Chronic resistance training activates autophagy and reduces apoptosis of muscle cells by modulating IGF-1 and its receptors, Akt/mTOR and Akt/FOXO3a signaling in aged rats

Li Luo a,b, A-Ming Lu a, Yan Wang b, An Hong a, Yulan Chen a, Juan Hu a, Xiaoning Li c, Zheng-Hong Qin b,*

a School of Physical Education and Sports Science, Soochow University, Suzhou 215020, China
b Department of Pharmacology and Laboratory of Aging and Nervous Diseases (SZS0703), Soochow University School of Pharmaceutical Science, Suzhou 215123, China
c School of Physical Education and Sports Science, Soochow University, Suzhou 215021, China

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Abstract

Resistance exercise training (RET) remains the most effective treatment for the loss of muscle mass and strength in elderly people. However, the underlying cellular and molecular mechanisms are not well understood. Recent evidence suggests that autophagic signaling is altered in aged skeletal muscles. This study aimed to investigate if RET affects IGF-1 and its receptors, the Akt/mTOR, and Akt/FOXO3A signaling pathways and regulates autophagy and apoptosis in the gastrocnemius muscles of 18–20 month old rats. The results showed that 9 weeks of RET prevented the loss of muscle mass and improved muscle strength, accompanied by reduced LC3-II/LC3-I ratio, reduced p62 protein levels, and increased levels of autophagy regulatory proteins, including Beclin 1, Akt5/12, Akt7, and the lysosomal enzyme cathepsin L. RET also reduced cytochrome c level in the cytosol but increased its level in mitochondrial fraction, and inhibited cleaved caspase 3 production and apoptosis. Furthermore, RET upregulated the expression of total AMPK, phosphorylated AMPK, and FOXO3A. Taken together, these results suggest that the benefits of RET are associated with increased autophagy activity and reduced apoptosis of muscle cells by modulating IGF-1 and its receptors, the Akt/mTOR and Akt/FOXO3A signaling pathways in aged skeletal muscles.

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1. Introduction

Aging is accompanied by a progressive loss of skeletal muscle mass and strength, leading to the loss of functional capacity and an increased risk for developing chronic metabolic diseases such as diabetes. Mitochondrial dysfunction and deregulation of apoptotic signaling have been implicated in the onset and progression of skeletal muscle aging (Marzetti et al., 2008a). Recent studies suggest that cellular quality control mechanisms such as autophagic signaling participate in the skeletal muscle aging process (Burks and Cohn, 2011; Combaret et al., 2009). Macroautophagy requires the synthesis of double membrane structure to form autophagosomes. Autophagosome formation is under the control of the autophagy-related proteins (ATC). ATC12–ATG5 complex participates in the formation of autophagosomal membranes (Rubinsztein et al., 2011). Atg7 is required for the formation and expansion of autophagosomes (Komatsu et al., 2005; Mizushima et al., 1998). Beclin 1 is essential partner in the autophagy interactor that signals the onset of autophagy (Cao and Klionsky, 2007; Yang and Klionsky, 2010). The microtubule-associated protein light chain, LC3, a mammalian homologue of yeast Atg8, is essential for the formation of the early autophagosomes (Ravikumar et al., 2010). p62, which is degraded by lysosomes together with autophagosome inclusions, indicates lysosomal degradation of autophagosomes (Bjorkoy et al., 2005, 2009).

Autophagy plays important roles in cell growth and development, organelle biogenesis and turnover, and in the control of the precise balance between protein synthesis and degradation (Rubinsztein et al., 2011). Autophagy plays a protective role in aging skeletal muscle of rats that were mildly calorie restricted (Wohlgemuth et al., 2010). Autophagy also contributes to the protective effects of chronic exercise on diabetes in a mouse model (He et al., 2012). The decline in autophagic potential in aging skeletal muscles has been demonstrated recently (Combaret et al., 2009; Gaugler et al., 2011; McMullen et al., 2009; Wohlgemuth et al., 2010). Resistance exercise training (RET) remains the most effective intervention for increasing muscle mass and strength in elderly people (Borst, 2004). However, the cellular and molecular mechanisms mediating training-induced adaptations are not yet completely understood. We hypothesized that some of the health benefits of RET on the elderly might be mediated by autophagy activation and apoptosis inhibition.

The IGF-1/Akt/mTOR signaling pathway is essential for muscle growth during development and regeneration (Schiaffino and Mammucari, 2011). Interestingly, IGF-1 has an inhibitory effect on autophagy through...
the activation of AKT and mTOR (Rubinsztein et al., 2011). Akt/mTOR signaling and Akt/FOXO3a signaling are major regulators in skeletal muscle autophagy (Mammucari et al., 2008; Zhao et al., 2007). We speculated that IGF-1 and its receptors, the Akt/mTOR and Akt/FOXO3a signaling pathways may be modulated by resistance exercise, and the regulation of these pathways leads to the promotion of autophagy and the inhibition of apoptosis. Aged skeletal muscle is characterized by specific type I muscle fiber atrophy (Koopman and van Loon, 2009) and 92% of the white portions of gastrocnemius muscle are type II fibers (Delp and Duan, 1996). Therefore, in this study we chose white gastrocnemius muscles as the model to investigate how RET affects the regulation of autophagy and apoptosis as well as the regulatory signaling pathway in the muscles of aged rats.

### 2. Materials and methods

#### 2.1. Animals and treatments

Old (18–20 month) male Sprague Dawley rats (554 ± 39 g) were obtained from the Experimental Animal Center of Soochow University (certificate no. 20020008, grade II). One week after arriving at the facility, rats were randomly assigned to two groups (n = 9): (1) sedentary control group (SC), and (2) resistance training group (RET). The rats were kept in individual cages with standard food and water ad libitum in a temperature (22 ± 2.5 °C) and light-controlled (12:12 h light–dark cycle) environment. The study protocols were approved by the Animal Care Ethical Committee of Soochow University.

#### 2.2. Resistance exercise training

The resistance training protocol for aged rats was established according to previous studies (Duncan et al., 1998; Grossman et al., 1997; Yang et al., 2006). Climbing of a one-meter ladder with 2 cm grid ladder inclined at 85° with weights attached to the rat tail was used as resistance exercise. In the first week, rats were familiarized with climbing up to the top with or without weight on their tails. After familiarization, the resistance training began using lead weights attached to the base of the tail. The rats were positioned at the bottom

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sedentary control group</th>
<th>Resistance training group</th>
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<tbody>
<tr>
<td>BW (g)</td>
<td>554 ± 39 a</td>
<td>489 ± 32 a</td>
</tr>
<tr>
<td>MW (g)</td>
<td>1.75 ± 0.05b</td>
<td>1.87 ± 0.06b</td>
</tr>
<tr>
<td>MW/BW (mg/g)</td>
<td>3.2 ± 0.03c</td>
<td>3.8 ± 0.044c</td>
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**Fig. 1.** Chronic resistance exercise training improves the morphology and strength of aged skeletal muscles. A. Histological cross-section of the white gastrocnemius muscle in the sedentary control (SC) group. B. Histological cross-section of the white gastrocnemius muscle in the resistance training (RET) group. C. Mean minor axis diameters of the white gastrocnemius muscles in the SC and the RET groups. D. Latency to fall by the wire suspension test in the SC group and the RET group. H&E staining. Asterisks indicate significant differences between the two groups (**P < 0.01)**.
of the climbing apparatus and motivated to climb the ladder by a grooming action to the tail. The initial weight attached was 10% of their body weight and gradually increased in the 9 weeks of training period. Ten percent of body weight was added to the prior weight every week. The resistance training consisted of 10 repetitions every day, and when the rats reached the top of the ladder, they were allowed to recover in the resting area for 10–20 s. The rats in the RET group were trained 3 days/week (Monday, Wednesday, and Friday) for 9 weeks.

Fig. 2. Chronic resistance exercise promotes autophagy in aged skeletal muscles. Protein levels of Beclin 1 (A), Atg5/12 (B), Atg7 (C), LC3 (D), P62 (E), and cathepsin L (F) in the white gastrocnemius muscle in the aged rats from sedentary control (SC) group and the resistance exercise training (RET) group were detected by Western blot analysis. Asterisks indicate significant differences between the two groups (***P < 0.01).
2.3. Wire suspension test

Wire suspension test was used to determine the muscle strength of aged rats as described previously (Shukitt-Hale et al., 1998). Rats were raised to an elevated taut horizontal wire (55 cm above the table top) and the forepaws of each rat were placed on the wire. Each rat was given one trial, with the total hanging time in seconds recorded (max scores: 60 s). To protect the falling rat from being hurt, a water basin was placed underneath the wire.

2.4. Tissue processing

Forty-eight hours after the last training, the animals were anesthetized with pentobarbital sodium (2.5%, 0.2/100 ml/g). After shaving and cleaning the skin, gastrocnemius muscles were dissected and weighed, the white (superficial) portions of the gastrocnemius muscle were separated into two equal parts, one for histological and immunohistochemical analyses, and the other for enzyme-linked immunosorbent assay (ELISA) and Western blot analysis. The rats in the SC group were anesthetized and sampled in the same way as those in the RET group. The tissues for histological and immunohistochemical analyses were immediately trimmed and fixed by immersion in 10% PBS buffered formalin for 24 h. The blocks were dehydrated in a graded series of ethanol and embedded in paraffin wax. The tissues for ELISA and Western blot analysis were stored at −80 °C until use.

2.5. Morphometric evaluation of the gastrocnemius muscle

Muscles were cut at 4 μm using a microtome (Leica-RM 2135, Germany). Serial sections were stained with hematoxylin and eosin (H&E) and analyzed with a light microscope. The minor axis diameters (i.e., the smallest diameter) of the myofibers in each of the images were measured using the image analysis software Image Pro Plus under ×200 magnification.

2.6. Enzyme-linked immunosorbent assay (ELISA)

The amount of total IGF-1 and IGF-1R protein in white gastrocnemius muscle was measured using rat IGF-1 and IGF-1R ELISA kits (#DRE20761, #DRE20814, Fengxiang Biotechnology, Shanghai, China). Frozen gastrocnemius muscle tissue was dissolved in nine volumes of lysis buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 10% glycerol) supplemented with protease inhibitor cocktail tablet (Roche Diagnostics), homogenized with a glass homogenizer and incubated on ice for 20 min. The lysates were centrifuged at 12,000 g for 30 min at 4 °C to obtain the cytosol (supernatant) and mitochondria (pellet) fractions. The supernatant was centrifuged at 100,000 g for 30 min at 4 °C to obtain the mitochondrial fraction. The amount of protein in each sample was subjected to centrifugation at 600 g for 10 min at 4 °C, then centrifuged at 12,000 g for 20 min at 4 °C. The supernatant was subjected to centrifugation at 100,000 g for 30 min at 4 °C to obtain the mitochondrial fraction. The amount of protein in each sample was determined using the Bradford protein assay kit (BioRad, CA, USA). The proteins were separated using 10% SDS-PAGE and transferred to nitrocellulose membranes, and incubated with the appropriate primary antibodies, LC3-II (#ab62721, Abcam, Cambridge, MA, USA), Caspase 3 (#AC031, Beyotime Institute of Biotechnology, China), FoxO3a (#2497, CST, USA), phospho-AMPK (Ser2448) (#2971, CST, USA), phospho-Akt (Ser473) (#AA329, Beyotime Institute of Biotechnology, China), mTOR (#2983, CST, USA), and GAPDH as loading control.

2.7. Preparation of mitochondrial and cytosolic fractions

The mitochondrial and cytosolic fractions were prepared from gastrocnemius muscles using a commercially available cytosol/mitochondria fractionation kit according to the manufacturer’s protocol (Beyotime Institute of Biotechnology, Nantong, China). Briefly, gastrocnemius tissues were homogenized by using a glass homogenizer on ice. The homogenate was subjected to centrifugation at 600 g for 10 min at 4 °C. Then, the supernatant was collected and centrifuged again at 12,000 g for 30 min at 4 °C to obtain the cytosol (supernatant) and mitochondria (pellet) fractions. The supernatant was centrifuged at 100,000 g for 1 h at 4 °C to generate the cytosolic fraction. Samples of the cytosol and mitochondria were dissolved in lyses buffer and subjected to Western blot analysis.

2.8. Western blot analysis

Frozen samples were homogenized (1:9 w/v) in ice-cold buffer (20 mM Tris–HCl, 1 mM EDTA, 150 mM NaCl, 2% Triton X-100, pH 7.5) supplemented with phosphatase inhibitors (PhosSTOP tablet; Roche Diagnostics, Indianapolis, USA) and protease inhibitors (Complete tablet; Roche Diagnostics). The homogenates were incubated at 4 °C for 30 min, and then centrifuged at 12,000 g for 20 min at 4 °C. The supernatant was collected for protein concentration determination using Bradford protein assay kit (BioRad, CA, USA). Forty-five micrograms of protein from each sample was subjected to 6%, 10%, or 15% SDS-PAGE, then transferred to nitrocellulose membranes, and incubated with the following primary antibodies, LC3-II (#ab62721, Abcam, Cambridge, MA, USA), Atg5/12 (#8540, CST, USA), Atg7 (#AA820, Beyotime Institute of Biotechnology), Beclin 1 (Santa Cruz Biotech, Santa Cruz, CA, USA), cathepsin L (#ab6314, Abcam, Cambridge, MA, USA), P62 (#PW1860, Enzo Life Science, Exeter, UK), Akt (#4691, CST, USA), phospho-Akt (Ser473) (#AA329, Beyotime Institute of Biotechnology, China), mTOR (#2983, CST, USA), phospho-mTOR (Ser2448) (#2971, CST, USA), caspase 3 (#AC031, Beyotime Institute of Biotechnology, China), AMPK (#2603, CST, USA), phospho-AMPK (#2535, CST, USA), FOXO3a (#2497, CST, USA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, #AG019, Beyotime Institute of Biotechnology, Nantong, China), and Cox VI (#AC610, Beyotime Institute of Biotechnology) for 1 h at 37 °C. Membranes were washed and incubated with horseradish peroxidase-conjugated second antibody (anti-mouse, anti-rabbit) for 1 h at 37 °C. Immuneactivity was detected with Super-Signal-West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL, USA) according to the manufacturer’s instructions, and analyzed with Sigma Scan Pro 5 with GAPDH as loading control.

2.9. TUNEL assay

The apoptosis was detected by using TdT-mediated dUTP nick end labeling (TUNEL) kit (Roche Molecular Biochemicals, Pleasanton, CA, USA) according to the manufacturer’s recommendations. Briefly, white gastrocnemius muscle cross sections were cut at 4 μm using a microtome (Leica-RM 2135, Germany), dewaxed and rehydrated according to standard protocols, blocked in 3% H2O2 in 100%
methanol at room temperature for 30 min, and permeabilized in 0.1% Triton X and 0.1% sodium citrate. TUNEL reaction mix was added and the sections were incubated in a humidified chamber at 37 °C for 1 h in the dark. Negative control experiments were performed in which the terminal deoxynucleotidyl transferase (TdT) enzyme was not added to the TUNEL reaction mixture. Sections were reacted with fluorescein-conjugated secondary antibody for 30 min at 37 °C, and substrate was added for color development. TUNEL-positive nuclei were counted and the data were expressed as TUNEL index, which was calculated by counting the number of TUNEL-positive nuclei divided by the total number of the nuclei. The TUNEL index for each muscle was calculated from six random, non-overlapping fields with an objective magnification of ×40.

2.10. Statistical analysis

Data were expressed as mean ± standard deviation (SD). The groups were compared using one way analysis of variance (ANOVA), and the Newman–Keuls test was used to determine the significance of differences among all experimental groups. Data were analyzed using GraphPad Prism 5.0 statistical software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. RET prevents the muscle loss and improves the strength of aged skeletal muscles

Age-related muscle loss is characterized by the loss of muscle fibers and a decrease in fiber cross-sectional area primarily in type II fibers (Adamo and Farrar, 2006). Nine weeks of RET significantly increased the size of the gastrocnemius muscle fibers in aged SD rats. The gastrocnemius muscle mass was significantly greater in the RET group than that in the SC group (P<0.01, Table 1). Muscle mass/body mass ratio for the gastrocnemius muscles was significantly greater in the RET group than that in the SC group (P<0.01, Table 1). The minor axis diameters (i.e., the smallest diameters) of the white gastrocnemius myofibers in the RET group were significantly larger than that in the SC group (P<0.01) (Fig. 1A and B, ×200 magnification; Fig. 1C). Moreover, the wire suspension test showed that RET led to a significant increase in the strength of forearms (P<0.01, Fig. 1D).

3.2. RET increases autophagic activity in aged skeletal muscles

Next, we analyzed the autophagic markers in aged skeletal muscles. The results showed that Beclin-1, Atg5/12 and Atg levels were significantly higher in the RET group than that in the SC group (P<0.01, Fig. 2A–C). However, the LC3-II/LC3-I protein ratio was significantly lower in the RET group than that in the SC group (P<0.01, Fig. 2D), which was mainly due to a decrease in expression of LC3-II. In addition, the protein level of an autophagy substrate p62 was significantly lower in the RET group compared to the SC group (P<0.01, Fig. 2E). RET also significantly increased the level of active cathepsin L (P<0.01, Fig. 2F). Collectively, these data indicate that RET increases autophagic activity in aged skeletal muscles.

3.3. RET inhibits the apoptosis of aged skeletal muscles

Mitochondria-mediated apoptosis represents a central process driving age-related muscle loss. Activation of apoptotic cascade includes the release of mitochondrial cytochrome c and the activation of caspase-3 (Marzetti and Leeuwenburgh, 2006; Marzetti et al., 2008b). The present study examined the apoptosis in white gastrocnemius muscles with TUNEL, and the results showed that muscles from the SC group showed more TUNEL-positive nuclei than those from the RET group (P<0.01, Fig. 3). Then, we measured mitochondrial cytochrome c release and the results showed that cytochrome c levels in the cytosol were significantly reduced while its levels in the mitochondrial fraction were significantly increased in the RET group compared to the SC group (P<0.01, Fig. 4A–B). Finally, we detected the activation of caspase 3, a marker of apoptosis. Western blot analysis showed that RET significantly downregulated the levels of cleaved caspase 3 (P<0.01, Fig. 4C). Taken together, these results demonstrate that RET inhibits the apoptosis of aged skeletal muscles.

3.4. RET modulates the expression of IGF-1 and its receptors, Akt, mTOR, AMPK and FOXO3a in aged skeletal muscles

ELISA results showed that protein levels of IGF-1 and IGF-1R were significantly higher in the RET group compared to the SC group (P<0.01, P<0.01, respectively, Fig. 5A–B). Western blot analysis showed that the phosphorylation of Akt was significantly lower in RET compared to SC (P<0.01, Fig. 5C). Both mTOR and FOXO3a are downstream targets of Akt. The phosphorylation of mTOR was significantly lower in RET compared to SC (P<0.01, Fig. 5D), and FOXO3a was significantly higher in RET compared to SC (P<0.01, Fig. 5G). We also detected the levels of total and phosphorylated AMPK, which are also implicated in the modulation of mTOR signaling. Western blot analysis showed that RET increased both total and phosphorylated AMPK levels in the muscles (Fig. 5E–F).

4. Discussion

In this study, we found that the rats in the RET group exhibited larger muscle mass and greater mean of minor axis diameters of the myofibers than those in the SC group. Chronic RET also improved the muscle strength of the aged rats. Activation of autophagic pathway and inhibition of apoptosis were observed, and these changes were associated with the alterations in IGF-1 and its receptors, the Akt/mTOR and Akt/FOXO3a pathways.

4.1. Chronic resistance exercise activates autophagic pathway

The reduced expression of Atg proteins, as well as other proteins required for autophagy induction, has been shown in aged tissues (Rubinsztein et al., 2011). Studies in rat muscles suggested an age-related decline in autophagic degradation and a concomitant age-related increase in oxidative damage and apoptosis, both of which correlate negatively with autophagy (Wohlgemuth et al., 2010). In this study, we found that exercise resulted in a decrease in the LC3-II/LC3-I ratio in the RET group. Meanwhile, there was an increase in Beclin 1, Atg5/12, Atg7, and lysosomal cathepsin L levels and a decline in the autophagy substrate protein p62. An increase in LC3-II (or autophagosomes) may represent an autophagy induction, or a blockade in degradation of autophagosomes (Klionsky et al., 2012). Macroautophagy decreases with aging due to a decreased formation of autophagic vacuoles combined with an even more marked delay of fusion of autophagic vacuoles with lysosomes (Terman, 1995). An accumulation of autophagic vacuoles in aging tissues is evident even though the autophagic flux is diminished (Cuervo and Dice, 2000). Therefore, the decrease in the LC3-II/LC3-I ratio we observed in the present study could be caused by an increased autophagic degradation process. This could be supported by the evidence of increased active lysosomal cathepsin L and decreased autophagic substrate p62.

Skeletal muscle requires a rapid and efficient system for the removal of abnormal organelles, the elimination of dysfunctional proteins, and the disposal of toxic products that may lead to cell death (Paolo Grumati, 2012). Inactivation of Atg5 and Atg7 in mice skeletal muscle induces a severe muscle loss associated with ultrastructural alterations of cytosolic organelles, such as mitochondria, and myofiber degeneration (Masiero et al., 2009; Raben et al., 2008). Wu et al. reported that cells derived from Atg7−/− mice exhibited altered metabolic profile
characterized by decreased resting mitochondrial oxygen consumption and a compensatory increase in basal glycolytic rates, and increased steady state levels of reactive oxygen species (Wu et al., 2009a). Grumati et al. demonstrated that the induction of autophagy in the skeletal muscles post-exercise was able to prevent the accumulation of damaged organelles and maintain cellular homeostasis (Grumati et al., 2011).

Although the important link between autophagy and physical exercise has been proposed, there is a limited understanding about the autophagic process in aging skeletal muscles and how the regulation of autophagy is altered via different modes of exercises in aged skeletal muscle. Several lines of evidences indicate that the effects of exercise on autophagy activity depend on the mode of exercise (e.g., intensity, duration, frequency), and the types of tissues. A single bout of exercise increased the autophagic activity in the vastus lateralis in men (Jamart et al., 2011), decreased autophagic activity in mouse skeletal muscles (Kim et al., 2012), or produced a biphasic change in autophagic activity in rat cardiac muscles during the recovery period (Ogura et al., 2011). Further studies will be important to fully clarify the alterations of autophagy and underlying molecular mechanisms by exercise in aged muscles.

There is growing evidence that autophagy may account for some of the benefits of exercise (Garber, 2012; He et al., 2012; Jamart et al., 2011, 2012). Previous studies demonstrated that chronic aerobic exercise could trigger the autophagic signaling in muscles (Wohlgemuth et al., 2011). Impaired autophagy leads to the accumulation of damaged proteins and dysfunctional contractile proteins, which might produce less force and contribute to impaired muscle quality (Lee et al., 1998; Wohlgemuth et al., 2010). Therefore, autophagic activity may be a critical determinant of skeletal muscle structure and function, and its modulation will be a potential strategy against skeletal muscle aging (McMullen et al., 2009; Paolo Grumati, 2012; Wohlgemuth et al., 2010).

4.2. Chronic resistance exercise inhibits apoptosis in aged skeletal muscle

Mitochondria-mediated apoptosis represents a central process driving age-related muscle loss and fatigability. Enhanced ROS production increases mitochondrial apoptotic susceptibility and reduces transcriptional drive for mitochondrial biogenesis, which contribute to the progressive loss of skeletal muscle mass and strength (Chabi et al., 2008; Marzetti et al., 2008b). The basal rate of cytochrome c release in subsarcolemmal mitochondria was 3.5-fold higher in senescent compared to young animals (Chabi et al., 2008). In this study, we found that chronic resistance exercise decreased the release of cytochrome c from the mitochondria to the cytosol, and the level of cleaved caspase 3. Furthermore, a low level of TUNEL index was observed in the RET group. These results suggest that chronic resistance exercise inhibits mitochondria-mediated apoptosis in aged skeletal muscle.
Fig. 5. Chronic resistance exercise modulates Akt/mTOR signaling in aged skeletal muscle. Protein levels of IGF-1 (A), IGF-1R (B), Akt phosphorylation at Ser473 (C), mTOR phosphorylation at Ser2448 (D), AMPK (E), phosphorylated AMPK (F), and FOXO3a (G) in the white gastrocnemius muscle in the aged rats from the sedentary control (SC) group and the resistance exercise training (RET) group were detected by Western blot analysis. Asterisks indicate significant differences between the two groups (**P<0.01).
4.3. Chronic resistance exercise increases the expression of IGF-1 and its receptors

IGF-1 plays a critical role in cell proliferation and differentiation (Schiaffino and Mammucari, 2011). IGF-1 regulates satellite cell function by boosting both proliferation and differentiation during muscle regeneration (Jang et al., 2011) and restores satellite cell proliferative potential in old muscle (Chakravarty et al., 2000). Interestingly, IGF-1 can promote mitochondrial biogenesis and mitochondrial DNA replication independent of Akt/mTOR signaling (Echave et al., 2009). In addition, IGF-1 inhibits apoptosis (Li et al., 2012b). It is known that the decline of the IGF-1 level contributes to the progress of muscle atrophy in senescence (Li et al., 2003; Musaro et al., 2001; Winn et al., 2002). Chronic resistance exercise has been shown to restore the levels of IGF-1 and its receptors in aged skeletal muscles (Adamo and Farrar, 2006; Kim et al., 2005; Urso et al., 2005). Our results are consistent with these previous studies.

4.4. Chronic resistance exercise modulates Akt/mTOR and Akt/FOXO3a signaling

Akt has been known to negatively regulate autophagy through activation of mTOR (Mammucari et al., 2008; Rubinsztein et al., 2011) and inactivation of FOXO3a by phosphorylation of these proteins (Zhao et al., 2007). However, the role of Akt in the response of aged muscle to mechanical load is less clear. A number of studies on Akt activity in aged muscles reported divergent results ranging from a marked decline, little change, to a relative and absolute increase (Gaugler et al., 2011; Li et al., 2003; Paturi et al., 2010; Wu et al., 2009b). The effects of resistance exercise on Akt activity have not reached a consensus (Haddad and Adams, 2006; Mayhew et al., 2009; Williamson et al., 2010). These discrepancies may result from the diversity of the animal species examined, the mode of the resistance exercise, the type of muscles surveyed, the ages and sex used for comparison, the time of harvesting muscle, and the Akt phosphorylation site detected. In the present study, we found that Akt phosphorylation at Ser473 decreased after chronic resistance exercise, which indicates that Akt is involved in the exercise-induced decrease in mTOR phosphorylation.

mTOR is a serine/threonine protein kinase that regulates cell growth and proliferation in response to growth factors, nutrient signals, and energy status (Oldham and Hafen, 2003). mTOR also negatively regulates autophagy, which plays an important role in aging (Jia et al., 2004). The inhibition of the IGF-1/PI3K/mTOR pathway and the induction of autophagy were associated with the delay of the onset of age-related disorders [Blagosklonny, 2010; Demidenko et al., 2009]. In this study, we found that mTOR phosphorylation at Ser2448 was significantly lower after chronic resistance exercise, indicating that the inhibition of mTOR signaling may lead to the activation of autophagic pathway and contribute to the beneficial effects of chronic resistance exercise on skeletal muscle.

Several studies suggested that age-related inhibition of mTOR signaling after an exercise bout or training is related to the limited capacity of hypertrophy in aged individuals (Faini et al., 2006; Thomson and Gordon, 2006). However, a recent study showed that aged skeletal muscle conserved the potential of muscle hypertrophy induced by exercise (Koopman and van Loon, 2009). Mayhew et al. found that after 16 weeks of RET, muscle mass, type II myofiber size, and voluntary strength were similar in young and old men. At the level of translational signaling, there was no evidence of impaired responsiveness among older adults (Mayhew et al., 2009). Drummond et al. also reported that muscle protein synthesis and mTOR signaling in response to resistance exercise were similar between young and old men, although the response was delayed with aging (Drummond et al., 2008).

It is important to note that AMPK also regulates mTOR phosphorylation in the cells. Aging-associated reduction in AMPK activity may contribute to the reduced mitochondrial function and dysregulated intracellular lipid metabolism (Reznick et al., 2007). Older men had decreased AMPKα2 activity and lower phosphorylation of AMPK and its substrate acetyl-CoA carboxylase (ACC) (Li et al., 2012a). The activation of AMPK and the inhibition of mTOR after a single bout of exercise or exercise training have been observed in animal studies as well as human studies (Drummond et al., 2008; Thomson and Gordon, 2006). In the present study, we showed that the phosphorylation of AMPKα at Thr172 was increased in aged skeletal muscle after 9 weeks of resistance exercise, confirming the results of the previous studies.

Akt/FOXO3a is known to be one of the central signaling pathways modulating autophagy in skeletal muscles by activating LC3 (Mammucari et al., 2008). Demontis et al. revealed that muscle aging is characterized in Drosophila by the progressive accumulation of protein aggregates that associate with impaired muscle function and FOXO plays a key role in delaying muscle functional decay (Demontis and Perrimon, 2010). The present study showed the activation of FOXO3a by RET in aged skeletal muscle, which may participate in the activation of autophagy.

The regulation of the autophagic machinery is highly complex and involves several signaling pathways which often crosstalk with each other (Paolo Grumati, 2012). In the present study, we provide evidence that multiple autophagy signaling molecules are activated by chronic resistance exercise.

5. Conclusions

In conclusion, this study demonstrates that chronic resistance exercise is a newly defined stimulus that induces autophagy and inhibits apoptosis in aged skeletal muscles, which may contribute to the exercise-induced beneficial effects. Mechanistically, our results suggest that the modulation of Akt/mTOR and Akt/FOXO3a signaling is implicated in the regulation of autophagy by chronic resistance exercise.

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