Thesis of Master of Science

Effects of resistance exercise on SPARC expression in skeletal muscle of aging mice

저항성 운동이 노화 쥐 골격근의 SPARC 발현에 미치는 영향

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ABSTRACT

Backgrounds: Sarcopenia is age-related loss of muscle mass and the consequent loss of muscle strength and quality is closely related to mobility and functional impairment as it increases the prevalence of lifestyle-related diseases such as diabetes and cardiovascular disease. SPARC is a novel myokine which inhibits adipogenesis but promotes myogenesis and it has been suggested as a therapeutic target to overcome sarcopenia. However, there is no study that shows how the exercise effects on SPARC expression during the aging process. Therefore, the purpose of this study is to describe the effect of 12 weeks resistance exercise on SPARC expression in skeletal muscle of aging mice.

Methods: Nineteen-month-old male wild-type C57BL/6 mice were randomized Old-CON, sedentary aging mice group (n=6); Old-REX, resistance exercise aging mice group (n=7). Old-REX mice performed 12 weeks of resistance ladder climbing exercise after taking non-weight bearing adaptation period for one week. The body composition was scanned by using DEXA and SPARC expression in each muscle such as soleus (SOL), extensor digitorum longus (EDL), tibialis anterior (TA), and gastrocnemius (GAS) muscles were detected using ELISA. Moreover, muscle strength, endurance capacity and mobility were measured after the last resistance exercise.

Results: SPARC protein levels in EDL, TA and GAS muscles were significantly increased in Old-REX mice compare to Old-CON mice, while there was no difference between groups in circulating SPARC level was found. The lean body mass in Old-REX

mice was significantly increased without a change in body weight. Additionally, a significant increase in soleus muscle wet weight was observed. The mobility was significantly improved in Old-REX group and grip strength AUC was also increased.

Conclusion: 12 weeks of resistance exercise increased the expression of SPARC in aging skeletal muscle. This is the first study that shows descriptive SPARC expression in various skeletal muscle of aging mice. In addition, resistance exercise increased muscle mass, but also improved physical functions. These observations suggest that decreased SPARC expression in aging skeletal muscle and declined muscle mass could be improved through resistance exercise and SPARC might be a potent contributor to overcome sarcopenia.

Key words

Aging mice, Sarcopenia, SPARC, Resistance ladder climbing exercise, Skeletal muscle

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LIST OF ABBREVIATIONS

SPARC	Secreted protein acidic and rich in cysteine
siSPRAC	small interfering Secreted protein acidic and rich in cysteine
Old-CON	Old sedentary Control
Old-REX	Old resistance Exercise
SOL	Soleus
EDL	Extensor Digitorum Longus
TA	Tibialis Anterior
GAS	Gastrocnemius
ELISA	Enzyme Linked Immune Sorbent Assays
AUC	Area Under the Curve
DEXA	Dual Energy X-ray Absorptiometry
BW	Body Weight
IACUC	Institutional Animal Care and Use Committee

I. INTRODUCTION

1.1. Significance of the study

Sarcopenia is age-related loss of muscle mass and the consequent loss of muscle strength and quality is closely related to mobility and functional impairment (Fiatarone et al., 1994; Foldvari et al., 2000; G. B. Forbes & Reina, 1970; Janssen, 2006). These alterations of physiological phenomena start to change after the age of 50 and it leads to increased prevalence of lifestyle-related diseases, such as diabetes, cardiovascular disease(Morley, Baumgartner, Roubenoff, Mayer, & Nair, 2001; Sehl & Yates, 2001). Exercise promotes numerous adaptations in skeletal muscle and it may help to prevent or attenuate sarcopenia(S. C. Forbes, Little, & Candow, 2012). Specially, resistance exercise may be the most effective strategy to combat sarcopenia through increased muscular strength and power(de Vos et al., 2008; Miszko et al., 2003). Moreover, resistance training can stimulate muscle fiber hypertrophy in all fiber types in older individuals(Larsson, 1982; Pyka, Lindenberger, Charette, & Marcus, 1994) and subsequent fiber type transitions have also been reported(Häkkinen et al., 1998). Therefore, resistance exercise is an effective intervention to improve aging muscle mass and strength, and these gains may lead to significant enhancement in function and overall quality of life (Fiatarone et al., 1994).

Secreted protein acidic and rich in cysteine (SPARC), also known as BM-40 is a nonstructural and matricellular glycoprotein and it functions in cell adhesion, angiogenesis,

interactions with growth factors, and cell differentiation(Brekken & Sage, 2001; Kos & Wilding, 2010). At the initial stage of research, SPARC was known as osteonectin secreted from bone(Termine et al., 1981). And then, numerous studies found that SPARC mRNA were expressed in various cell type including osteoblasts, macrophages, fibroblasts, endothelial cells, and smooth muscle cell(Hirota et al., 1993; Lane & Sage, 1994). Since then, SPARC was investigated in many kinds of pathological conditions such as obesity, diabetes, cancer, Alzheimer and aging (Kos & Wilding, 2010).

Newly, SPARC was identified to be an age-related protein as it decreases with aging while exercise increases SPARC expression in aging mouse at the same time. They reported that SPARC as a novel exercise induced myokine, secreted from muscle cells. In addition, exercise induced increase in SPARC level appeared to be muscle specific in comparison to other organ where SPRAC is abundant, such as adipose tissue, testis, liver, and colon in a mouse. Also a single bout of exercise increased the level of serum SPARC in young healthy men, which is consistent with the results of the mouse experiments(Aoi et al., 2013).

According to Nakamura et al 2013, injection of siSPARC cause myofiber atrophy in mouse and increase in adipose tissue without significant differences in body weight. These findings suggest that attenuated SPARC is likely the major contributor muscle atrophy(Nakamura, Nakano, Miyoshi, Yamanouchi, & Nishihara, 2013).

Although SPARC was known to a novel myokine, the descriptive measurement of SPARC in various muscles, particularly during skeletal muscle aging process, have not been estabilished through other studies. Therefore, it is necessary to study

the effect of resistance exercise on SPARC expression in various skeletal muscles of aging mice.

1.2. Purpose of the study

This study aimed to investigate the effect of 12 weeks resistance exercise on SPARC expression in skeletal muscle of aging mice.

1.3. Research Hypothesis

1) There would be significant differences in fat mass and lean body mass between exercise and control group.

2) There would be significant differences in mobility, grip strength, and muscular endurance capacity between exercise and control group.

3) There would be significant differences on SPARC expression in various skeletal muscles between exercise and control group.

1.4. Limitations

This study is limited in the following manner:

1) This study did not have young control group.

2) Lean body mass was used instead of skeletal muscle mass.

3) This study could not completely control food intake.

II. LITERATURE REVIEW

2.1. Sarcopenia

2.1.1. Sarcopenia

The increased prevalence of age-realated disease has been one of the public health issues in Korea. According to 「Statistic Korea 2010」, the ratio of elderly people greater than 65 years old has been constantly increased from 3.1% in 1970 to 11.8% in 2012, and it is expected to reach 24.3% by the year 2030. With advancing age, the phenomena of several age-related changes such as sarcopenia, that is loss of muscle mass, have been on the rise(Brooks & Faulkner, 1994). Maximal muscle mass is usually reached at about 20 years of age and fat mass peaks at the ages between 60 and 70 years(Baumgartner, Stauber, McHugh, Koehler, & Garry, 1995; Gallagher et al., 1997). Particularly after the age of 70 years, there is a redistribution of body fat and fat free mass(Beaufrere & Morio, 2000). By the effect of insulin resistance, it occurs a reduction in peripheral skeletal muscle mass and an increase in intramuscular and intrahepatic fat(Cree et al., 2004).

Muscular strength alone is associated with functional ability in the elderly(D. Buchner & De Lateur, 1991; Hyatt, Whitelaw, Bhat, Scott, & Maxwell, 1990). The progressive loss of muscular strength and muscle mass with age(Larsson, Grimby, & Karlsson, 1979) has important health consequences(Young & Skelton, 1994) such as an increased susceptibility to disability(D. M. Buchner & Wagner, 1992; Rantanen et al., 1999), an

increased risk of falls(Campbell, Borrie, & Spears, 1989) and hip fractures(ANIANSSON, ZETTERBERG, HEDBERG, & HENRIKSSON, 1984), a decrease in bone mineral density(SINAKI, McPHEE, HODGSON, MERRITT, & OFFORD, 1986), and an increase in glucose intolerance(Bloesch, Schutz, Breitenstein, Jequier, & Felber, 1988). These changes can lead the elderly to an increased risk for a variety of diseases and disabilities(Rantanen et al., 1999).

The molecular mechanisms of sarcopenia were not fully understood while several mechanisms are thought to be involved in the pathophysiology of sarcopenia, including age-related endocrine changes, chronic inflammation, malnutrition, sedentary lifestyle and chronic diseases(Kamel, Maas, & Duthie Jr, 2002; Morais, Chevalier, & Gougeon, 2005; Roth, Metter, Ling, & Ferrucci, 2006; Roubenoff, 2003).

2.1.2. Effect of exercise on Sarcopenia

Although aerobic exercise does not improve muscular for production(Klitgaard et al., 1990; Thompson, Crist, Marsh, & Rosenthal, 1988), resistance exercise promotes numerous adaptations in skeletal muscle and many of which may help preventing or reverseing sarcopenia(S. C. Forbes et al., 2012). For example, Candow et. Al., 2011 showed that 22 weeks of whole-body resistance exercise training (3 days per week) in healthy older males (60–71 years) was sufficient to overcome the age-related deficits in whole-body lean tissue mass, regional muscle size, and upper and lower body strength. These parameters were found to be equally meaningful in untrained young males(Candow, Chilibeck, Abeysekara, & Zello, 2011). In addition, heavy resistance exercise in 65 to 75 years old men and women improved their 1 repetition maximum

(1RM) values, about more than 30% within the first couple of months(Lemmer et al., 2000). Therefore, age-induced muscle mass loss can be reversed with only about few months of resistance exercise(Hurley & Roth, 2000).

The mechanisms responsible for resistance exercise which induces an increases in strength in the elderly are not entirely understood, but an increase in motor unit firing frequency and in maximal motor unit recruitment rates are likely to be the contributors to the substantial increase in strength after short term resistance exercise(Enoka, 1997; Leong, Kamen, Patten, & Burke, 1999).

Resistance exercise can also stimulate muscle fiber hypertrophy in all fiber types in older individuals(Larsson, 1982; Pyka et al., 1994) and fiber type transitions have also been reported(Häkkinen et al., 1998). These results suggest that the muscles of older individuals can adapt to a resistance exercise stimulus such that age-related muscle fiber atrophy may be completely reversed in some individuals(Larsson, 1982).

2.2. SPARC

2.2.1. SPARC

Secreted protein acidic and rich in cysteine (SPARC), also known as BM-40 is a nonstructural and matricellular glycoprotein and it functions in cell adhesion, angiogenesis, interactions with growth factors, and cell differentiation(Brekken & Sage, 2001; Kos & Wilding, 2010). At the initial stage of research, SPARC was thought to be secreted from bone and it was called as osteonectin(Termine et al., 1981) and then later, numerous

studies found that SPARC mRNA were expressed in various cell type including osteoblasts, macrophages, fibroblasts, endothelial cells, and smooth muscle cell(Hirota et al., 1993; Lane & Sage, 1994). Since then, SPARC has been investigated in many kinds of pathological conditions such as obesity, diabetes, cancer, Alzheimer and aging(Kos & Wilding, 2010). Specially in a case of cancer conditions, tumor growth has been reported to be increased in SPARC-null mice(Puolakkainen, Brekken, Muneer, & Sage, 2004). In addition, SPARC dysregulation has been found to be associated with a wide range of obesity-related disorders, including type 2 diabetes mellitus and its complications, renal and liver disease, cardiovascular disease and obesity-associated cancer(Kos & Wilding, 2010). It turned out to be that SPARC may strengthen bone as SPARC-knockout mice had a low-turn over osteopenia. This suggests that SPARC may also have a protective role against osteoporosis in diabetes patients. In a recent study, SPARC is age-related protein, loss of SPARC caused myofiber atrophy in mouse and increased in adipose tissue without significant differences in body weight(Nakamura et al., 2013). Nevertheless, So far, there are only few studies that investigated SPARC in age-related muscle atrophy.

2.2.2. Effect of exercise on SPARC expression

Myokine is a protein that is synthesized by skeletal muscle tissue and is increased in response to exercise(Pedersen, 2009; Pedersen & Febbraio, 2008; Raschke & Eckel, 2013). Myokine can regulate the functions of other organs through an endocrine, autocrine or paracrine manner(Pedersen & Febbraio, 2008; Tamura et al., 2010). SPARC is identified as a novel exercise-induced myokine that is secreted from muscle cells. They

showed that cyclic stretching increased SPARC in both cells and medium, suggesting that mechanical contraction accelerates the secretion of SPARC from muscle cells into the extracellular media by stimulating protein translation(Aoi et al., 2013). Moreover, regular treadmill exercise induced an increase in SPARC level and this is appeared to be muscle specific compare with other organ such as adipose tissue, testis, liver and colon in a mouse where SPRAC is abundant. According to a time-course analysis, the plasma levels of SPARC was increased immediately after a single bout of exercise, and then gradually returned to the pre-exercise level(Aoi et al., 2013).

Although SPARC was known to be a novel exercise induced myokine, which is a potent therapeutic target for sarcopenia, there are only few studies that have investigated it. Moreover, the descriptive measurement of SPARC in various muscles, particularly, during skeletal muscle aging process has not been established. Therefore, it is necessary to study the effect of resistance exercise on SPARC expression in circulating level and various skeletal muscles of aging mice.

III. METHODS

3.1. Experimental animal

Nineteen-month-old male wild-type C57BL/6 mice (Biomedical mouse resource center, Korea) were used for this experiment. All mice were housed in a controlled environment with a 12:12-h light-dark cycle with room temperature maintained at 22℃. All mice were provided with water and food ad libitum. The animals were taken cared in accordance with the Guide for the Care and Use of Laboratory Animals issued by Institute of Laboratory Animal Resources, USA, 1996, and with a protocol that was approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University. All of the experiments were conducted to minimize the number of animals utilized and the suffering caused by the procedures of the present study.

3.2. Experimental design

3.2.1. Experimental design

To investigate the effect of resistance exercise on SPARC expression in skeletal muscle of aging mice, nineteen-month-old male C57BL/6 mice were randomized each of the following groups: Old-CON, sedentary aging mice (n=6); Old-REX, resistance exercise aging mice (n=7). All mice were provided a chow containing 12.5% of calories provided from fat, 24.5% from protein, and 63% from carbohydrates (Purina rodent chow 5057, Purina Korea) and food intake was measured weekly. Old-REX group was forced to climb a vertical ladder (1-m ladder with 1.5-cm grid and inclined at 85°) 3 times a week for 12 weeks. 48 hours after exercise training, mice were anesthetized and sacrificed. Following the sacrifice, soleus (SOL), extensor digitorum longus (EDL), tibialis anterior (TA), and gastrocnemius (GAS) muscles were surgically removed and weighted. After the muscles' weight was measured, the muscles were frozen in liquid nitrogen and stored at -80℃.

To analyze the expression of SPARC, all muscle tissues were homogenized in 1.0 ml extraction buffer (RIPA). The extracts were then centrifuged at 13,000 rpm at 4℃ for 15 min to remove insoluble materials. SPARC (catalog# SEA791Mu) was measured using commercial enzyme linked immune sorbent assays kit (ELISA, Uscn Life Science Inc. USA).

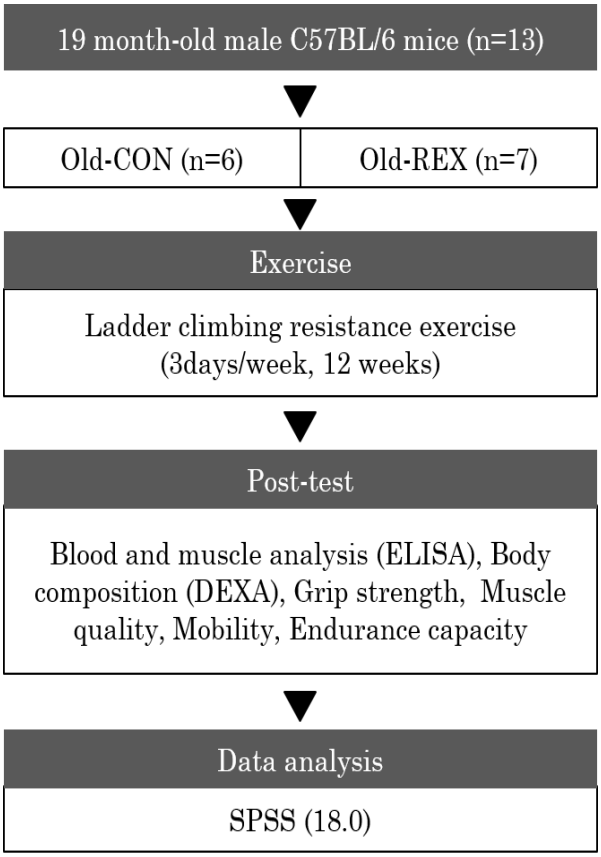


Figure 1. Experiment design

3.3. Exercise protocol

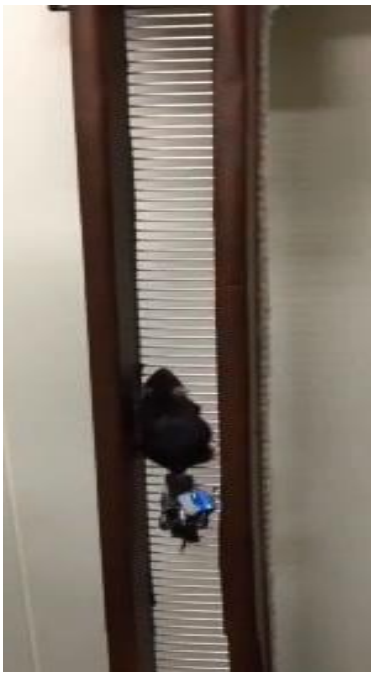
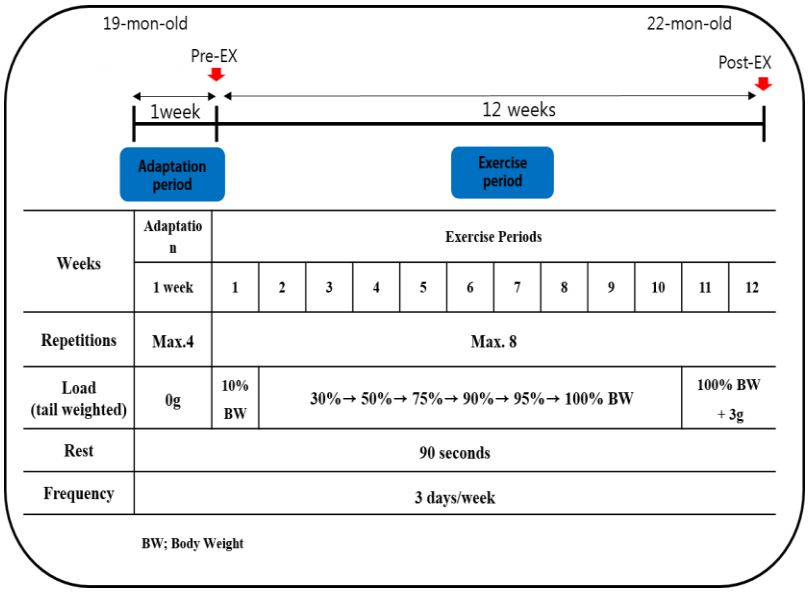


Figure 2. Ladder climbing resistance exercise

The mice performed climbing the vertical ladder 3 day/wk for 12 weeks. Exercise was accomplished utilizing a 1-m ladder with 1.5-cm grid and inclined at 85°. Initially, the mice performed climbing with free weight for a week in order to be accustomed. Neither food reward nor any tortuable stimulation such as electric shock or forced air was provided to encourage the performance.

For the first training session, 10% of body weight (BW) was attached to the base of their tail, and the resistance was progressively increased to 30%, 50%, 75%, 90%, and

100% during 12 weeks. Either 3.0 g added resistance or additional two trails without increcreasing the weight were perfomed when the mice succeeded to climb 100% of their BW. Failure was defined as the inability to climb the ladder. Following a failure, the previously lifted weight was put on the mouse and made it climb the ladder. Each bout consisted of eight repetitions, but no more than ten repetitions were used. When the mice reached the top of the ladder, they were allowed to rest for 90 seconds. The training session was stopped when the mice succeeded to climb the ladder for 8 repetitions.



Weeks	Adaptation	Exercise Periods											
	1 week	1	2	3	4	5	6	7	8	9	10	11	12
Repetitions	Max.4	Max. 8											
Load (tail weighted)	0g	10% BW	30%→ 50%→ 75%→ 90%→ 95%→ 100% BW									100% BW + 3g	
Rest	90 seconds												
Frequency	3 days/week												

BW; Body Weight

Figure 3. Exercise program

3.4. Grip Strength

The grip strength was measured using a Grip Strength Meter (Bioseb, France), and a modification of a previously reported method (Meyer et al., 1979). Grip strength test was performed by allowing the animals to grasp a grid attached to the force gauge. This was followed by pulling the animal away from the gauge until the combined hind and forelimb released the grid. This provides a value for the force of maximal grip strength (g). The force measurements were recorded in three separate trials, and the maximum strength results were used in analyses. The grip strength was measured once every other week. The result was presented as area under the curve (AUC).

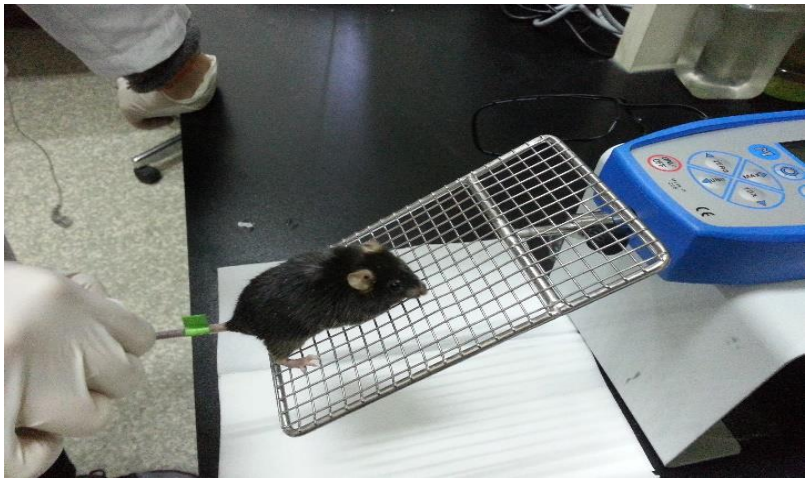


Figure 4. Grip strength

3.5. Mobility

Mobility was measured to observe physical activity of mice. Physical activity chamber was made 100-cm x 100-cm x 16-cm with transparent acrylic panels and it has 21 holes in the cover plate for the ventilation. To observe general activity levels of all mice, 10-cm x 10-cm lined-square was drawn on cover plate. Two days after the last experiment, all mice were adapted to activity chamber over 1 hour. Each group of mice was put in the chamber and recorded their activity by a video camera fixed above the device (in a dimmed-room light setting) for 30 minutes without a supervisor present in the same room. Activity score of 1 was counted when the body of a mouse crossed the line drawn above the device and a diagonal crossing movement was counted as 2 in activity score. The average counted number of each groups was used for an analysis.

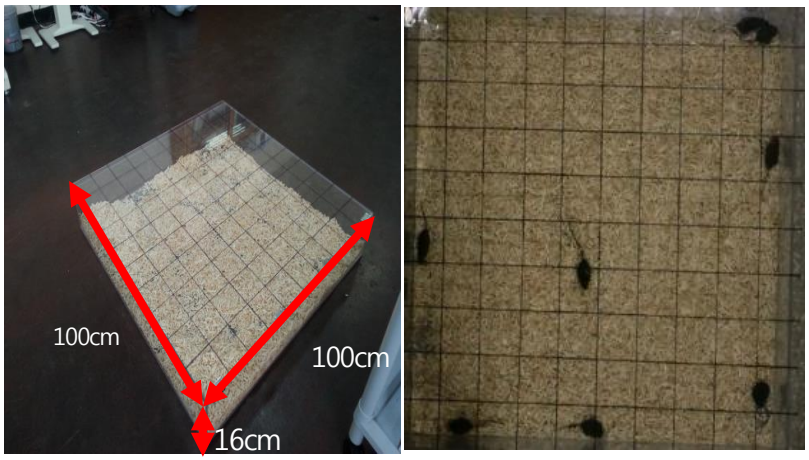


Figure 5. Physical activity

3.6. Body composition

DEXA (Dual Energy X-ray Absorptiometry) scanning was performed to measure the skeletal muscle mass of mice using a Hologic Discovery DEXA instrument (Hologic, USA). As DEXA provides the values of whole body fat mass including visceral fat and other tissues and lean body mass, it was limited to obtain the accurate skeletal muscle mass. Therefore, the values of fat and lean mass from fore and hind limb were used for this study. All mice were anesthetized right before the sacrifice and their leg composition such as lean mass, fat mass, fat percentage and lean percentage was measured.

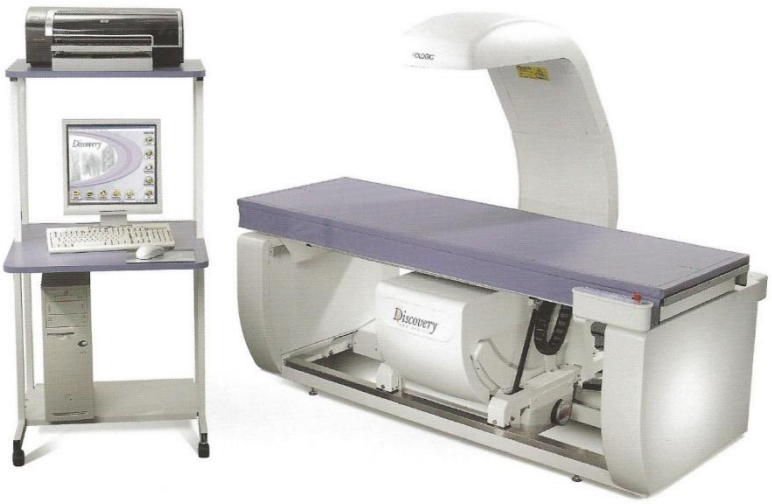


Figure 6. DEXA

3.7. Muscular endurance capacity

The wire hanging test was performed to examine the muscular endurance capacity of mice. It began with the position that an animal being put on to the top of the elevated grid. The mice were placed on the top of the grid with 10 g weight attached to their tail. Then, the grid was inverted and suspended above the home cage in order to prevent any possible damage happening to the animal. The animal's hanging time was recorded until the animal fell. When the mice fell within 10 seconds, additional trials were performed. The test was performed once a week.

Figure 7. Wire hanging test

3.8. Statistical Analysis

Statistical analysis was performed using the SPSS 18.0 software (SPSS Inc.) Data were analyzed using independent t-test to examine the body weight, wire hanging test, physical activity, muscle wet weight, body composition, and SPARC levels between Old-CON and Old-REX. Values were expressed as mean ± S.E.M.

IV. RESULTS

4.1. Body weight and food intake

In the present study, body weight was checked weekly to observe a change However, both initial and final body weights of the Old-CON and Old-REX mice were not significantly different. In addition, in the recorded amount of food intake per mice, there was no statistical difference in food intake over time between the Old-CON and Old-REX.

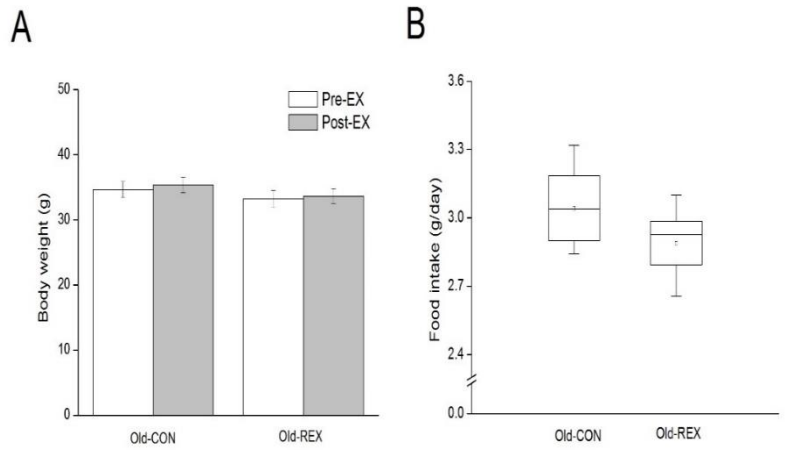


Figure 8. Effect of 12 weeks of resistance exercise on (A) body weight and (B) food intake. Values are mean ± standard error of mean for (A) and values are median for (B). Old-CON, sedentary aging mice (n=6); Old-REX, resistance exercise aging mice (n=7).

4.2. Exercise intensity (tail-attached weight)

Exercise intensity was progressively increased during the 12 weeks of training session. At the last week of training, Old-REX mice lifted weight of 45 g, approximately 1.3 fold their body weight. Therefore, our exercise intensity was increased not only in the absolute weight, but also in the relative intensity during whole experiment periods.

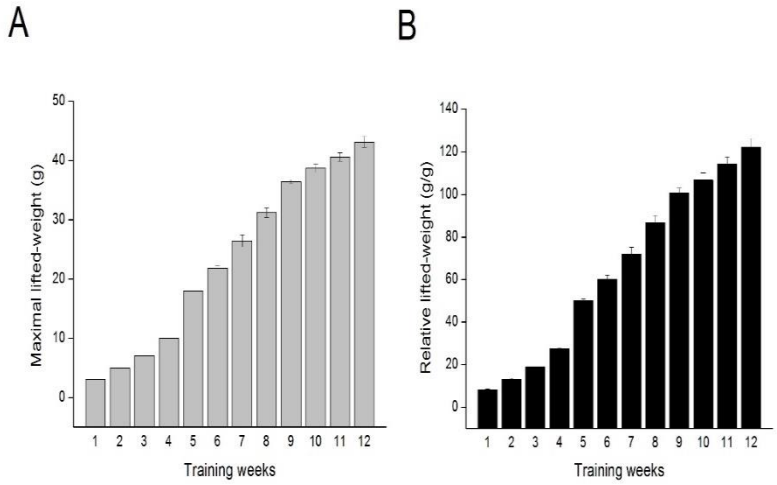


Figure 9. Weekly change of resistance exercise performance.

Table 1. Exercise performance

Variables	1	2	3	4	5	6	7	8	9	10	11	12
	Mean ± S.E.M.											
Maximum weight lifted (g)	3 ± 0.0	5 ± 0.0	7 ± 0.0	10 ± 0.0	18 ± 0.0	21.8 ± 0.5	26.4 ± 1.0	31.2 ± 0.8	36.4 ± 0.4	38.7 ± 0.7	40.6 ± 0.7	43.1 ± 0.9
Maximal weight lifted/body weight (g/g)	0.08 ± 0.0	0.13 ± 0.0	0.19 ± 0.0	0.27 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.71 ± 0.0	0.87 ± 0.03	1.01 ± 0.02	1.07 ± 0.03	1.14 ± 0.04	1.22 ± 0.04

Values are mean ± standard error of mean.

4.3. Muscular endurance capacity

Wire hanging test with 10 g tail weight was performed to test an improvement of muscular endurance capacity of mice. Although a significant difference between Old-CON and Old-REX mice was not observed, it showed a small tendency of increased hanging time in Old-REX mice.

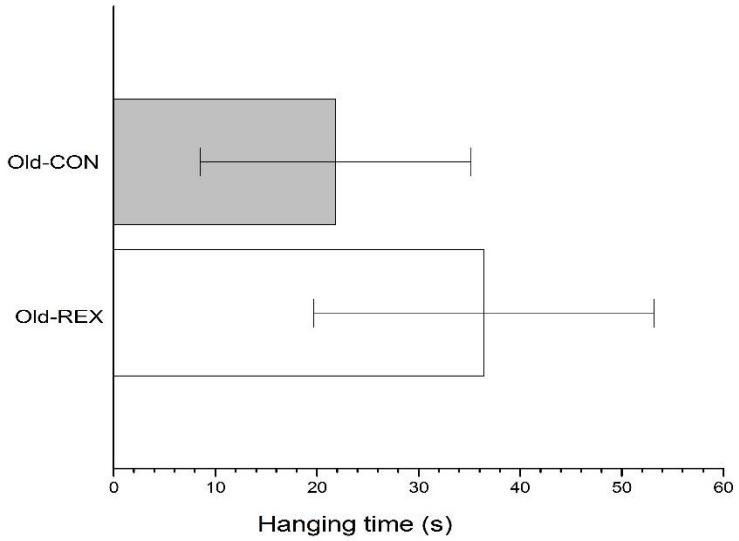


Figure 10. Effect of 12 weeks of resistance exercise on muscular endurance capacity.

4.4. Mobility

Physical activity of mice was measured 24 hours after the last exercise to observe their mobility. A comparison of mobility between Old-CON and Old-REX mice showed a statistically significant difference ($P<.05$). The mobility of Old-REX mice was resulted to be 3.3 fold higher than that of Old-CON mice.

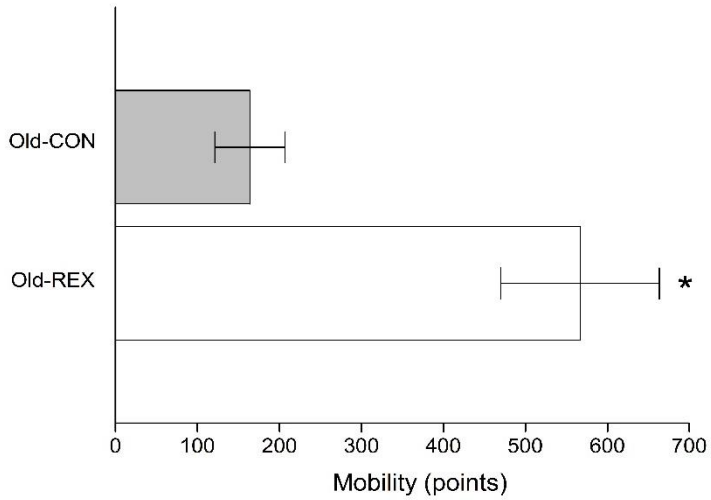


Figure 11. Effect of 12 weeks of resistance exercise on physical activity.

*$P<.05$ compared with Old-CON.

4.5. Muscle wet weight changes

Muscle wet weight was measured right after the dissection of each muscle. The weight was shown in Table 2. Only the SOL muscle wet weight was significantly changed between Old-CON and Old-REX mice ($P<.05$), whereas no statistical differences in EDL, TA and GAS muscles were found. Additionally, significant differences between the groups were not found in relative muscle wet weight, however, the relative muscle wet weight of SOL muscle tended to have higher weight in Old-REX mice.

Table 2. Characteristics of muscle

Variables		Old-CON	Old-REX	P value
		Mean ± S.E.M.		
Muscle wet weight (mg)	SOL	20.01±0.47	21.87±0.60	0.039*
	EDL	25.70±0.92	24.16±0.58	0.618
	TA	108.12±3.80	108.7±2.19	0.893
	GAS	281.28±8.90	279.06±5.74	0.833
Relative Muscle wet weight (g/body weight)	SOL	5.69±0.19	6.41±0.32	0.080
	EDL	7.27±0.07	7.36±0.30	0.772
	TA	30.61±0.62	31.19±1.13	0.405
	GAS	79.64±1.18	81.61±3.01	0.580

Values are mean ± standard error of mean. *P<.05 compared Old-CON with Old-REX.

4.6. Body composition (DEXA)

The lean mass, fat mass, percentage of lean mass and percentage of fat mass from a mouse's limb were screened using DEXA after 12 weeks of resistance exercise. Although there were no significant changes between the groups in the percent of lean mass, fat mass and percent of fat mass, the lean mass of Old-REX was statistically increased compare with Old-CON group ($P<.05$).

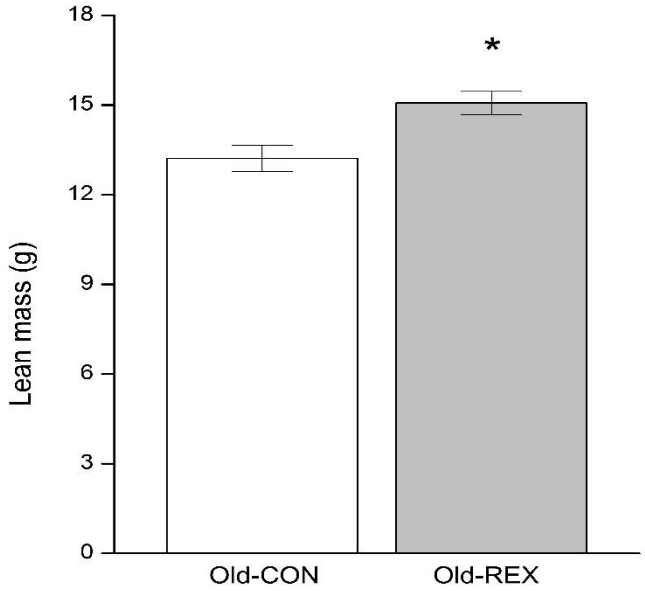


Figure 12. Effect of 12 weeks of resistance exercise on lean mass.

*$P<.05$ compared with Old-CON.

4.7. Muscle strength and muscle quality

Grip strength test was performed to measure the muscle strength and the values were presented as area under the curve (AUC). Grip strength AUC between Old-CON and Old-REX mice showed a significant difference ($P<.05$). Old-REX group was 107% higher than Old-CON group. However, the muscle quality was not statistically different between Old-CON and Old-REX group.

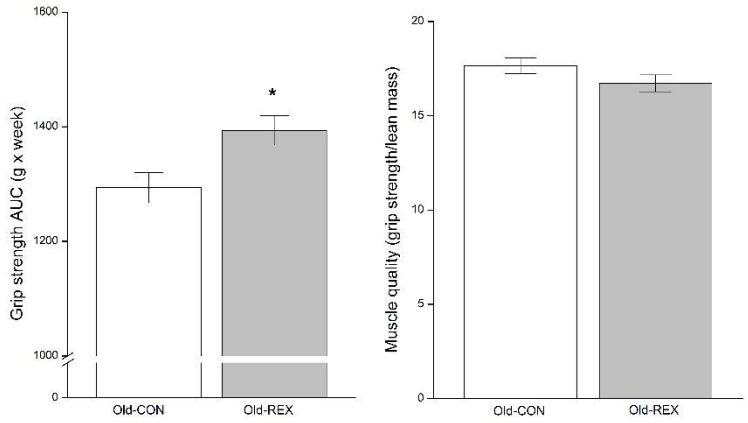


Figure 13. Effect of 12 weeks of resistance exercise on grip strength AUC and muscle quality. *$P<.05$ compared with Old-CON.

4.8. Change in circulating SPARC levels

As myokine was secreted from muscle and it exerts either paracrine or endocrine effects, we measured the serum levels of SPARC protein. A comparison of circulating SPARC levels was not statistically different between Old-CON and Old-REX mice after 12 weeks of resistance exercise.

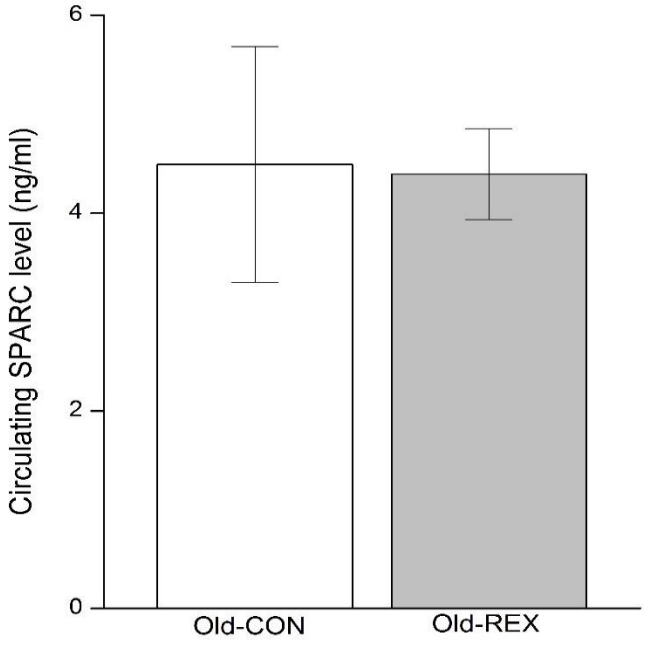


Figure 14. Circulating SPARC level between Old-CON and Old-REX group.

4.9. Expression of SPARC in muscle tissue

The expressions of SPARC in SOL, EDL, TA and GAS muscles were measured at the end of the 12 weeks resistance training and the results were shown in Figure 15. There were significant differences on SPARC expression between Old-CON and Old-REX mice in SOL, EDL, TA and GAS muscles, respectively (*P*=.072, *P*=.017, *P*<.001, *P*<.001).

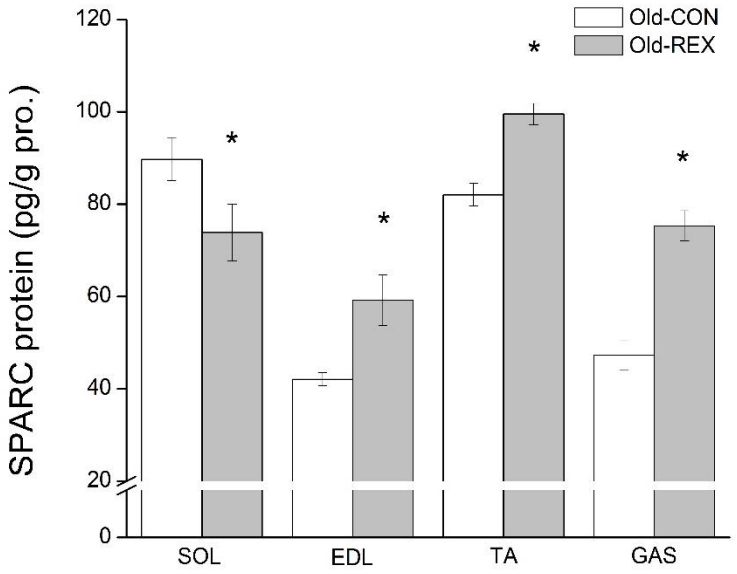


Figure 15. Effect of 12 weeks of resistance exercise on SPARC expression in SOL, EDL, TA and GAS muscle. **P*<.05 compared with Old-CON.

V. DISCUSSION & CONCLUSION

In the current study, we investigated the effect of 12 weeks resistance exercise on expression of SPARC in aging skeletal muscles. Our data demonstrated that SPARC proteins in EDL, TA and GAS muscle were significantly different between Old-CON and Old-REX mice. In addition, the lean body mass, measured by DEXA was significantly increased in Old-REX mice. Specially, a significant increase of soleus muscle wet weight was observed. In this study, several functional tests were examined to evaluate the functional movement of aging mice. The mobility was significantly improved in Old-REX group compared to Old-CON group. Moreover, grip strength AUC between groups was statistically significantly different.

Resistance exercise alters lean body mass without a change in body weight.

Body weight between Old-CON and Old-REX was not significantly different, whereas the lean body mass was significantly increased in Old-REX mice. In addition, the amount of food intake was measured in every week and there was no statistical difference found in food intake between the groups. These results indicated that resistance exercise could increase muscle mass without a significant change in weight. This is consistent with a study that reported no change in body weight due to increased muscle mass in older women who performed resistance exercise for 16 weeks(Treuth et al., 1995). In a more recent study, a resistance exercise intervention of three times per week reported no

change in body weight but a decrease in fat mass and an increase in muscle mass following 12 weeks of training(Iglay, Thyfault, Apolzan, & Campbell, 2007). Thus, these data support our results that resistance exercise intervention was effective to increase the lean mass even in an aging model.

Resistance exercise increases muscle weight and muscle strength in aging mice.

With increasing age, muscle mass declines up to 40% in their 80s compared to their 20s(Adamo & Farrar, 2006). An animal study reported that during the aging process, 5.6% of soleus muscle and 4% of EDL muscle were declined (Daw, Starnes, & White, 1988). However, resistance exercise could induce muscle hypertrophy and it is recommended as an effective strategy to overcome sarcopenia. In our study, soleus muscle wet weight was significantly increased in Old-REX mice. This result is consistent with the result from a study that weight bearing ladder climbing exercise increased soleus muscle mass in hypophysectomized rats(Roy et al., 1996). Also, it was shown that 12 weeks of ladder climbing exercise increased FHR muscle wet weight in middle age SD rat in our unpublished lab data.

Furthermore, to investigate whether muscle strength would be improved after resistance exercise, grip strength was measured in every 2 weeks. There was no significant change resulted in grip strength between the groups. However, grip strength AUC in Old-REX mice was increased compared with Old-CON. AUC, area under the curve, includes all points of grip strength areas bellow the curve (2, 4, 6, 8, 10, 12 week), so variation of grip strength within/between mice was adjusted. In addition, relatively, Old-

CON mice were not accustomed to ladder climbing exercise, inaccurate values were adjusted as we used grip strength AUC instead of grip strength. (Meaning needs to be articulated, revision needed.) Taken together, our results imply that 12 weeks of resistance exercise positively affected muscle weight and it may induce improved muscle mass and strength in aging mice.

Resistance exercise improves physical activity in aging mice.

The level of physical activity in which individuals are engaged at any point in the lifespan reflects a complex interaction of biological, psychological, and sociological factors(Sallis & Hovell, 1990). According to Ingram 2000, with increasing age, physical activity declines in various species. Consistent with this previous study, we observed a significant decline of mobility in Old-CON mice, but a significant increase in Old-REX mice. The mechanism of this phenomena was not fully understood and the search for neural mechanisms of the age related change in physical activity has pointed a major involvement of the dopamine neurotransmitter system(Ingram, 2000). A previous studies in rats have indicated that treadmill exercise over 6 months that acts to release dopamine or stimulates dopamine receptors could increase the concentration of D2 receptors and metabolism of dopamine in the striatum(MacRae, Spirduso, Walters, Farrar, & Wilcox, 1987). The effect of exercise on dopamine synthesis might be activated by increasing calcium levels in the brain through stimulation of an enzyme system known as calmodulin(Sutoo & Akiyama, 1996).

Resistance exercise increases SPARC expression in skeletal muscle of aging mice.

Up to date, this is the first study that shows the expression of exercise induced SPARC, a novel myokine, in various skeletal muscle of aging animals. SPARC expressions in EDL, TA and GAS muscle were significantly increased in Old-REX mice, which is consistent with Aoi et al 2013. They reported that SPARC was significantly increased in gastrocnemius muscle of treadmill exercised mice(Aoi et al., 2013). Additionally, there was no significant difference between Old-CON and Old-REX mice in measuring circulating SPARC level. Aoi et al 2013 reported that regular exercise did not affect the circulating level of SPARC and they suggested that regular exercise can enhance the secretory capacity of SPRAC in response to muscle contraction by increasing the amount of SPARC in muscle tissue in the resting state(Aoi et al., 2013).

Although this study established a new perspective that resistance exercise induces an increase of SPARC in aging animals, several limitations to the experiment still remains. First, young sedentary mice should have been included in this experimental design to estimate the effect of resistance exercise on aging mice. In addition, instead of providing the accurate skeletal muscle mass, the lean body mass was analyzed using DEXA. Lastly, this study was a descriptive experiment so that it was only able to explain the role and mechanism of SPARC in skeletal muscle. Therefore, further experiment should be designed to investigate the mechanism of SPARC in skeletal muscle as a therapeutic target to improve sarcopenia.

In conclusion, this study demonstrated that 12 weeks of resistance exercise increased

the expression of SPARC, a novel myokine, in aging skeletal muscle. In aging mouse model, resistance exercise significantly increased lean body mass and muscle wet weight. Furthermore, grip strength and mobility were improved following exercise. These observations suggest that decreased SPARC expression in aging skeletal muscle and declined muscle mass could be improved through resistance exercise and conclusively, SPARC would be a potent contributor to overcome sarcopenia.

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국 문 초 록

12주간의 저항성 운동이 노화 쥐 골격근의 SPARC 발현에 미치는 영향

최 미 정

서 울 대 학 교 대 학 원

체 육 교 육 학 과

근감소증은 노화로 인한 근육, 근력, 근육질 감소 현상을 일컫는 것으로, 이는 노인들의 움직임 또는 신체수행기능적 장애와 밀접한 관련이 있다. 또한 근감소증은 제 2형당뇨, 심혈관 질환과 같은 생활관련 질병률을 증가시킨다고 보고 되었다. 스파크는 지방분화를 억제하고 근육분화를 촉진하는 물질로, 최근 새로운 마이오카인으로 밝혀졌다. 특히 골격근 내 스파크의 감소는 근감소를 야기시킬 수 있다고 보고 되면서 근감소증을 극복 할 수 있는 물질 중 하나로 제시되고 있다. 따라서 본 연구의 목적은 12주간의 저항성 운동이 노

화 쥐 골격근의 스파크 발현에 미치는 영향을 분석하는 것이다. 본 연구에 사용된 노화 쥐는 19개월령으로 통제군(Old-CON, n=6), 운동군 (Old-REX, n=7)으로 분류되었으며, 운동군은 총 12주간 주 3회 저항성 사다리 운동을 실시하였다. 매주 체중과 식이섭취량 변화를 관찰하였으며, 운동 종료 후 모든 쥐의 신체조성은 DEXA를 통해 측정하였다. 또한 골격근 내 스파크의 발현량을 확인하기 위해 모든 쥐의 가자미근, 장지신근, 전경골근, 비복근을 적출하여 ELISA 실험을 진행하였다.

그 결과, 12주간의 저항성 운동은 장지신근, 전경골근, 비복근의 스파크 발현량을 증가시켰다. 하지만, 운동을 통한 혈중 내 스파크 발현량은 관찰할 수 없었다. 또한 저항성 운동을 통해 노화 쥐의 제지방량이 유의하게 증가되었으며, 움직임 및 근력 또한 유의하게 향상되었다.

따라서, 노화 쥐 골격근의 감소된 스파크 발현량과 제지방량은 저항성 운동을 통해 향상될 수 있으며, 스파크가 근감소증을 극복 할 수 있는 물질로 제시 될 수 있을 것으로 사료된다.

주요어: 노화 쥐, 근감소증, SPRAC, 저항성 사다리 운동, 골격근

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