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Physical inactivity induces the atrophy of skeletal muscle of rats through activating AMPK/FoxO3 signal pathway

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Abstract. – OBJECTIVE: Long-term physical inactivity can cause the atrophy of skeletal muscle. The aim of this study is to explore the underlying mechanisms of physical inactivity-induced atrophy of skeletal muscle.

MATERIALS AND METHODS: 14 Sprague-Dawley (SD) male rats were divided into 2 groups including normal control (NC) and hindlimb suspension (HS) groups. After two weeks of HS stimulation, the ratio between skeletal muscle weight and body weight, and cross-sectional area (CSA) of skeletal muscle fibers, were measured. Western blot was applied to evaluate the expression of proteins associated with atrophy and autophagy. The transmission electron microscope was used to observe the ultra-microstructure and the mitochondrial quality of skeletal muscle.

RESULTS: The rats subjected to 2-week HS treatment presented an evident atrophy of the skeletal muscle with a significantly reduced ratio between skeletal muscle weight and body weight, and smaller cross-sectional area (CSA) of skeletal muscle fibers when compared with control rats. Meanwhile, HS stimulation resulted in the damage of mitochondria, the increased expression of MuRF1 and Atrogin-1/MAFbx, and enhanced apoptosis, as well as dysfunctional autophagy in skeletal muscle.

CONCLUSIONS: HS-induced skeletal muscle atrophy involves the activation of AMPK/FoxO3 signal pathway, evidenced as AMPK phosphorylation, FoxO3 activation, and Atrogin-1 and MuRF1 up-regulation. FoxO3-mediated autophagy plays an important regulatory role in HS-induced skeletal muscle atrophy.

Key Words:
Hindlimb suspension, Autophagy, Skeletal muscle atrophy, Apoptosis, Mitochondrial quality control.

Introduction

Skeletal muscle disuse atrophy (SMDA) is one of the important research topics in the fields of clinical medicine and rehabilitation medicine. It mainly refers to physiological, biochemical, morphological and functional changes of skeletal muscle under the states of hypokinesis, immobilization, and weightlessness[1-3]. Clinically, many diseases including paralysis and pulled muscle and therapeutic measures, such as skeletal fixation, are often accompanied with hypokinesis or immobilization. The idea that mitochondrial dysfunction contributes to disuse muscle atrophy has been described over 40 years ago[4]. Many studies have documented the changes in shape, number, and function of mitochondria in skeletal muscle under the condition with long-term physical inactivity[5]. Moreover, recent studies reveal that increased mitochondrial fragmentation due to fission is a required signaling event to activate AMP-activated kinase-Forkhead box O3 (AMPK-FoxO3) signal axis during denervation-induced muscle atrophy, thereby inducing the expression of atrophy genes, protein degradation, and final atrophy of skeletal muscle[6]. Numerous experiments[5,7,8] have shown that immobilization-induced skeletal muscle atrophy is attributed to the decrease of protein synthesis and the increase of proteolysis. The ubiquitin-proteasome system (UPS), and the autophagy-lysosome pathway (ALP) are major proteolytic executors for regulating the half-life time of a majority of cellular proteins. Exploring molecular mechanisms to modulate muscle mass is one of the important research topics.

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AMPK, as a metabolic sensor, can trigger the phosphorylation and nuclear translocation of FoxO3, thereby leading to the up-regulation of MuRF1 and myotube atrophy[9,10]. AMPK-FoxO3 signaling can partially govern muscle mass maintenance and myofiber hypertrophy. AMPK activation during muscle unloading can inhibit protein synthesis[11], further accelerating muscle protein loss. FoxO proteins, as the downstream targets of AMPK, act as the critical mediators of myofiber atrophy during muscle disuse, because they orchestrate the induction of an atrophic gene program in both UPS- and ALP-mediated catabolism. Based on above factors, we hypothesized that long-term unloading could activate autophagy and apoptosis. To test this hypothesis, the hindlimb suspension (HS) of the rats was used to establish a disuse muscle atrophy model. The effects of HS stimulation on the functional status of autophagy and mitophagy in skeletal muscle were explored. Results showed the critical regulatory role of the AMPK-FoxO3 signal pathway in excessive autophagy and apoptosis of HS-induced model rats with skeletal muscle atrophy.

Materials and Methods

Animal Study Design

A total of 14 male SD rats with the age of 8 weeks were purchased from Experimental Animal Research Center of Hubei Province (Certificate No.: SCXK20080005) and housed at SPF-grade environment with room temperature of 22 ± 3°C, relative humidity of 50-70%, and light-dark cycle of 12 h-12 h, as well as the accessibility to food and water *ad libitum*. All experimental protocols were reviewed and approved by Institutional Animal Care and Use Committee at Wuhan Sports University. The rats were divided into 2 groups (n = 7 per group) including hindlimb suspension (HS) and non-suspension as the normal control (NC) groups. HS stimulation was performed for 14 days as previously described[12]. Briefly, the rats were fed separately, and their tails were suspended over the cages. Their hind legs were off the ground of the cage, while their forelegs grounded in the cage, thus forming an angle of 30-45° between their body and the ground. The rats can rotate for 360° angles, and access to food and drink water freely. The suspension was examined carefully during the experiment. After HS stimulation for 14 days, the rats were euthanized with sodium pentobarbital (100 mg/kg). Gastrocnemius and soleus muscle were carefully dissected, weighed, frozen in liquid nitrogen, and stored in -80°C freezer until analysis. The ratio between skeletal muscle weight and body weight was calculated as the atrophy rate of skeletal muscle.

HE Staining

The samples of gastrocnemius and soleus muscle were fixed in 10% neutral buffered formalin (Beyotime Institute of Biotechnology, Jiangsu, China) and cut into transverse sections with the thickness of 5 μm. Then, the sections were dewaxed and stained with hematoxylin and eosin (HE). After dehydrated and mounted, sections were observed under a light microscope (Olympus, Tokyo, Japan) at a magnification of 40×. The cross-sectional area of selected 100 skeletal muscle fibers in stained sections was measured and calculated using ImageJ software (NIH, MD, USA).

Transmission Electron Microscopy

The samples of gastrocnemius and soleus were harvested at the volume of 1 mm^3, and were sequentially fixed in phosphate buffered saline (PBS) containing 2.5% glutaraldehyde (Beyotime Institute of Biotechnology, Jiangsu, China) for 2 hours. They were rinsed with 1 mmol/L phosphoric acid solution, and were fixed in 1% osmium tetroxide (Beyotime Institute of Biotechnology, Jiangsu, China) for 2-3 h. After acetone was used for step-by-step dehydration, the block was cut carefully into ultrathin sections at the thickness of approximately of 70 nm. The sections were stained with 3% uranyl acetate and lead citrate. Next, they were examined under a JEOL JEM1400 electron microscope (JEOL, Tokyo, Japan) at Wuhan Institute of Virology, Chinese Academy of Sciences, to examine the changes of myofibril, myofilament, mitochondria as well as autophagosomes.

Western Blot

40 mg of gastrocnemius muscle or soleus muscle samples were subjected to the lysis using 0.6 mL RIPA buffer (Beyotime Institute of Biotechnology, Jiangsu, China) containing protease inhibitor, phenylmethylsulfonyl fluoride (PMSF) (Beyotime Institute of Biotechnology, Jiangsu, China). After homogenization, ultrasonic treatment, and centrifugation at 6,700 g for 10 min at 4°C, the supernatant was collected, and total protein concentration was measured by BCA kit (Walterson Biotechnology Inc., Beijing, China).

The proteins were sequentially subjected to the denaturation in the presence of sample buffer at 95°C water bath for 5 min, and separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred to nitrocellulose membrane. The target protein was probed by primary antibody, including Atg7, LC-3, Bax, Drp1, Beclin1, Caspase-3, Bcl-2, Fbx-32, or GAPDH (1:1000, Cell Signaling Technology, Danvers, MA, USA), and secondary antibody (1:8000) (Abcam, Cambridge, MA, USA). Next, it was visualized by enhanced chemiluminescence (ECL) reagent (Thermo Scientific, Waltham, MA, USA) and imaged by ultra-sensitive fluorescence/chemiluminescence imaging system ChemiScope6300 (CLiNX Science Instruments, Shanghai, China).

Statistical Analysis

All data were expressed as mean ± standard deviation (M±SD). Statistical analysis for the comparison between groups was conducted using nonparametric one-way ANOVA followed by post-hoc analysis through SPSS 17.0 curative statistical processing software (IBM SPSS Inc., Chicago, IL, USA); the statistically significant difference was considered at $p < 0.05$.

Results

HS-induced Damage on Skeletal Muscle Fibers

After being suspended for 14 days and compared with the rats from normal control group, the ratio between soleus muscle and body weight and the ratio between gastrocnemius muscle and body weight of the rats from HS group, revealed a significant reduction (Figure 1A), suggesting that physical inactivity or HS stimulation could result in the significant atrophy of skeletal muscle. To characterize the internal structure of skeletal muscle fibers, we examined structural change of soleus muscle and gastrocnemius muscle through HE staining. Under normal conditions, myofilaments displayed an orderly and neat arrangement, while skeletal muscle fibers in HS-induced group had an evident damage with loose structure and disordered arrangement. Due to the mass loss of gastrocnemius muscle and soleus muscle, the CSA of gastrocnemius and soleus muscle in rats from HS group was significantly smaller than those in the rats from the normal control group (Figures 1C and 1D). In addition, to better explore the effect of HS stimulation on the atrophy of skeletal muscle, transmission electron microscope was used to examine the ultra-microstructure of skeletal muscle fibers. The clear I-band, H-band, Z line without any thickening and warping, and orderly-organized myofibrils and myofilaments without warping or broken, were observed in gastrocnemius muscle and soleus muscle of the rats from normal control group. In contrast, after being suspended for 14 days, the Z line of skeletal muscle was thickened and warped, and I-band and H-band became vague. The myofibril was disorganized and partly broken, and even part of myofilaments disappeared, with irregular band and uneven-length myotome (Figure 1B). These results indicated that HS stimulation could lead to the loss of skeletal muscle fibers in rats, suggesting that physical inactivity can induce the atrophy of skeletal muscle.

Effect of Hindlimb Suspension on Structure and Function of Mitochondria

Compared with the rats from the normal control group, the expression of Drp1, Parkin and Bnip3 in skeletal muscle of the rats from HS group increased significantly, while the expression of Mfn2 revealed a sharp reduction. Compared with the rats from the normal control group, the decreased expression of Mfn2 and increased expression of Parkin1 in the rats from HS group were observed (Figure 2A). In addition, based on transmission electron microscopic observation, the mitochondria in the rats from the normal control group revealed the equal size and even distribution as well as circular or oval shape. The mitochondrial membrane and cristae were clearly visible without any fracture. Its matrix was well distributed without typical autophagosome structure. Compared with the rats from the normal control group, the rats from HS group experienced the changes in mitochondrial size and shape, and revealed the characteristics of swelling, cristae breaking, reduced density, and vacuolation of mitochondria. Mitochondrial structure wrapped by double-layer membrane or multi-layer membrane was also observed (Figure 2B), suggesting that HS significantly damaged the ultra-microstructure of gastrocnemius and soleus muscle.

Hindlimb Suspension Aggravated Protein Degradation in Skeletal Muscle

To evaluate whether UPS presents an increase in skeletal muscle during HS stimulation, we

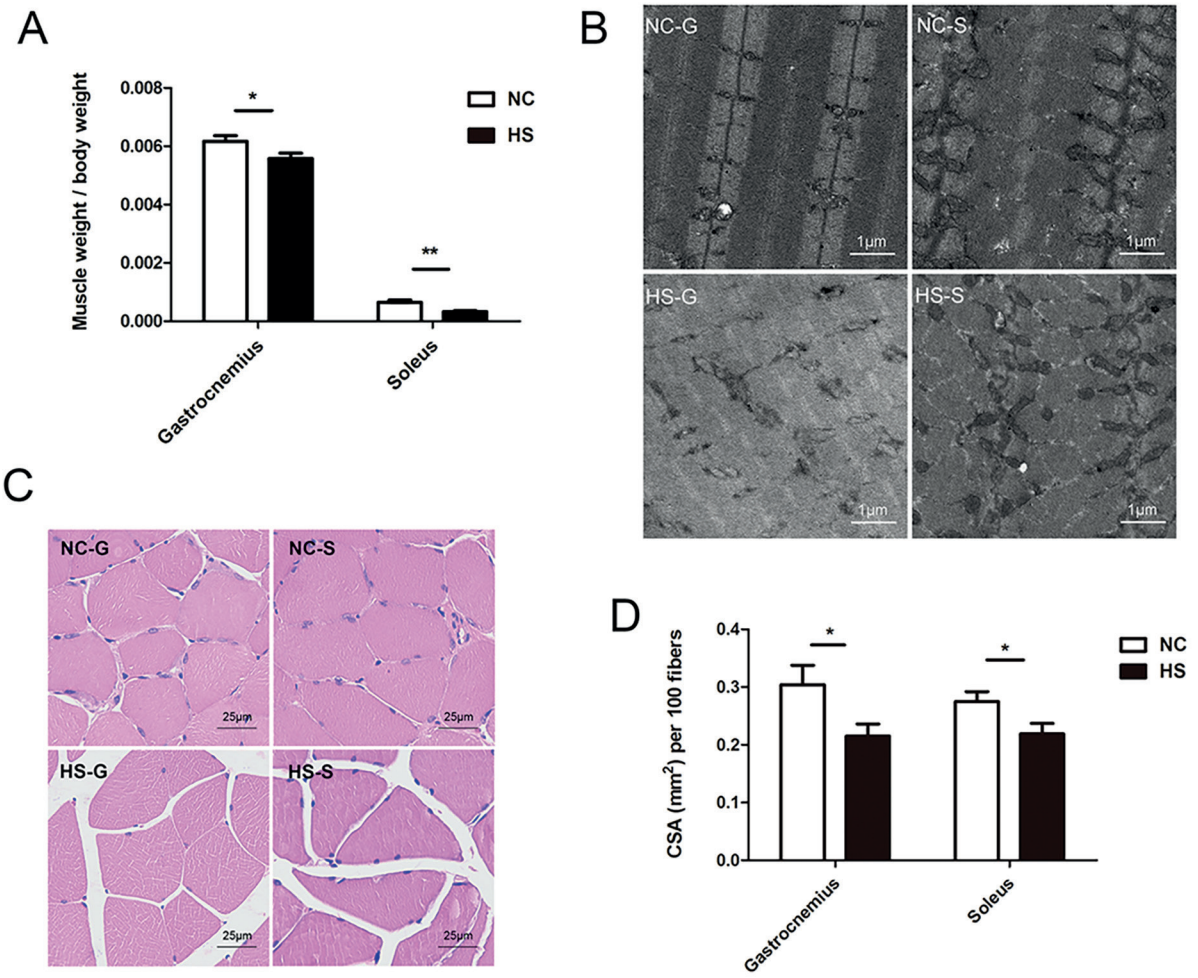

Figure 1. Hindlimb suspension induced the damage of skeletal muscle fibers. All rats were assigned into normal control (NC) and hindlimb suspension (HS) groups. The HS-induced atrophy of skeletal muscle was evidenced as reduced muscle weight/body weight for gastrocnemius muscle and soleus muscle ***(A)***, respectively. HS stimulation damaged the ultra-microstructure of skeletal muscle examined by transmission electron microscope ***(B)***, and the morphology of skeletal muscle fibers examined by H&E staining ***(C)***. Cross-sectional area (CSA) of skeletal muscle per 100 fibers ***(D)*** was determined through ImageJ software. NC-G: Gastrocnemius muscle from NC group; NC-S: Soleus muscle from NC group; HS-G: Gastrocnemius muscle from HS group; HS-S: Soleus muscle from HS group. *p <0.05, **p < 0.01, and ***p < 0.001 compared with NC group.

detected two important biomarkers such as Atrogin-1 and MuRF1 responsible for UPS degradation pathway. Results showed that Atrogin-1 and MuRF1 were significantly up-regulated in skeletal muscle of the rats subjected to HS stimulation when compared with the rats from the normal control group (Figure 3A). AMPK, as a metabolic sensor, can trigger the phosphorylation and nuclear translocation of FoxO3, and also can lead to an increase in MuRF1 expression and myotube atrophy. So we evaluated AMPK and FoxO3 in skeletal muscle. As compared with the normal control group, the skeletal of the rats from HS stimulation group demonstrated higher p-AMPK/AMPK ratio and up-regulated expression of FoxO3 (Figure 3B). Caspase-3 is the specific regulator of mitochondria-mediated apoptotic signal pathway. The experimental results indicated the significant activation of Caspase-3 in skeletal muscle of the rats from HS stimulation group (p < 0.01) when compared with the normal control group. In addition, Bcl-2 plays an important regulatory role via adjusting the permeability and completeness of mitochondrial outer membrane. There are two types of Bcl-2 family members. The first type is anti-apoptotic Bcl-2. The other is pro-apoptotic Bax. Compared with the normal control, HS stimulation could promote the down-regulation

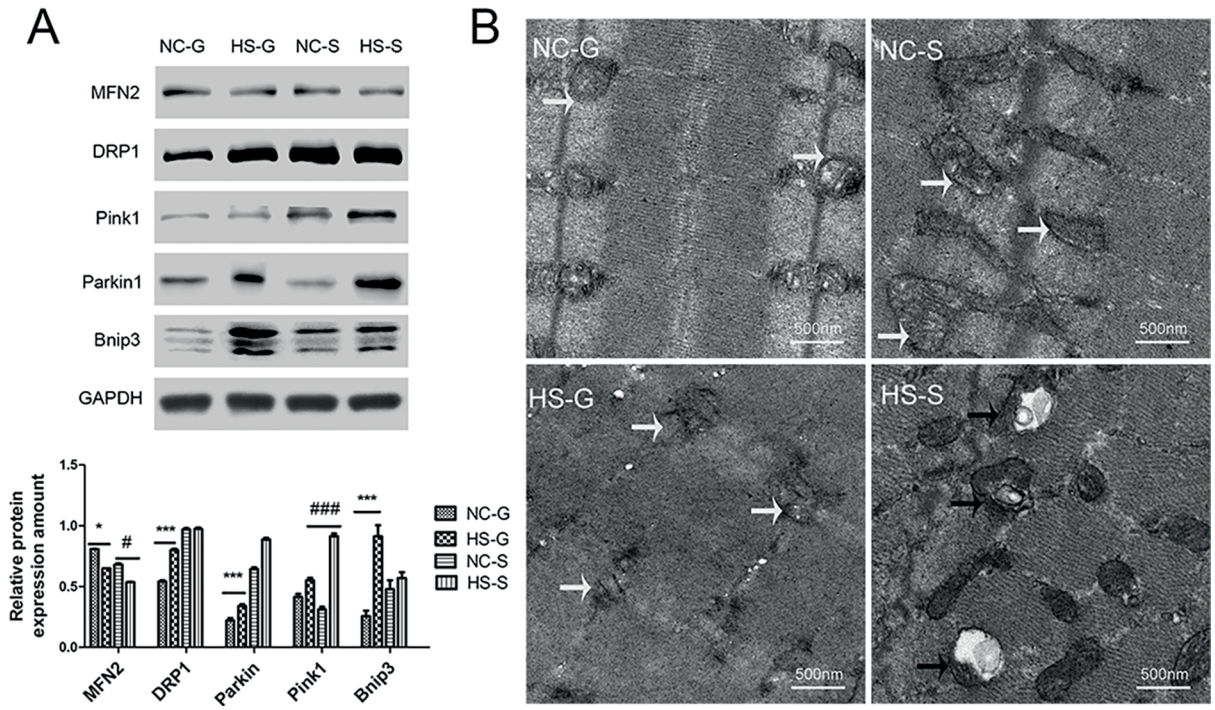

Figure 2. Hindlimb suspension damaged the structure and function of mitochondria. The expression of proteins associated with the quality control of mitochondria was evaluated by Western blot ***(A)*** and HS-induced the damage of ultra-microstructure of mitochondria in skeletal muscle was examined by transmission electron microscope ***(B)***. White arrows represent mitochondria and black arrows represent autophagosomes. The data were expressed as M ± SD from at least three independent experiments using GAPDH as the loading control of equal protein amount. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with NC-G; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with NC-S.

of Bcl-2, but result in the up-regulation of Bax (Figure 3C), suggesting that the activation of apoptosis upon HS stimulation can induce the mass loss of skeletal muscle.

LC3-II/LC3-I ratio is used as an indicator to evaluate the activation of autophagy or the functional status of autophagy. The p62 protein is known to be segregated into autophagosomes and degraded when autophagosomes fuse with lysosomes. Beclin1 and Atg7 are two proteins closely associated with autophagy. Compared with the normal control group, HS stimulation resulted in enhanced LC3-II/LC3-I ratio, and up-regulated Beclin1 and Atg7, as well as the reduced p62 expression (Figure 3D), indicating the activation of autophagy companied with the excessive apoptosis can accelerate the atrophy of skeletal muscle.

Discussion

The commonly used index for evaluating the degree of disuse atrophy is the ratio between skeletal muscle weight and body weight[13,14]. By comparing body weight and skeletal muscle quality, both skeletal muscle weight and the ratio between skeletal muscle weight and body weight reveal a sharp reduction upon HS stimulation for 2 weeks. Under examination by transmission electron microscope, compared with the skeletal muscle in the rats from the normal control group, Z line of skeletal muscle in rats from HS group was thickened and warped. The myofibrils were disorganized and partly broken, and even some myofilaments disappeared, evidenced as vague brightness zones, irregular bands, uneven-length myotomes, and swelled and uneven-size mitochondria. Therefore, HS stimulation could result in the loss of skeletal muscle fibers of the rats. The mitochondrial quality control system is a regulatory system to keep the quality and quantity of mitochondria within a certain scope. It mainly includes the reconstruction of mitochondrial energetics, mitochondrial biosynthesis, mitochondrial fusion, chondriokinesis, mitochondrial DNA restoration, and mitophagy. Mitochondrial dysfunction can result in disuse atrophy[4]. Muscle immobilization can lead to mitochondrial dys-

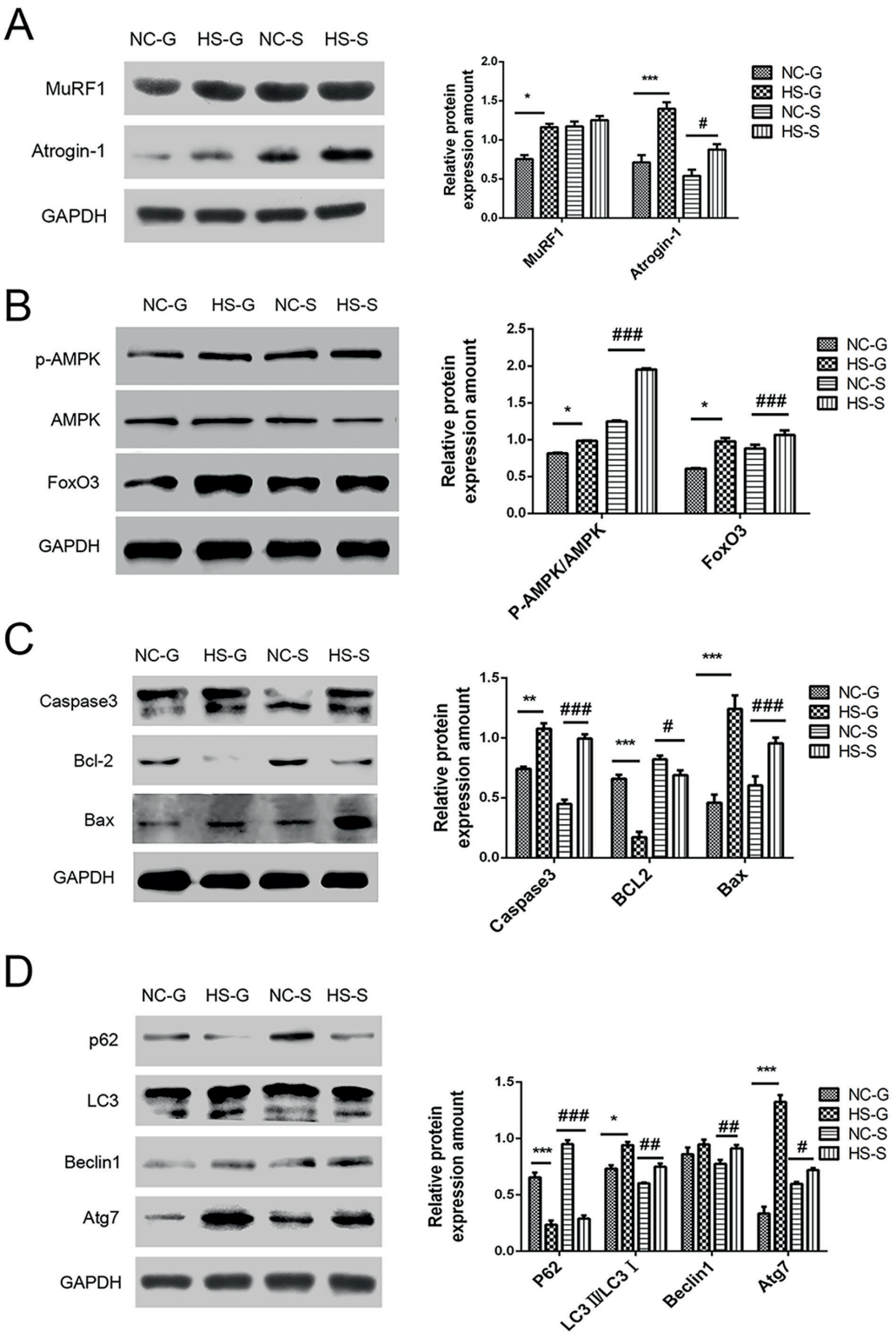

Figure 3. Hindlimb suspension aggravated the protein degradation process in the skeletal muscle. HS stimulation up-regulated muscle-specific E3 ligases such as Atrogin-1 and MuRF1 ***(A)***, activated AMPK/FoxO3 signal pathway ***(B)***, and enhanced the expression of apoptosis-associated proteins including Bax and Caspase-3 ***(C)***, and autophagy-related proteins including LC3, Beclin1 and Atg7 ***(D)*** in skeletal muscle. The data were expressed as M ± SD from at least three independent experiments using GAPDH as the loading control of equal protein amount. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared with NC-G; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with NC-S.

function to propel disuse muscle atrophy. Recent researches have demonstrated that the increase of mitochondrial fission in cells is closely related to the intensified apoptosis, and it can improve skeletal muscle fiber atrophy[15,16]. For example, the suppression of Drp1 activity can delay the apoptosis of Caspase-induced cells[17]. In addition, the suppression of Fis1 can also prevent apoptosis, and the over-expressed Fis1 can promote the death of apoptotic cells[18]. Similarly, the functional loss of Mfn1/2 will cause mitochondrial dysfunction and muscle atrophy[19,20]. Therefore, mitochondrial fusion in skeletal muscle is critical in maintaining the stability of mitochondrial DNA and preventing mitochondrial DNA mutation. In the present study, 2-week HS stimulation led to increased Drp1 and reduced Mfn2, which indicates the enhanced mitochondrial dissociation and the declined mitochondrial fusion. During muscle disuse, mitochondria dynamically shift toward fission and autophagy is over-activated. ROS generation by mitochondria is increased, which combines with the up-regulation of fission for stimulating apoptosis[21]. Therefore, excessive fission may be conducive to the development of acute muscle wasting. The enhanced fission and reconstruction of the mitochondrial network have been detected in murine muscles atrophied by denervation or fasting. Pink1 can be used as the biomarker for the identification of mitochondrial damage and the initiation of mitophagy. It works by transferring endochylema into depolarized mitochondria, collecting Parkin to impaired mitochondria and then mediating autophagosomes to wrap up mitochondria[22]. Bnip3 is anchored in the mitochondrial outer membrane via the trans-membrane domain of carboxyl terminal. Its amino-terminal, facing the endochylema, can interact with autophagy key protein LC3 to mediate mitophagy[23]. By using denervation to simulate skeletal muscle disuse model, the quality of skeletal muscle in mice is reduced, and the orientation of p62 and Parkin to mitochondria reveals an evident improvement, indicating that skeletal muscle disuse can result in mitochondrial damage and the initiation of mitophagy[24]. The degradation mechanism will be probably prevented later so as to mediate skeletal muscle atrophy. In the mouse model of denervation-induced skeletal muscle atrophy, LC3-II is up-regulated accompanying by increased localization of this autophagic mediator to both intermyofibrillar mitochondria (IFM)[25] and subsarcolemmal mitochondria (SSM)[26], suggesting that mitophagy may be activated during the development of acute atrophy of skeletal muscle. Our study has also demonstrated that the expression of Pink1, Parkin, and Bnip3 can be improved significantly in the rats with skeletal muscle atrophy stimulated by HS stimulation, suggesting that HS stimulation can promote mitophagy of skeletal muscle, and Pink1/Parkin signal pathway can play a certain role in mitophagy. However, further study on its specific mechanism is still needed.

Skeletal muscle atrophy is caused by the imbalance between the rates of protein synthesis and protein degradation[27]. Autophagy lysosomal pathway and ubiquitin-proteasome pathway are involved in protein decomposition in skeletal muscle. A previous research[28] indicates that the reduction of protein synthesis is likely to play a significant role in initiating skeletal muscle atrophy. However, in the later period of disuse, the intensified proteolysis is probably critical in skeletal muscle atrophy and its progression[29]. UPS is correlated not only with intracellular protein degradation, but also with intracellular protein modification and transduction regulation of intracellular and extracellular signals. Further studies have found that the ubiquitination level of proteins in skeletal muscle is improved during the pathological process of atrophy. The expression of mRNA of the genes associated with increased UPS signaling further confirms the important role of UPS signal pathway in the atrophy of skeletal muscle[30]. Consistent with previous reports, Atrogin-1 and MuRF1 are significantly up-regulated in skeletal muscle when compared with the normal control group in our study. Atrogin-1 and MuRF1 are important muscle-specific E3 ligases that play a major role in the degradation of muscle contractile proteins via the ubiquitin-proteasome system. Numerous studies have demonstrated that these ubiquitin ligases are activated in various atrophy states including prolonged muscle inactivity, sarcopenia, and cancer cachexia[31]. The deletion of MuRF1 in mice is resistant to skeletal muscle atrophy induced by hindlimb unloading[32]. Moreover, the knockdown of Atrogin-1 prevents the mass loss of skeletal muscle during fasting[33]. In the current study, our findings have confirmed that the expression of Atrogin-1 and MuRF1 is increased after l4 days' unloading, further suggesting that ubiquitin-proteasome pathway plays an important role in HS-induced atrophy of skeletal muscle (Figure 4). Excessive or insufficient autophagy is detrimental for the homeostasis of skeletal muscle[34]. Some genetic models have ver-

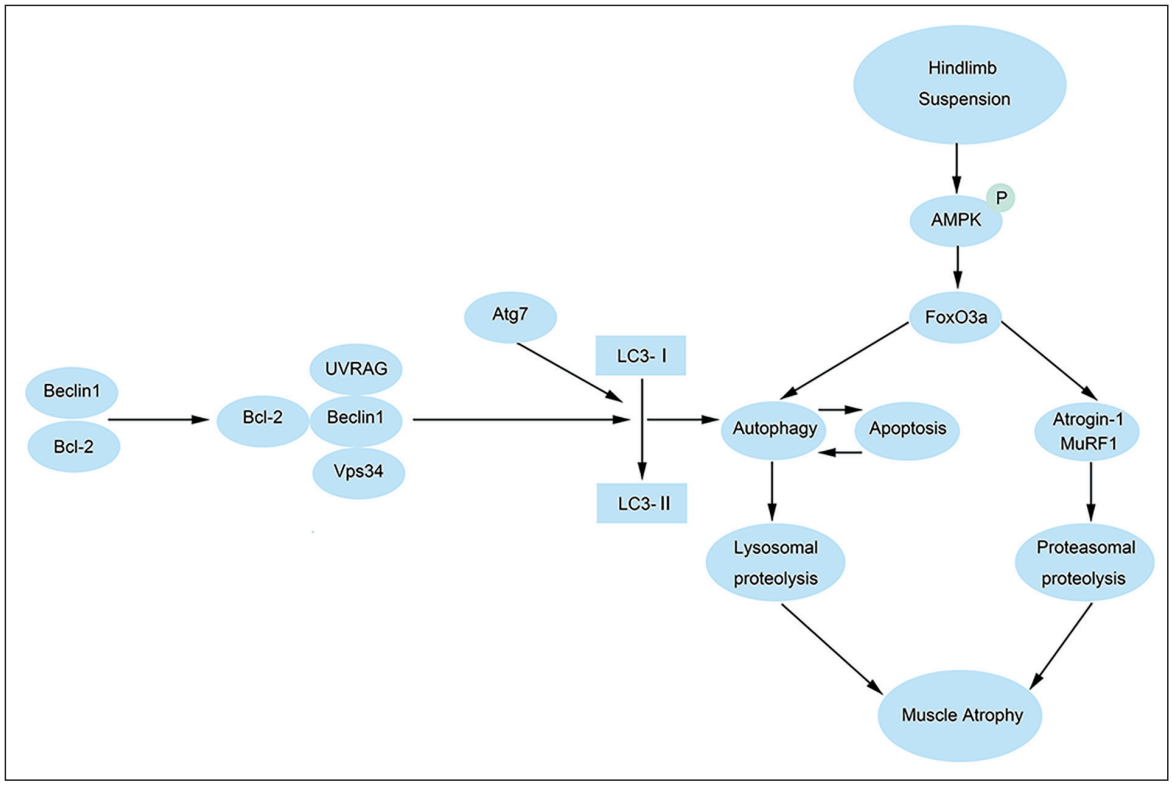

Figure 4. Signal pathways involved in HS-induced atrophy of skeletal muscle. HS stimulation induced the phosphorylation of AMPK and then activated AMPK-FoxO3 signal pathway. The activation of FoxO3 promoted the expression of MuRF1 and Atrogin-1, thus leading to the increased proteasomal proteolysis that contributed to protein degradation in skeletal muscle due to the excessive autophagy and apoptosis.

ified the role of autophagy during skeletal muscle atrophy. It is well known that myofiber atrophy is induced *in vivo* by overexpression of constitutively active FoxO3. FoxO3-mediated muscle loss is partially blocked when the vital gene LC3 is knocked down by RNAi[35]. The specific mutation of $SOD1^{G93A}$ gene in skeletal muscle can cause muscle atrophy and muscle weakness due to the activation of autophagy. Reduced autophagy flux by the expression of shRNAs against LC3 can reduce mass loss of skeletal muscle in transgenic $SOD1^{G93A}$ mice[36]. Therefore, suitable functional status of autophagy plays a crucial role in the maintenance of skeletal muscle quality. When autophagy appears, LC3-I, after the ubiquitin-like processing and modification, combines with PE in the surface of cells to form LC3-II. LC3-II has always been in the membrane of intracellular autophagosomes, and its content is in proportion to the quantity of autophagic vacuoles. Thus, the expression amount of LC3 is closely correlated with the activity of autophagy[37]. In the current research, an evident increase in LC3-II/LC3-I ratio can be the biomarker for autophagy activation. On the other hand, p62, as the bridge for connecting LC3 and ubiquitinated protein, makes use of USP to transfer receptors including polymerized protein, impaired mitochondria and invasive bacteria into autophagosomes for degradation. Generally, the intracellular expression of p62 is negatively correlated to the activity of autophagy[38]. Atg7 is the key gene in the upper ubiquitin-like protein pathway. It plays a positive role in activating LC3-I, promoting the combination of LC3-I and PE to form LC3-II, and shaping essential component for autophagic vacuoles [39]. Beclin1 is used to adjust the position of other ATG proteins in autophagy precursor and regulate the functional status or activity of autophagy after combining with phosphatidylinositol 3-kinase III (PI3K-III). In the present study, LC3-II/LC3-I ratio, Atg7 and Beclin1 in skeletal muscle of suspended rats, were increased, while the content of p62 was reduced, indicating that HS-induced autophagy in skeletal muscle can probably result in the excessive digestion of cells and the

excessive degradation of key cellular components, so as to lead to cell death. As some proteins modulate both autophagy and apoptosis, there exists a crosstalk between them. For instance, the antiapoptotic protein Bcl-2 can bind to Beclin1 and segregate Beclin1 away from class III PI3K, thus leading to an inhibited autophagy. To reveal the underlying molecular mechanisms of unloading-induced skeletal muscle atrophy, we have also detected apoptosis-related proteins. Caspase-3 is a predominant effector caspase in apoptosis and Bax is the most widely studied pro-apoptotic protein in Bcl-2 family. Hindlimb suspension could reduce the expression of Bcl-2 and Caspase-3, but result in the increased expression of Bax. The Beclin1-Bcl-2 complex is probably used to regulate the change of HS-induced autophagy and apoptosis in skeletal muscle, which serves as the intracellular rheostat to maintain autophagy level in the range of physiological stability and not to exceed non-physiological range to induce cell death[40]. Bcl-2, with the function of modulating autophagy, can help to keep autophagy within a reasonable range[41]. In our HS-induced model with skeletal muscle atrophy, once autophagy is activated, the expression of Bcl-2 is significantly reduced, indicating that the induction of autophagy is probably beyond the normal physiological range, so it can also result in cell death and skeletal muscle atrophy.

AMPK is recognized as the energy receptor of cells, and it will be activated when the intracellular AMP is elevated. Generally, the activation of AMPK will initiate the metabolic pathway to generate ATP, and restrain ATP-consumed cell proliferation and biosynthesis. Recently, AMPK is reported to imply in controlling muscle mass by regulating protein degradation through the ubiquitin-proteasome system and autophagy[42]. In the present study, AMPK is activated in skeletal muscle after hindlimb suspension. Similarly, previous evidence also suggests that AMPK activation can regulate myofibrillar protein degradation through transcription factor FoxO[43]. The FoxO family including FoxO1, FoxO3, FoxO4, and FoxO6 in mammals plays a crucial role in the regulation of diverse cellular functions such as apoptosis, differentiation, metabolism, proliferation, and cell survival[44]. FoxO transcription factors integrate cellular signals emanating from insulin, growth factors, cytokines, and oxidative stress[45]. AMPK can directly phosphorylate FoxO3[46]. In the present investigation, we have found that AMPK activation is accompanied by an increase of FoxO3, and then the phosphorylation of FoxO3 may regulate MuRF1 and Atrogin-1 expression, thus leading to the atrophy of skeletal muscle. In addition, FoxO family is also involved in autophagy. FoxO3 is a potent transcriptional activator responsible for the induction of autophagy-related genes, thus leading to increased autophagy[47], as evidenced by enhanced LC3-II/LC3-I ratio, Atg7, and Beclin1, and declined p62 in skeletal muscle during HS-induced rat model with skeletal muscle atrophy. Taken together, HS-induced autophagy through AMPK-FoxO3 signal pathway and FoxO3-mediated autophagy play a vital role in HS-induced atrophy of skeletal muscle.

Conclusions

Hindlimb suspension can induce the atrophy of skeletal muscle via the signal pathway involving the activation of AMPK, the phosphorylation of FoxO3, and the induction of Atrogin-1 and MuRF1. At the same time, FoxO3-mediated autophagy plays an important role in HS-induced atrophy of skeletal muscle.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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