



Short Communication

Resistance exercise reduced the expression of fibroblast growth factor-2 in skeletal muscle of aged mice

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ABSTRACT

Background: Fibroblast growth factor-2 (FGF-2) protein level has been shown to be elevated in aged mice muscle compared to adult mice. It activates the satellite cell quiescence, which leads to satellite cell depletion and may accelerate aging process. The purpose of this study was to see the effect of resistance exercise on skeletal muscle FGF-2 protein level in aged mice.

Methods: This study included eight young adult control C57BL/6 male mice (age 12 weeks, YCON group) and 14 aged C57BL/6 male mice (age 19 months), randomly divided into two groups (old control, OCON, $n=7$; old resistance exercise, ORT, $n=7$). Resistance ladder climbing exercise was conducted 3 d/wk for 12 weeks. Soleus and tibialis anterior muscles were collected for body composition, relative grip strength, and muscle wet weight and for enzyme-linked immunosorbent assay protein analysis.

Results: Relative soleus muscle wet weight and hindlimb lean mass showed a significant increase in ORT group compared to OCON group ($p=0.013$ and $p=0.015$, respectively). In relative grip strength, both OCON and ORT showed a significant decrease compared to YCON ($p<0.001$ and $p=0.011$, respectively). However, ORT showed a significant increase compared to OCON ($p=0.02$). OCON showed a significant increase in skeletal muscle FGF-2 protein level compared to YCON in soleus ($p=0.035$), and ORT showed a significant decrease compared to OCON in soleus muscle ($p=0.045$). FGF-2 protein level was significantly decreased in tibialis anterior muscle in the ORT group compared to OCON ($p=0.022$). Correlation analysis showed a negative correlation between FGF-2 protein level and soleus and tibialis anterior muscle weight ($r^2=0.514$, $p=0.0035$; $r^2=0.312$, $p=0.025$, respectively).

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Conclusion: Twelve weeks of resistance exercise reduced soleus and tibialis anterior muscle FGF-2 protein level in aged mice with improvement in appendicular lean mass and muscle strength. Thus, resistance exercise could be an efficient intervention for preventing age-related increase of FGF-2 in skeletal muscle.

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1. Introduction

Aging is a natural phenomenon that all living thing creatures experience with time. With aging, various physiological changes occur, although muscle aging is prominent.¹ Age-related muscle loss can be characterized as decrease of muscle mass, motor unit, and muscle cross sectional area, and finally decrease of muscle quality.^{1–3} Muscle loss in particular can lead to a decrease of functional capacity of muscle and increase risk of developing age-related problems, such as chronic diseases.⁴

Recently, muscle has been shown to secrete various cytokines and growth hormones during contraction.⁵ Because muscle-derived cytokines and growth hormones induce various systemic changes on muscle and/or other tissues, numerous studies have investigated the effect of these cytokine and growth hormones in muscle from aged individuals.⁶ Among these muscle-derived cytokines and/or growth factors, fibroblast growth factor 2 (FGF-2) regulates muscle regeneration and maintenance.⁷ Clarke and Feeback⁸ demonstrated an increase in cytoplasmic secretion of FGF-2 by disruption of plasma membrane homeostasis, which is induced by chronic mechanical stress on the muscle cells. This muscle-derived FGF-2 influences muscle growth and regeneration by increased proliferation of satellite cells.⁸

However, recent study has demonstrated elevation of muscle fiber FGF-2 protein level in homeostasis of aged mice compared to younger adult mice, which induced a negative effect on muscle aging.⁹ Normally, satellite cells are in quiescence to maintain the stem cell pool¹⁰; however, chronically increased FGF-2 in muscle fiber activates satellite cells and increased the satellite cell cell-cycle.⁹ An increase of satellite cell cell-cycle leads to satellite cell depletion, and it may cause acceleration of muscle aging.^{9,10} This study suggested that it is necessary to regulate the muscle FGF-2 to prevent age-related muscle loss and/or muscle capacity loss.

Numerous studies recommend exercise as one of the strategies to improve strength and muscle mass, and it causes numerous positive physiological changes.^{5,11,12} With one of the muscle's important characteristics, plasticity,³ muscle can adapt to environment throughout life, and trainability of skeletal muscle and muscle improvement has been demonstrated in aging.⁶ Also, exercise accelerates production of various cytokines and growth factors or regulates homeostasis.⁵ In particular, numerous studies have demonstrated increased FGF-2 protein level due to mechanically induced cell wound or exercise, and it leads to muscle regeneration and/or muscle hypertrophy.^{13,14} In addition, there is developing evidence that FGF-2 influences muscle

hypertrophy and angiogenesis in human and rodents throughout resistance exercise.^{7,15} FGF-2 level was significantly increased from the baseline at 2 weeks of strength training in human.¹⁶ Also, the FGF-2 level of healthy older people was appeared to a sudden vigorous resistance exercise.¹⁵ Although basal FGF-2 protein level was shown to be elevated in aged individuals, no study yet has reported the result about exercise-induced changes in FGF-2 protein level of aged muscle. Therefore, in this study, we investigated the effect of resistance exercise on change of muscle FGF-2 protein level in aged mice. We hypothesized that resistance exercise may decrease FGF-2 protein level in skeletal muscle of aged mice with increase in muscle mass, muscle quality compared with untreated aged mice.

2. Methods

2.1. Animals

Eight young male C57BL/6 mice (age, 12 weeks) and 14 aged male C57BL/6 mice (age, 19 months) were obtained from Biomedical Mouse Resource Center, Korea. All mice were housed in a controlled environment with a 12:12 light–dark cycle with room temperature maintained at 22°C, and provided with adequate food and water. The animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals issued by Institute of Laboratory Animal Resources, Purina, St. Louis, MO, USA, 1996, and the protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University, Seoul, Korea.

2.2. Experiment design

The old mice were randomly assigned to two groups—old control group (OCON, $n = 7$) and old resistance exercise group (ORT, $n = 7$)—and the younger mice were assigned as young control group (YCON, $n = 8$). Weight of chow consumed was measured weekly to observe the food intake. Twelve weeks of resistance ladder climbing exercise was performed in the ORT group by adding weight on the tail. During this exercise intervention, the strength of mice was assessed by measuring their grip strength every 2 weeks using a Grip Strength Meter (Bioseb, Vitrolles, France). The median values in 12 weeks of results were taken for analysis as their motivation or actual strength was affected by their daily conditions due to their age. Forty eight hours after the last exercise session, after overnight fasting, all animals were anesthetized with 20% urethane and body composition was measured by using dual energy X-ray absorptiometry (DXA; Discovery W; Hologic, Marlborough, MA,

USA). All animals were sacrificed and tibialis anterior muscle and soleus muscles were surgically removed and weighed. After the extractions, the muscles were frozen in liquid nitrogen and stored at -80°C until protein analysis.

2.3. Exercise protocol

For the ORT group, resistance ladder climbing exercise was performed 3 d/wk for 12 weeks. Ladder climbing exercise was conducted by using a 1-m ladder with a 1.5-cm grid. The ladder was set at 85° from the ground. One week's adaptation was conducted by letting mice to climb up the ladder without any resistance. Outsource stimulus such as food reward or electrical stimulation were not given to the mice during ladder climbing exercise. When mice reached the top of the ladder, a 90-second rest was given before the next trial of ladder climbing. After the 1-week adaptation, resistance (10% of body weight) was given to the mice by adding weight on the tail, and the loads were increased gradually as the exercise sessions proceeded. To progressively increase exercise intensity, 2 g of additional weights were applied after four successful trials.¹⁷

2.4. Body composition and grip strength

Forty eight hours after the last exercise session, body composition was measured by using DXA (Discovery W, Hologic). Whole body small animal DXA modulation was used to measure body composition. By using standard software (QDR for Windows XP Operating System, Hologic), hindlimb regions were carefully selected and analyzed, because DXA cannot differentiate body organs and vascular smooth muscles. Grip strength was measured by modifying a method that had been used previously.¹⁸ The mice were allowed to grasp steel wired grill attached to the force gauge and were pulled away from the gauge. The force when the mice released the grill was recorded. For each measurement, five trials were conducted, and median values were taken for analysis.

2.5. Protein extraction and muscle FGF-2 protein quantification

After the DXA measurement, anesthetized mice's left and right tibialis anterior and soleus muscle were rapidly removed and stored at -80°C until the protein analysis. To extract the protein from muscles, tibialis anterior and soleus muscles were homogenized in 500 μL of extraction buffer with protease inhibitor. The extracts were then centrifuged at 2,000 g at 4°C for 15 minutes to remove insoluble material. Protein concentrations in the supernatants, were determined using the Bradford assay kit (Bio-Rad, Hercules, CA, USA). Quantification and measurement of FGF-2 protein of quantified soleus and tibialis anterior muscle sample by Bradford assay was performed by using FGF basic mouse enzyme-linked immunosorbent assay kit (ab100670; Abcam, Cambridge, CA, USA).

2.6. Data analysis

Statistical analysis was performed using SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA), and results are

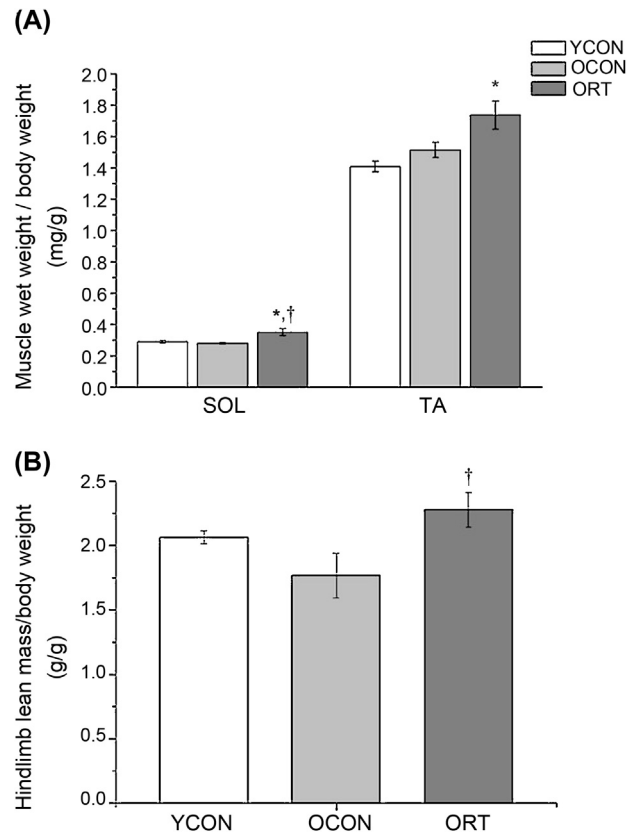


Fig. 1 – Hindlimb lean mass and muscle wet weight. (A) Muscle wet weight of soleus muscle and tibialis anterior muscle of young control (YCON), old control (OCON), and resistance exercise group (ORT). (B) Dual energy x-ray absorptiometry data of hindlimb lean mass of YCON, OCON, and ORT group.

* $p < 0.05$ compared to YCON.

† $p < 0.05$ compared to OCON.

expressed as mean \pm standard error. One way analysis of variance (ANOVA) was performed to examine the difference between groups in body weight, grip strength, lean mass, fat mass, muscle wet weight, and FGF-2 level. Posthoc analysis was conducted to determine the existence of mean difference of each group. Also, Pearson correlation analysis was used to assess the correlation between hindlimb lean mass and muscle FGF-2 protein level. The level of significance was set at $p < 0.05$.

3. Results

Although absolute wet weight of soleus and tibialis anterior muscles were not significantly different among three groups (data not shown). In relative wet weight, both soleus ($p = 0.013$) and tibialis anterior ($p = 0.003$) of ORT showed a significant increase in muscle wet weight compared to YCON. Relative muscle wet weight of soleus of ORT showed a significant increase compared to OCON ($p = 0.008$; Fig. 1A). From the DXA result, the whole body composition among three groups was not different including fat mass and percentage body fat.

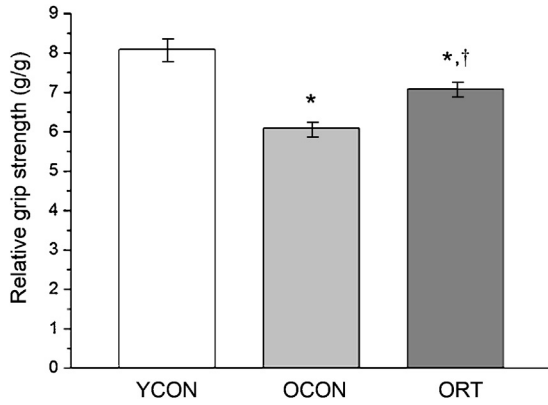


Fig. 2 – Relative grip strength; grip strength divided by body mass of young control (YCON), old control (OCON), and resistance exercise group (ORT).

* $p < 0.05$ compared to YCON.
 † $p < 0.05$ compared to OCON.

However, not only was there a significant difference among three groups in individual muscle wet weight, but hindlimb lean mass was also significantly different among the groups ($p = 0.02$), and hindlimb lean mass of ORT showed a significant increase compare to OCON ($p = 0.015$; Fig. 1B).

In relative grip strength, significant less relative grip strength was measured in both OCON ($p < 0.001$) and ORT ($p = 0.011$) compare to YCON. However, ORT showed a significantly higher relative grip strength than OCON ($p = 0.02$; Fig. 2).

FGF-2 protein level for both soleus ($p = 0.02$) and tibialis anterior muscle ($p = 0.001$) showed a significant difference among three groups. In soleus muscle, FGF-2 protein level of OCON showed a significant increase compared to YCON ($p = 0.035$); however, there was no difference between YCON and ORT. Moreover, a significant decrease in ORT compared to OCON was shown in FGF-2 protein level of soleus muscle ($p = 0.045$; Fig. 3A). In tibialis anterior muscle, there was a significant decrease in ORT compared to YCON ($p < 0.001$) and OCON ($p = 0.022$); however, there was no significant difference between YCON and OCON in FGF-2 protein level ($p = 0.076$; Fig. 3A).

Finally, to determine the relationship between muscle FGF-2 protein level and lean mass, Pearson correlation analysis of muscle FGF-2 level and hindlimb of old mice was conducted. The data not only demonstrated negative correlation between hindlimb lean mass and soleus muscle FGF-2 protein level ($r^2 = 0.514$, $p = 0.0035$), but also between hindlimb lean mass and tibialis anterior muscle ($r^2 = 0.312$, $p = 0.025$; Fig. 3B and 3C).

4. Discussion

In this study, resistance exercise reduced FGF-2 protein level of soleus and tibialis anterior muscles of aged mice. In addition to these changes, increase of muscle wet weight, hindlimb mass, and muscle quality with resistance exercise were observed. To our knowledge, this was the first attempt to investigate

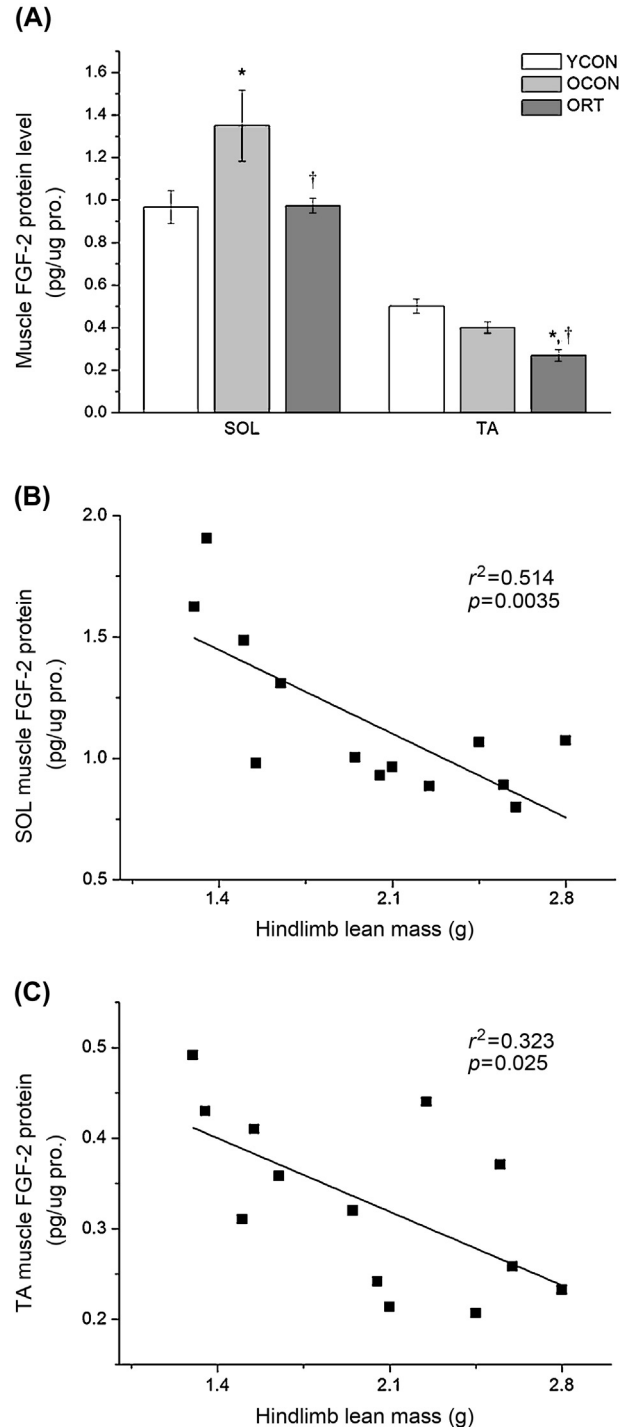


Fig. 3 – Muscle fibroblast growth factor-2 (FGF-2) protein level and correlation between muscle FGF-2 protein level and hindlimb lean mass. (A) Muscle FGF-2 protein level of soleus muscle and tibialis anterior muscle of young control (YCON), old control (OCON), and resistance exercise group (ORT). (B) Correlation between soleus muscle FGF-2 protein level and hindlimb lean mass among old mice. (C) Correlation between tibialis anterior muscle FGF-2 protein level and hindlimb lean mass among old mice.

* $p < 0.05$ compared to YCON.
 † $p < 0.05$ compared to OCON.

the effect of resistance exercise in relation to the age-related elevation of muscle FGF-2.

The strength of mice over 12 weeks was assessed by measuring grip strength. Over 12 weeks of exercise intervention period, grip strength was measured every 2 weeks. Due to their age, daily conditions of individual mice affected either their motivation or actual strength, the median values among 12 weeks were taken for analysis. As a result, the relative grip strength of ORT group was increased compared to OCON group. This not only indicated that the resistance exercise conducted for 12 weeks had sufficient intensity to observe exercise induced changes in muscle but also that resistance exercise was an efficient treatment to prevent age-related loss of strength.¹⁹

It is known that resistance exercise increases functional capacity, however, increase in lean mass, and decrease in fat mass in aged individuals is still in debate.^{20,21} In this study, the whole body lean mass in aged individuals did not show a remarkable difference in response to resistance exercise. From our previous studies, the food intake affected the whole body composition greatly. To minimize the effect of other factors that might affect the body composition, food intake per day was recorded and did not show a significant difference among three groups throughout the 12 weeks of intervention period (data not shown). However, interestingly, in DXA results analyzed by appendicular body parts, the body composition differences were shown to be significant in hindlimb lean mass between ORT group and OCON. This result was similar to the previous study, which demonstrated the effectiveness of resistance exercise in increasing lean mass of the affected body parts.¹⁹ In addition, relative muscle wet weight of soleus muscle in ORT group showed a significant increase compared to OCON; however, there was no difference found between OCON and ORT in tibialis anterior muscle wet weight. Previous reports demonstrated less responsiveness of tibialis anterior muscle to exercise²² and the result showed similar trends.

Muscle FGF-2 level was found to be important for satellite cell proliferation, and it promotes muscle regeneration.⁸ However, in aged mice, muscle fiber FGF-2 protein level was dramatically increased compared to that of younger adult mice and it induced satellite cell depletion.⁹ In this study, similar results were shown in soleus muscle. In soleus muscle, the FGF-2 protein level was significantly increased in OCON group compared to YCON group. However, this trend was not shown in tibialis anterior muscle, which indicates that muscle type difference might have been a factor of this result. Thus, muscle type should be considered for future studies.

The main purpose of this study was to investigate the effect of resistance exercise on muscle FGF-2 protein level in aged mice. In a previous study, it was suggested that regulation of elevated FGF-2 protein level in muscle might have positive effects on muscle aging by maintaining its regenerative capacity.⁹ This study followed the suggestion of a previous study, and treated one of the strategies that might have preventive effect on muscle aging. As resistance exercise is known to be a strategy that gives beneficial effect on maintaining muscle strength, mass, and quality in aged individuals,²³ it is applied to investigate the regulation of

FGF-2 in muscle of aged mice via exercise. In our study, the ORT group showed a significant decrease of FGF-2 protein level in both soleus and tibialis anterior muscle compared to OCON group. In addition, we observed a concomitant negative correlation between muscle FGF-2 protein level and hindlimb lean mass. Our results imply that resistance exercise-induced reduction of FGF-2 protein level might be a preventive effect on losing hindlimb lean mass of aged mice. However, the result of this study is a descriptive, mechanistic explanation of the reasons for the elevation of FGF-2 and effect of resistance exercise in relation to FGF-2 in aged individuals are required for the future studies.

Conflicts of interest

All authors have no conflicts of interest to declare.

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