPK can also directly phosphorylate Forkhead box O3 (FoxO3) on its regulatory sites and induce the mitochondrial fission cascade. By regulating Unc-51 like autophagy activating kinase 1 (ULK1), AMPK can also modulate the autophagy-lysosome system in mitochondria, named mitophagy.

PGC-1 α , a crucial protein in the oxidative metabolism in skeletal muscles (McGee and Hargreaves, 2010). PGC-1 α upregulates nuclear respiratory factors and other enzymes in the electron transport system, thus promoting mitochondrial respiration and biogenesis (Cheng *et al.*, 2018). For instance, PGC-1 α activates nuclear respiratory factor-1 through mitochondrial transcription factor A promoter, which regulates mitochondrial DNA replication and transcription (Scarpulla *et al.*, 2012). During exercise, AMPK activity inhibition in skeletal muscle decreases cellular NAD⁺ levels, resulting in the impairment of PGC-1 α deacetylation and inhibition of mitochondrial gene expression (Canto *et al.*, 2009). Regulation of the expression of mitochondrial genes by AMPK is mostly dependent on PGC-1 α .

Together, these studies indicate that AMPK promotes glucose uptake and fatty acid oxidation in skeletal muscle by direct or indirect regulation of related enzymes.

Targeting 5'-Adenosine Monophosphate-Activated Protein Kinase for the Treatment of Skeletal Muscle Atrophy

Although many studies have investigated the AMPK signaling pathway, the response of AMPK in skeletal muscle atrophy is still unclear, and conflicting results have been reported. Rodent models of disused skeletal muscle atrophy established by hindlimb unloading were reported to show increased (2–13 weeks) (Hilder *et al.*, 2005) or decreased (3–7 d or 4 weeks) (Cannavino *et al.*, 2015; Liu *et al.*, 2012) AMPK phosphorylation levels. Other studies showed no effect of hindlimb unloading on AMPK activity and AMPK-

related enzymes (Egawa *et al.*, 2015). Vilchinskaya *et al.* (2015) analyzed signaling pathways in the proteolysis in human soleus muscle during gravitational unloading and found significant changes in AMPK phosphorylation. Later, a summary of the mechanisms for the dysregulated autophagy-mediated sarcopenic obesity indicated the involvement of AMPK and PGC1 α signaling pathways in this process (Ryu *et al.*, 2020). Increased activity of AMPK was observed in denervated mice or rats (Gao and Li, 2018; Guo *et al.*, 2016; Ribeiro *et al.*, 2015), but its activity was also reported to be unchanged in muscle (Dreyer *et al.*, 2008). The difference in results in denervation models may be due to different handling times. AMPK phosphorylation was shown to increase in the early stage of denervation and decrease in later stages (Stouth *et al.*, 2018). The activity of AMPK dynamically changes in skeletal muscle atrophy, at least in denervation models. Targeting AMPK might be an effective approach for the treatment of skeletal muscle atrophy. Furthermore, AMPK is involved in most existing treatments for skeletal muscle atrophy. The roles of AMPK in the treatment of skeletal muscle atrophy-related diseases, which mostly involve AMPK activation, are listed in Table 1.

Exercise

Exercise is considered an effective recovery approach for skeletal muscle atrophy, especially atrophy caused by disuse. Exercise, especially physical exercise throughout life, plays an important role in maintaining a healthy life, including that in elderly individuals. The upregulation of skeletal

TABLE 1

The role of AMPK in the treatment of skeletal muscle atrophy-related diseases

	AMPK subunit target	Type	Application	Following cascades	Reference
Exercise	$\alpha 1, \alpha 2$	Physically	Muscle disuse, aging	Glucose uptake, mitochondrial biogenesis, energy homeostasis, fatty acid oxidation, autophagy, insulin	(Gariballa and Ali, 2020; Morales-Alamo and Calbet, 2016; Parker <i>et al.</i> , 2017; Richter and Ruderman, 2009; Treebak <i>et al.</i> , 2007)
AICAR	Mainly $\alpha 2$	Direct AMPK activator	Duchenne muscle dystrophy	Autophagy, oxidation	(Bolster <i>et al.</i> , 2002; Choi <i>et al.</i> , 2019; Ljubicic and Jasmin, 2013; Musi <i>et al.</i> , 2001; Paulsen <i>et al.</i> , 2001; Pauly <i>et al.</i> , 2012)
Metformin	Mainly $\alpha 2$	Indirect AMPK activator	Burn injury, tumor cachexia	Insulin, glucose uptake, protein synthesis	(Musi <i>et al.</i> , 2002; Oliveira and Gomes-Marcondes, 2016; Oshima <i>et al.</i> , 2015; Rena <i>et al.</i> , 2017; Yousuf <i>et al.</i> , 2020; Zhou <i>et al.</i> , 2001)
Low-lever lasers	–	Physical method	Doxorubicin-induced skeletal muscle atrophy	AMPK/SIRT1/PGC-1 α pathway	(Ou <i>et al.</i> , 2021)

Note: AMPK, 5'-adenosine monophosphate-activated protein kinase; AICAR, 5-amino-4-imidazolecarboxamide ribonucleoside; SIRT1, sirtuin 1; PGC-1 α , peroxisome proliferator-activated receptor- γ coactivator-1 α .

muscle glucose uptake is a well-known response to exercise and is mediated by improving insulin sensitivity and maintaining glucose homeostasis in both direct and indirect methods (Parker *et al.*, 2017). Exercise also promotes energy consumption, decreases ATP levels, and increases AMP levels in cells, which induces AMPK phosphorylation and activation, strengthening glucose uptake in skeletal muscle (Radhakrishnan *et al.*, 2019). Nonetheless, the role of AMPK activation in stimulating glucose uptake under physiological conditions is still under investigation.

Exercise for different durations and intensities can influence the different subunits of AMPK. Under exercise of mild intensity and 1 h of duration, $\alpha 1\beta 2\gamma 1$ and $\alpha 2\beta 2\gamma 1$ are the main activated forms of AMPK (Treebak *et al.*, 2007). Under intensive exercise for 20 min, $\alpha 2\beta 2\gamma 3$ is activated (Richter and Ruderman, 2009). The $\alpha 2$ subunit can be phosphorylated in prolonged exercise, and LKB1 is the primary kinase for the phosphorylation of the AMPK $\alpha 2$ subunit (Richter and Ruderman, 2009). The increase in intracellular Ca^{2+} that induces CaMKK phosphorylation is another mechanism for AMPK activation. Stress-induced reactive oxygen species increase in the endoplasmic reticulum and muscle temperature increase may also contribute to AMPK activation during physical exercise (Gariballa and Ali, 2020; Morales-Alamo and Calbet, 2016). Different modalities of exercise also show different effects on AMPK phosphorylation. Strength- and endurance-trained subjects showed different responses to AMPK phosphorylation levels in the body after cycling (Coffey *et al.*, 2005). Furthermore, AMPK activity in exercise is dependent on the level of AMP but not on the duration and intensity of exercise, but the level of AMP in the body is higher during physical exercise than in resting conditions until exhaustion (Morales-Alamo and Calbet, 2016).

A regular habit of exercise is of great benefit to elderly individuals, since it can also induce AMPK activity. In

addition to skeletal muscle atrophy, exercise-induced glucose uptake and AMPK activation can exert beneficial effects on insulin secretion and hemodynamic changes in aging people. The following cascades of AMPK activation include PGC-1 α activation (for mitochondrial biogenesis, and energy homeostasis), peroxisome proliferator-activated receptor- α expression (for fatty acid oxidation), autophagy stimulation, and mTOR inhibition. However, the distinct exercise types in previous research should be considered. Especially in the recovery of skeletal muscle atrophy, one needs moderate exercise since excessive exercise can result in excessive autophagy, which can lead to skeletal muscle atrophy and related diseases (Xia *et al.*, 2021).

The role of 5-amino-4-imidazolecarboxamide ribonucleoside (AICAR)

AICAR has long been applied as an AMPK activator in skeletal muscle and other tissues (Corton *et al.*, 1995; Hardman *et al.*, 2014; Thomson *et al.*, 2008). After administration, AICAR is converted to its monophosphate form, ZMP, which is a mimic of AMP and can activate AMPK without any change in the levels of intracellular adenine nucleotide. In rat gastrocnemius, like prolonged exercise, AICAR can activate the $\alpha 2$ subunit of AMPK but not the $\alpha 1$ subunit (Bolster *et al.*, 2002). Thus, AICAR-induced glucose uptake is diminished in AMPK $\alpha 2$ knockout mice but not in AMPK $\alpha 1$ knockout mice (Choi *et al.*, 2019). However, AICAR can activate AMPK $\alpha 2$, as well as the AMPK $\alpha 1$ subunit *in vitro* (Musi *et al.*, 2001).

Based on the role of AMPK activation (inhibiting protein synthesis, promoting protein degradation), AICAR should, in principle, accelerate the loss of muscle mass. However, in a rat denervation model, AICAR injection did not exacerbate skeletal muscle atrophy (Paulsen *et al.*, 2001). In fact, AICAR improved muscle function in mdx mice, a mouse model of Duchenne muscular dystrophy (Ljubicic and

Jasmin, 2013; Pauly *et al.*, 2012). The mechanisms included enhanced autophagy, clearance of damaged components, and upregulation of oxidative muscle phenotype, probably through AMPK activation. More investigations of the AMPK-involved treatment for Duchenne muscular dystrophy are in progress.

The role of metformin

Metformin is a well-known drug for the treatment of diabetes and insulin resistance. It can alleviate hyperglycemia, independent of insulin. The metabolic effect of metformin is similar to that of AMPK, and some effects of metformin are independent of AMPK activation (Zhou *et al.*, 2001). Metformin inhibits oxidative phosphorylation in mitochondria, reduces the production of ATP, and results in energy stress in cells; all these events can indirectly activate AMPK (Rena *et al.*, 2017). AMPK $\alpha 2$ (but not $\alpha 1$) subunit is the target for metformin in skeletal muscle, and chronic administration of metformin can increase the activity of the AMPK $\alpha 2$ subunit (Musi *et al.*, 2002), an effect that can sustain even after the withdrawal of metformin. However, in other studies, both $\alpha 1$ and $\alpha 2$ subunits of AMPK were activated by metformin treatment, both *in vivo* and *ex vivo* (Oshima *et al.*, 2015). In skeletal muscle cells, metformin has been shown to impair muscle function and induce muscle atrophy via the AMPK-FoxO3a-HDAC6 pathway (Kang *et al.*, 2021). In burn injury patients, metformin treatment can activate AMPK $\alpha 2$ in skeletal muscle and lead to an increase in protein synthesis (Yousuf *et al.*, 2020). In a tumor cachexia animal model, metformin treatment can promote protein synthesis as well as decrease protein degradation when the treatment activates AMPK (Oliveira and Gomes-Marcondes, 2016). The improvement in muscle metabolism of metformin may be attributed to its effect on insulin sensitivity, which regulates glucose uptake and protein synthesis. However, whether AMPK activation has a role in metformin-induced muscle recovery needs further investigation.

The effect of low-level lasers

Ou *et al.* (2021) investigated the effect of low-level laser irradiation on muscle atrophy and the related mechanism in the doxorubicin (Dox)-induced skeletal muscle atrophy model. In both animal and cultured cells, irradiation with low-level lasers alleviated Dox-induced muscle wasting; Dox-induced mitochondrial dysfunction, cell apoptosis, and oxidative stress were inhibited through the AMPK/SIRT1/PGC-1 α pathway. The study not only provided evidence for the benefit of low-level lasers on Dox-induced muscle disease but also unveiled the role of AMPK activation in muscle atrophy treatment, in which the activation of the AMPK/SIRT1/PGC-1 α pathway exerts protection against Dox myotoxicity through preservation of mitochondrial homeostasis and alleviation of oxidative stress and apoptosis.

Discussion and Perspective

AMPK is a master regulator of kinase in the metabolism of skeletal muscles. It inhibits protein synthesis in the muscle by inhibiting mTORC1 activity both directly and indirectly.

AMPK can also stimulate protein degradation through the autophagy-lysosomal system, ubiquitin-proteasome system, and FoxO3a-dependent pathways, playing an important role in protein turnover. AMPK also enhances mitochondrial biogenesis and promotes glucose uptake and metabolism of fatty acids and cholesterol. Skeletal muscle is the tissue with an active metabolism, regardless of glucose and energy, making AMPK important in skeletal muscle. Modulating AMPK activity has been shown to contribute to the therapy of skeletal muscle atrophy. Therefore, it is important to understand the specific mechanisms that regulate AMPK and its downstream cascades to improve the current therapeutic strategies and develop new methods to protect against skeletal muscle atrophy. Considering the general role of AMPK activation in promoting muscle atrophy, it seems inhibition of AMPK should be a therapeutic strategy; however, many muscle atrophy treatments were shown to activate AMPK. Given the diverse effects of AMPK, inhibition of AMPK may increase the “quantity” of muscle but may result in poor “quality” of the muscle. The effect of these therapeutic means on AMPK and resultant signaling depends on the modality and duration. More detailed investigations are needed, considering the specific role of AMPK on specific types and stages of skeletal muscle atrophy. Notwithstanding, the crucial role of AMPK in skeletal muscle atrophy is incontrovertible, and targeting AMPK for developing new drugs with research on the mechanism for skeletal muscle atrophy and even other diseases, such as diabetes and cancer, is of great significance.

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