Thesis of Master of Science

The Effect of Rat Resistance Ladder Climbing Exercise on Skeletal Muscle Hypertrophy

쥐의 저항성 사다리 운동이 골격근 비대에 미치는 영향

By

Jang Hoe KIM

August 2014

Department of Physical Education

College of Education

Graduate School of Seoul National University

ABSTRACT

Backgrounds: Resistance exercise is one of the methods that are used to maintain skeletal muscle mass and to increase muscle strength. Muscle quality (MQ) is a force per unit muscle mass; it is used to represent one of the resistance exercise effects. Recent studies suggested that ladder climbing exercise as a resistance exercise and they reported the increase in flexor hallucis longus (FHL) skeletal muscle mass. In this study, ladder climbing apparatus was modified for rat to mimic human resistance exercise. The purpose of this study was to confirm and evaluate whether a ladder climbing exercise is an efficient resistance exercise for an animal model.

Methods: Male Sprague-Dawley rats (n=16) were divided into 2 groups; control group (CON; n=8) and exercise group (EX; n=8). Ladder climbing exercise was performed three times in a week, for 12 weeks (total 36 bouts, each bout consists of 10 climb ups). EX group climbed 100 cm high and 25 cm wide ladder angled at 85° with the weights secured to its tail. The intensity of ladder climbing exercise was progressively increased every week. Grip strength was measured at the first, fourth, eighth and the last week. Body weight was measured in every week. Skeletal muscles including biceps (BIC), soleus (SOL), gastrocnemius (GAS), tibialis anterior (TA), extensor digitorum longus (EDL), flexor hallucis longus (FHL) and plantaris (PLN) were dissected and their weights were measured. In addition, skeletal muscle cross sectional area was analyzed in EDL and TA. The muscle quality (MQ) was calculated by the ratio of grip strength (g) to the sum of skeletal muscle masses (g) in each muscle.

Results: There was no significant difference in body weight between CON and EX group, which were from ten-week-old to fifteen-week-old. However, from the point of sixteen-week-old to the end of experiment, CON group showed higher BW than EX group ($p<0.05$). In relative skeletal muscle weights of EX group, it was resulted that only the FHL muscle showed greater tendency than that of CON group ($p=0.065$). In CSA analysis, EX group showed bigger fiber size in EDL and TA muscles than those of CON group ($p<0.001$ each).

In the grip strength test, EX group had stronger grip strength than CON group after the 12th week of exercise training ($p=0.01$). In Muscle quality, EX group showed significantly higher values than CON group ($p=0.01$).

Conclusion: In this study, 12 weeks of progressive resistance laddering exercise 1) decreased body weight, 2) increased relative skeletal muscle weight in FHL muscle, 3) enhanced CSA in EDL and TA muscles, and 4) elevated MQ index. Therefore, the ladder climbing exercise and exercise protocols were found to be an appropriate, yet effective exercise program that lead toward skeletal muscle hypertrophy.

Key words

Ladder climbing exercise, Progressive resistance exercise, Skeletal muscle mass, Muscle strength, Muscle quality

Student number: 2011-21606

CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Skeletal muscle is one of the fundamental organs of body composition. There are four major roles of skeletal muscle. The functions of the skeletal muscle are to fulfill movements of the body, support the body, generate heat regulation of the body and maintain posture of the body. Various types of resistance exercise have been recommended to maintain skeletal muscle mass, strength and function in animal models (Lowe, D. A. and S. E. Alway 2002; Seo, Dae Yun et al. 2014). It was shown that the studies presented skeletal muscle hypertrophy by analyzing the increment of the muscle fiber size (Mikesky, ALAN E et al. 1991; Volek, JEFF S et al. 1999). Otherwise, other studies tend to show the changes of muscle contractile properties from the effect of resistance exercise (Norenberg, Kris M et al. 2004; Heinemeier, Katja Maria et al. 2007). Likewise, in many studies that executed resistance exercise, they tended to analyze the result of muscle hypertrophy by investigating the increment either in muscle fiber size or in contractile force.

However, in the mechanism of skeletal muscle hypertrophy, it is found to be that the aspect of protein synthesis takes a pivotal role in muscle anabolism. It showed that resistance exercise accelerates muscle anabolism, and muscle proteins gradually accumulate during exercise period (Ogasawara, R. et al. 2013). There were well-known factors that are responsible for protein synthesis in skeletal muscle. Skeletal muscle also has the remarkable plasticity to change its form through muscle fibers (Yarasheski, KE. et al . 1990; Pette, Dirk et al. 1997; Scott, Wayne et al. 2001; Shoepe, Todd C. et al. 2003). The purpose of this study was to investigate the effect of 12-week resistance ladder climbing exercise and to confirm whether the model is an effective exercise model.

I. LITERATURE REVIEW

1. Resistance Exercise Models of Animal

There have been various animal models that used birds, rodents, dogs and horses in order to complement human studies of skeletal muscle hypertrophy (Cholewa, Jason et al. 2013). The specific control of loading condition and targeted-muscle activation were more easily achieved in animal models than in humans. Moreover, extraneous variables such as environmental conditions and nutritional ingestion could be precisely regulated in laboratory animals, whereas it is difficult to control in human researches. In addition, skeletal muscles that were used to reveal hypertrophy were able to be obtained in any interested regions (Alway, Stephen E. et al. 2005). Likewise, these factors including control of environment, food intake, exercise intensities and diverse regions of muscle dissection area brought the increase in sensitivity and reproducibility of the experimental outcomes.

Among various types of resistance exercise in animal model, the enlargement in muscle that is induced by voluntary exercise was more human-like. There was one study (Yarasheski et al. 1990) investigated whether heavy-resistance exercise training alters the skeletal muscle fiber composition of young rats. The model of this resistance exercise used ladder climbing apparatus. The rats were trained to climb up with weights attached to their tail. They climbed up a 40-cm, 90 degree-inclined mesh 20 times per day, and 5 days per week with food rewards. The load was remained as equal to the each rat’s body weight for the first 5 days, and it was progressively increased to 60g on day 6 and then 30g was added every 3 days thereafter for total 8 weeks. They demonstrated an alteration in the muscle phenotype and a change in the muscle mass of the exercised animals, but only in the superficial region of the rectus femoris (RF), which contained a greater proportion of type IIb fiber was increased. In addition, Duncan et al. (1998) trained male Wistar rats to climb up a 40-cm vertical ladder (4 days/week) with lifting progressively heavier loads secured to their tails. The animals started training at the age of 3 weeks, and after 26 weeks of training, the rats were capable of lifting up to 800 g, which was 140% of their body mass for four sets of 12-15 repetitions per session. They also reported that exercise group had significantly bigger relative muscle mass than control group in extensor digitorum longus (EDL) and soleus (SOL) muscles. This study group also conducted skeletal cross sectional area (CSA) analysis which indicated that single fibers size of EDL, SOL and RF were greater in exercise group. Moreover, Hornberger Jr, T. A. and R. P. Farrar (2004) used 110 cm ladder (80° incline) to train Sprague-Dawley rats. The animals performed climbing up exercise with weights attached to their tail. The exercise protocol consisted of climbing 4 to 9 times per session and it was done once every 3 days for 8 weeks. They also readjusted initial weight load by adding weights in each session in order to fulfill progressive resistance exercise model. After the end of exercise period, the rats could lift up 287% of their body weight. The study reported 23% absolute increase in the weight of the flexor hallucis longus (FHL) in exercise group.

On the other hand, the resent study (Scheffer et al. 2012) analyzed the oxidative stress in skeletal muscles by using a similar ladder climbing exercise. The study consisted of 3 different resistance training protocols which were based on exercise concept: muscular resistance training (RT), hypertrophy training (HT) and strength training (ST). They used the same ladder climbing exercise model which was used in the study of Hornberger Jr, T. A. and R. P. Farrar (2004). In RT protocol, resistance exercise consisted of climbing the ladder with initial load of 10% of body weight, then progressively increased to 20%, 30%, 40%, and 50%, for 3 to 6 sets with 12 to 15 repetitions. On the other hand, HT program was composed of climbing the ladder with initial load of 25% of body weight, then progressively increased to 50%, 75% and 100%, for 3 to 6 sets with 8 to 10 repetitions. ST exercise was comprised of climbing the ladder with initial load of 25% of body weight, then progressively increased to 50%, 100%, 125%, 150%, 175%, and 200%, for 3 to 6 sets with 3 to 5 repetitions. Although each exercise program had the “progressive” concept in common, the study altered the exercise intensities as well as the repetitions. After 12 weeks of training, they reported that there was no difference among the three groups in body weight. Although this study mainly focused on oxidative stress in skeletal muscle, it was resulted to be a meaningful approach to show diverse exercise intensities and repetitions may alter oxidative parameters.

In a related study (Lee et al. 2004), the similar ladder climbing exercise model was also used (100cm ladder with 85° incline, with weight attached on tail). The experiments were executed on 2-month-age Sprague-Dawley rats and had a purpose to investigate the combined effect of resistance exercise with viral injection which composed of IGF-1. The resistance exercise started with 50% of animals’ body weight then, rats climbed up with 50, 75, 90, and 100% of maximal load from the previous exercise session. If a rat successfully climbed up the ladder, additional weight (30g) was added. They reported that 8 weeks of progressive resistance training brought 23.3% increase in muscle mass in the FHL muscle. Moreover, exercise intervention with viral injection produced a 31.8% increase in muscle mass. They concluded that the combination of resistance training and viral injection led greater skeletal muscle hypertrophy than either exercise alone or injection itself.

These results suggested that even though the same concept of progressive resistance exercise model was used on the same animal model, the outcomes might be different depending on the specific exercise protocol and the age of animal models.

II. METHODS

1. Animal Model

Sixteen male Sprague-Dawley rats were used for present experiment and were purchased from Orient Bio Co, Korea. All rats were age-matched in 9-week-old and housed in a controlled environment with a 12:12-h light-dark cycle with room temperature maintained at 22℃. All rats were provided with water and food ad libitum. Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University. To minimize crowdedness of cage and to reduce stressful environment, animals were housed with no more than 3 rats in each cage. The cage size was 40-cm length by 25-cm width and by 18-cm height. The hardwood chips were placed as bedding and spread out at least 2-cm thickness. In addition, animal experiment conductor was educated "Animal experiment workshop" by Institute of Laboratory Animal Resources (ILAR) of Seoul National University. The certification number is ILAR 12-01-038.

2. Experimental Design

The animals were randomly assigned to each of the following group: Control group (Con; n=8); and Exercise group (Ex; n=8). Grip strength test was conducted at the first, fourth, eighth and last week of training. Ex group was forced to climb a vertical ladder (length of 1m, incline of 85˚) 3 times a week for 12 weeks. 48 hours after exercise training, animals were sacrificed to minimize the effect of the last bout of exercise. Following sacrifice, skeletal muscle dissection was performed in various regions. The biceps (BIC), gastrocnemius (GAS), soleus (SOL), flexor hallucis longus (FHL), plantaris (PLN), tibialis anterior (TA), extensor digitorum longus (EDL) were rapidly removed and weighted. The TA and EDL muscles were dissected for cross sectional area analysis. The details were presented in below (Figure 1).

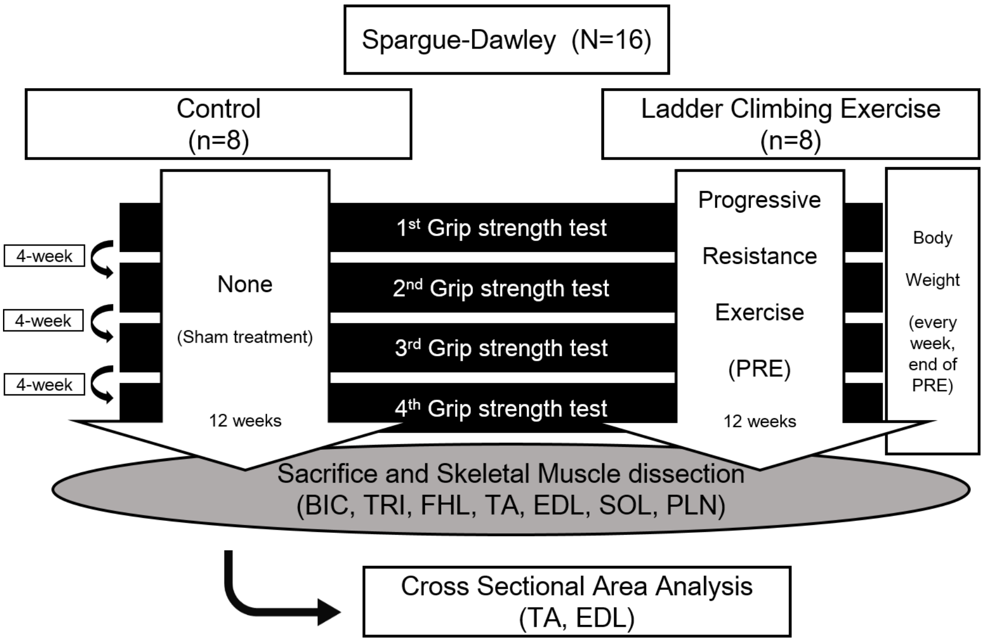
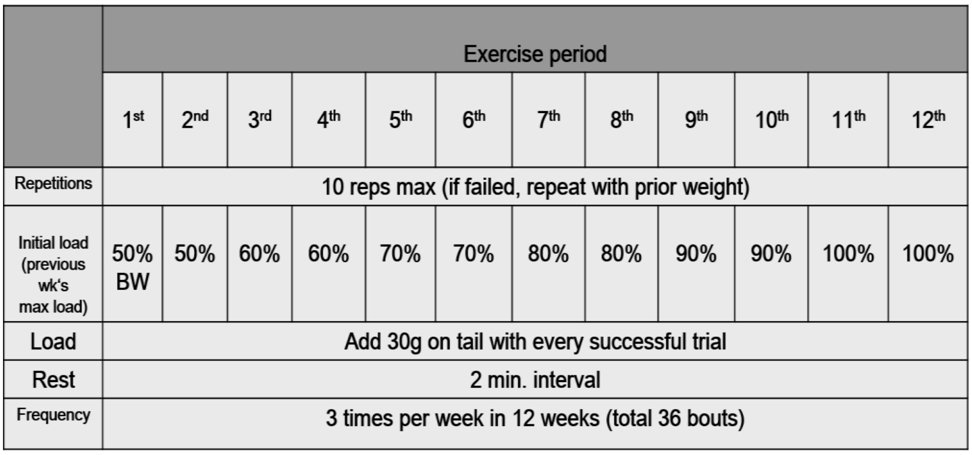


Figure 1. Experimental design

3. Exercise Apparatus and Protocol

The rats in the Ex group were forced to climb vertical ladder three times per week for twelve weeks. Training was accomplished by climbing up a 1-meter ladder with 25-cm wide and 2-cm grid steps, inclined at 85°. Initially, the rats were familiarized with the ladder by practicing climbing the ladder without weight from the bottom to the top for 5 days in the adaptation period which was conducted before the exercise period. There was no reward to motivate animals to exercise. The only encouragement was a gentle finger tapping on the animal's tail. The weight loads which were made by steel fragments in 50 ml conical tubes were attached by a clip. The clip's contact sides to a rat's tail were made by rubber to minimize stress and pain to animal. The initial load to each animal's tail was 50% of its body weight. The rats were placed at the bottom of the vertical ladder in fore-limb up position, and their instinctive characteristic drove themselves to climb up the apparatus. When the rats reached the top of the ladder, they were allowed to rest for 2 minutes. After the rest period, additional 30g were added on the weight, and the rats were returned to the bottom of the ladder for the next climb up. If a rat was not able to climb up the ladder with attached load, 2 minutes of rest time was given and then, the climbing up with the previous load was executed. This procedure was repeated until ten climbing ups were achieved. The second-week's initial exercise intensity was set at the 50 % of the maximal weight lifted in the previous week's record, and from the third to the last exercise period, initial exercise intensities were progressively increased (Figure 2 and 3). In addition, Con group was also treated in Sham control which was a familiarization of tail clipping by 10 seconds each of 10 repetitions during the experimental period.

Figure 2. Rat ladder climbing apparatus



	Exercise period											
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
Repetitions	10 reps max (if failed, repeat with prior weight)											
Initial load (previous wk's max load)	50% BW	50%	60%	60%	70%	70%	80%	80%	90%	90%	100%	100%
Load	Add 30g on tail with every successful trial											
Rest	2 min. interval											
Frequency	3 times per week in 12 weeks (total 36 bouts)											

Figure 3. Progressive resistance exercise

4. Grip Strength Test

Grip strength test was performed by allowing the animals to grasp a thin bar attached to the force gauge. This was followed by pulling the animal away from the gauge until the forelimb of a rat released the bar. This provided a value for the force of maximal grip strength. The force measurements were recorded in five separate trials while 2 minutes of rest interval was given between each trial. The maximum strength result was used in the analysis. The grip strength was measured using a grip strength meter (Model GS3, Bioseb), and a modification of a previously reported method (Meyer et al., 1979). The grip strength was measured at the last week of the exercise period which was not the exercise day. A rat's tail was pulled by an experimenter as soon as it grabbed the steel bar with its forelimb. The tail was gently but steadily pulled back from the beginning until its grip becomes lost. The maximum force (g) of grip strength was recorded (Figure 4).

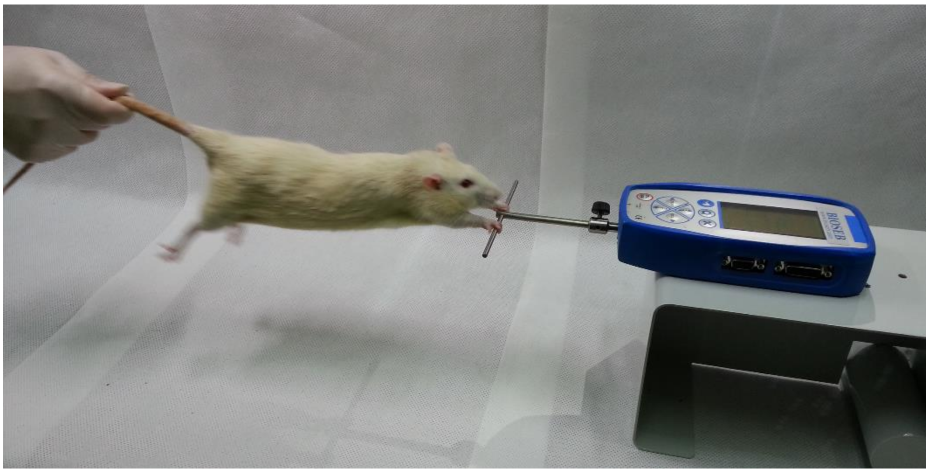


Figure 4. Grip strength test

5. Skeletal Muscle Dissection

Animals in each group were anesthetized with 30 mg/kg Zoletil 50 (Virbac, Carros). The cervical vertebral was dislocated followed by animal's anesthetic status. Then, groups of muscle which were biceps (BIC), gastrocnemius (GAS), soleus (SOL), flexor hallucis longus (FHL), plantaris (PLN), tibialis anterior (TA), extensor digitorum longus (EDL) were surgically removed and weighted. The procedure of muscle dissection was done within 15 to 30 minutes by 2 technicians and each person's performance remained the same throughout the entire procedure. The five regions (BIC, GAS, SOL, FHL and PLN) of skeletal muscle were immediately put into the 1.8 ml cryo-tube and stored in a -80℃ freezer. The muscle regions of TA and EDL which were used for histological analysis stored in paraformaldehyde (PFA) for fixation. The regions of skeletal muscle were briefly represented below (Figure 5).

Figure 5. Various parts of skeletal muscle dissection

6. Skeletal Muscle Cross Sectional Area

After muscle weight of TA and EDL was measured, the muscles were preserved in 4% paraformaldehyde (PFA) for fixation for more than a week. Then, the midsection, which was the thickest part of the muscle, was carefully cut. After that, the muscles were placed in the cassette and rinsed off gently in distilled water for 10 to 15 minutes. The all cassettes were then run in the processing machine (Shandon Citadel 1000, Thermo Fisher Co.). After the processing procedure, the cassettes were carried into embedding machine (Tissue-Tek® TEC® 5 Tissue Embedding Console System, Sakura) to make final paraffin blocks. The blocks were then cut into 4-μm thickness and placed on the slides. The microtome (20 Microsystems, Leica) was used for this procedure. The slides then were put to hematoxylin and eosin (H&E) staining which was a modification of the previously reported method (Stein, John M and Padykula, Helen A., 1962). The slides were taken through brief continual submergences in xylene, alcohol and water to hydrate the tissue. The slides were then stained with the nuclear dye (Hematoxylin, Sigma) and rinsed off, and then stained in the counterstain (Eosin, Sigma). After that, they were rinsed in a reverse order that is from water, alcohol to xylene. The stained skeletal muscle cross sections were placed on the coated glass and sealed with mounting gel (Canada Balsam, Kanto). Then, images were captured by computer-assisted processing system, INFINITY lite software (Innerview 2.0, Lumenera) with optical microscope (ECLIPSE E100, Nikon). The images were analyzed in 40x magnification to avoid miscalculation such as recognizing a broken fiber as two fibers, which were originally one fiber (Figure 6). In addition, as the magnifying power went up, there were incomplete fibers shown at the edge of images. In the same manner to reduce miscalculations, these fibers were not counted or measured.

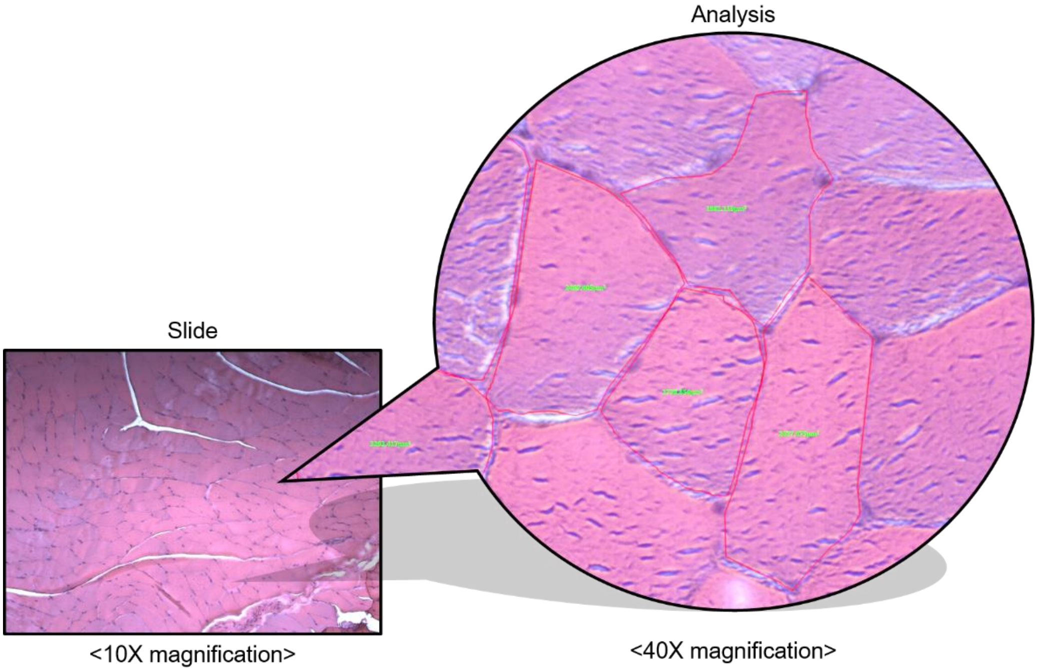


Figure 6. Analysis of CSA

7. Statistical Analysis

Statistical analysis were carried out on SPSS version 18.0 software. Values were expressed as means ± SD. Independent T-test was used to compare mean value of CON group and mean value of EX group in all results and paired T-test was used to compare the first grip strength test to other three tests in EX group. The criterion for significance was set the 0.05 α level.

III. RESULT

1. Exercise Performance

The exercise performance was recorded from the first week to the end of exercise session. The initial intensity was set at 50 % of a rat's body weight. Then, from the second week to the end of exercise session, the exercise intensities were progressively increased. In the second week of training, 50% of the maximal weight, which was accomplished in the previous week, was set as initial load (Figure 3). Likewise, the intensity was gradually increased and recorded as shown in Figure 7 and Table 1. In addition, the relative load which is maximal weight divided by a rat's body weight was represented in Figure 8 and Table 2. Interestingly, the maximal weights recorded as 1754±25 g, that was 3.7-fold greater than the rats average body weight (471±24 g).

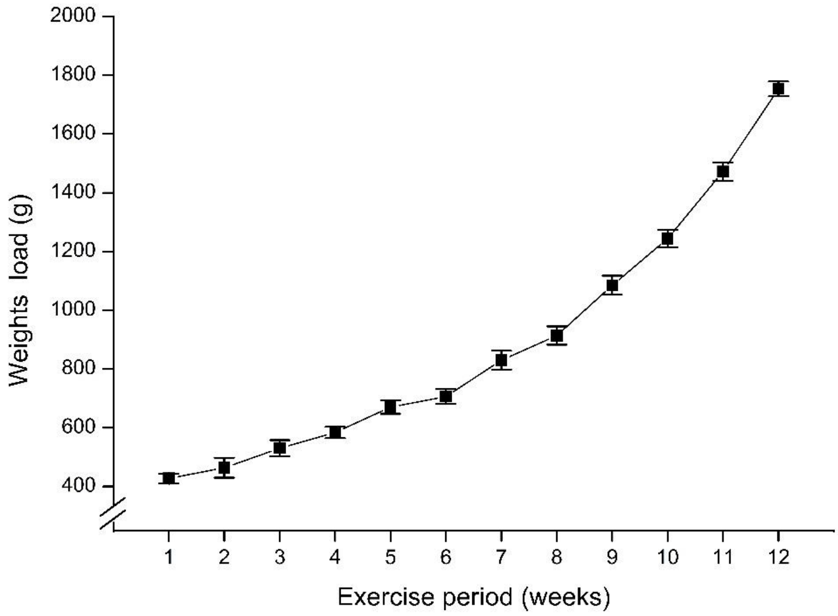


Figure 7. Maximal weight lifted per week

Table 1. Exercise performance record

Variable	Mean ± SD					
	1 WK	2 WK	3 WK	4 WK	5 WK	6 WK
Maximal load lifted (g)	428 ± 17	464 ± 34	530 ± 28	584 ± 19	670 ± 23	706 ± 25
	7 WK	8 WK	9 WK	10 WK	11 WK	12 WK
	830 ± 32	914 ± 31	1085 ± 33	1243 ± 30	1473 ± 31	1754 ± 25

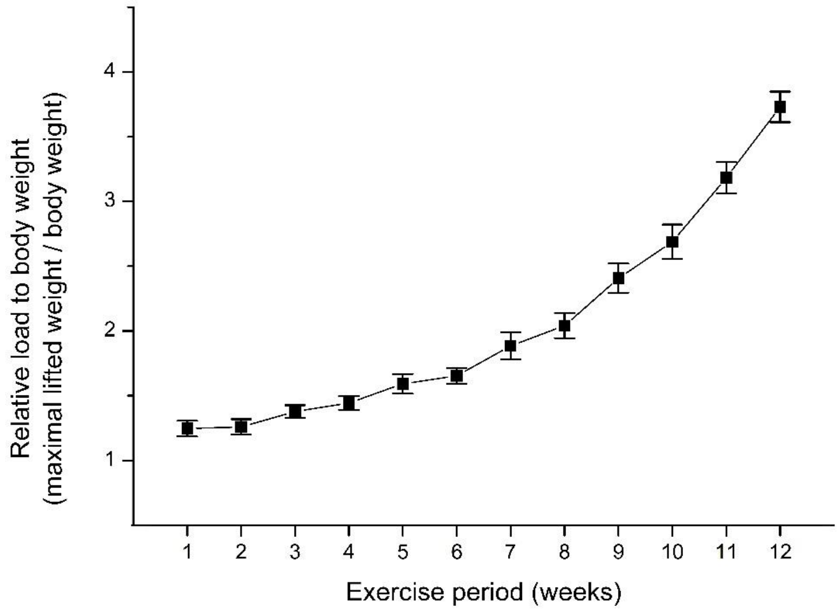


Figure 8. Maximal load adjusted to body weight

Table 2. Relative exercise performance adjusted to body weight

Variable	Mean ± SD					
	1 WK	2 WK	3 WK	4 WK	5 WK	6 WK
Maximal load adjusted to body weight	1.246 ± 0.06	1.259 ± 0.059	1.377 ± 0.049	1.443 ± 0.053	1.591 ± 0.075	1.653 ± 0.06
	7 WK	8 WK	9 WK	10 WK	11 WK	12 WK
	1.885 ± 0.103	2.041 ± 0.097	2.408 ± 0.113	2.688 ± 0.134	3.183 ± 0.123	3.73 ± 0.119

2. Body Weight Change

In the body weight from ten-week-old to fifteen-week-old period, there was no significant difference~~s~~ between Con group and Ex group. However, from sixteen-week-old to twenty first-week-old period, Con group showed higher body weight than Ex group ($p<0.05$) (Figure 9). The records of animals’ weight were represented in Table 3 for Con group and Table 4 for Ex group. In addition, the value of body weight gain showed a significant difference at the second, sixth, seventh, eighth and tenth point (Figure 10). The detailed amount of weight gain values was described below (Table 5 and Table 6).

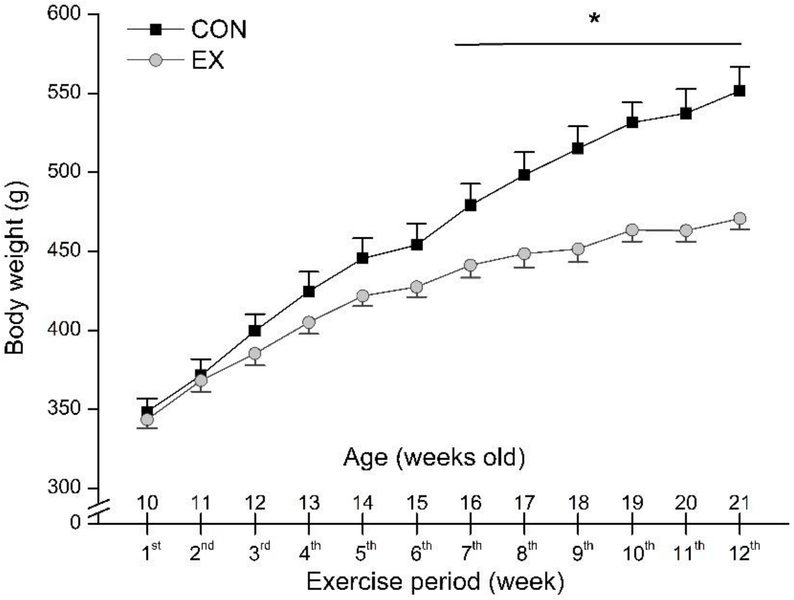


Figure 9. Record of body weight

Values are mean ± S.D.; n=8/group; * significantly different from Ex group ($p<0.05$).

Table 3. Weekly body weight record of Con group

Variable	Mean ± SD					
	1 WK	2 WK	3 WK	4 WK	5 WK	6 WK
Body weight	349 ± 24	372 ± 29	400 ± 31	425 ± 35	446 ± 36	454 ± 38
	7 WK	8 WK	9 WK	10 WK	11 WK	12 WK
	479 ± 38	498 ± 41	515 ± 39	532 ± 36	537 ± 43	552 ± 42

Table 4. Weekly body weight record of Ex group

Variable	Mean ± SD					
	1 WK	2 WK	3 WK	4 WK	5 WK	6 WK
Body weight	344 ± 15	368 ± 20	385 ± 21	405 ± 21	422 ± 19	428 ± 18
	7 WK	8 WK	9 WK	10 WK	11 WK	12 WK
	441 ± 22	449 ± 25	452 ± 24	464 ± 21	463 ± 20	471 ± 24

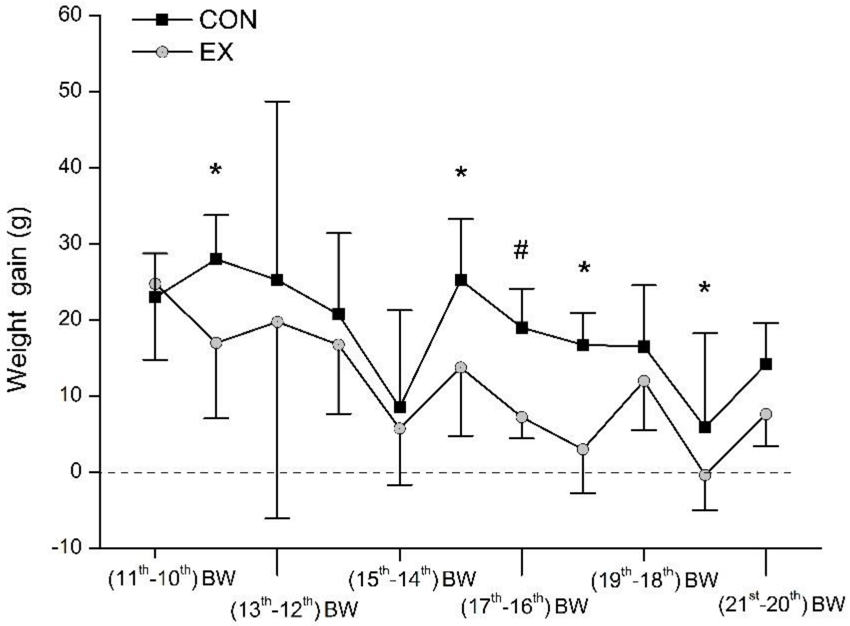


Figure 10. Difference in weekly body weight gain

Values are mean ± S.D.; n=8/group; * significantly different from Ex group ($p<0.05$).

significantly different from Ex group ($p<0.01$).

Table 5. Body weight gain value from weekly body weight record of Con group

Variable	Mean ± SD					
	(11th – 10th)	(12th – 11th)	(13th – 12th)	(14th – 13th)	(15th – 14th)	(16th – 15th)
Body weight gain	23 ± 5.8	28 ± 5.8	25.3 ± 23.5	20.8 ± 10.6	8.5 ± 12.8	25.3 ± 8
	(17th – 16th)	(18th – 17th)	(19th – 18th)	(20th – 19th)	(21st – 20th)	
	19 ± 5.1	16.8 ± 4.1	16.5 ± 8.1	5.9 ± 12.4	14.2 ± 5.4	

Table 6. Body weight gain value from weekly body weight record of Ex group

Variable	Mean ± SD					
	(11th – 10th)	(12th – 11th)	(13th – 12th)	(14th – 13th)	(15th – 14th)	(16th – 15th)
Body weight gain	24.8 ± 10	17 ± 9.9	19.8 ± 25.8	16.8 ± 9.1	5.8 ± 7.4	13.8 ± 9
	(17th – 16th)	(18th – 17th)	(19th – 18th)	(20th – 19th)	(21st – 20th)	
	7.3 ± 2.8	3 ± 5.8	12 ± 6.5	-0.4 ± 4.6	7.6 ± 4.2	

3. Skeletal Muscle Wet Weight

The skeletal muscle wet weight was measured in seven different regions which were BIC, SOL, GAS, EDL, FHL and PLN. The BIC, GAS and TA muscles were significantly heavier in Con group than those of Ex group (Figure 11). The other parts of skeletal muscle showed a similar tendency as Con group showed bigger weight, whereas the FHL muscle of Ex group showed the opposite prospective (Figure 11). The detail values were described in Table 7.

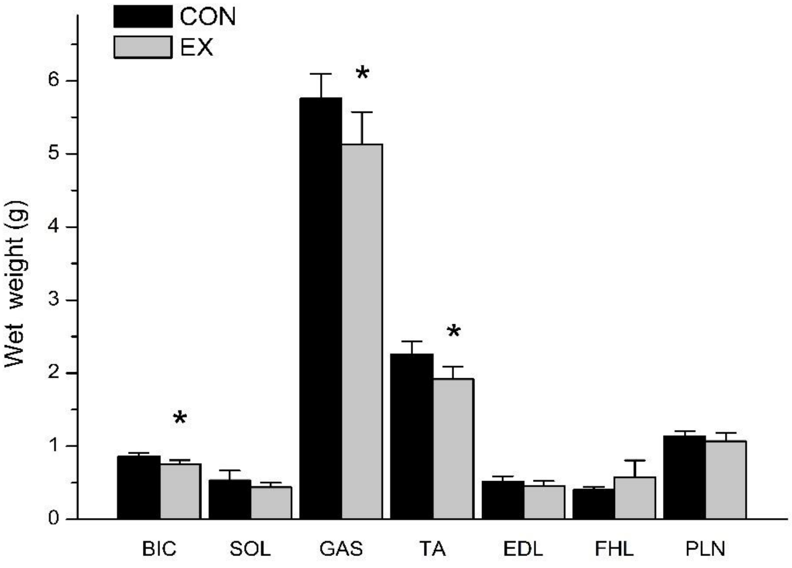


Figure 11. Skeletal muscle wet weight

Values are mean ± S.D.; n=6~8/group; *significantly different from Con group ($p<0.01$).

Table 7. Skeletal muscle wet weight

Variable	Group	N	Mean ± SD	*P* value
BIC	CON	8	0.86 ± 0.04	0.001
	EX	8	0.76 ± 0.05	
SOL	CON	8	0.53 ± 0.14	0.113
	EX	8	0.44 ± 0.06	
GAS	CON	8	5.76 ± 0.34	0.007
	EX	8	5.13 ± 0.44	
TA	CON	8	2.26 ± 0.18	0.002
	EX	8	1.92 ± 0.17	
EDL	CON	8	0.52 ± 0.07	0.103
	EX	8	0.46 ± 0.07	
FHL	CON	8	0.4 ± 0.04	0.12
	EX	6	0.58 ± 0.23	
PLN	CON	8	1.14 ± 0.07	0.18
	EX	8	1.07 ± 0.11	

4. Relative Skeletal Muscle Weight

The relative skeletal muscle weight was calculated by absolute skeletal muscles weight (mg) divided by the body weight (g). In relative skeletal muscle weight, there was no significant difference between Con group and Ex group. On the contrary, the tendencies shown in skeletal wet weight result, FHL weight was resulted to be greater in Ex group than that of Con group (Figure 12). The values were described in Table 8.

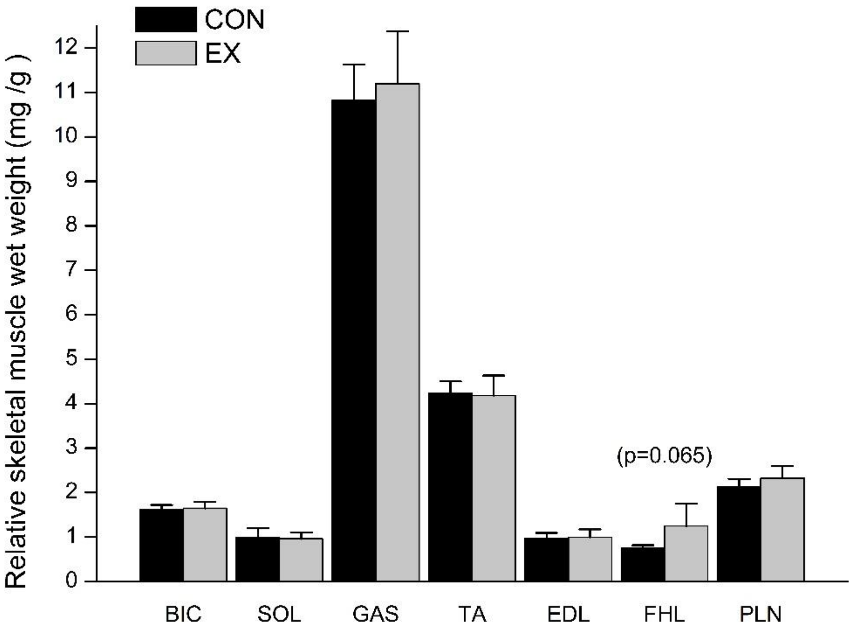


Figure 12. Skeletal muscle weight adjusted to body weight

Values are mean ± S.D.; n=6~8/group

Table 8. Relative skeletal muscle weight

Variable	Group	N	Mean ± SD	*P* value
BIC	CON	8	1.62 ± 0.96	0.695
	EX	8	1.65 ± 0.14	
SOL	CON	8	0.99 ± 0.21	0.734
	EX	8	0.96 ± 0.15	
GAS	CON	8	10.83 ± 0.8	0.483
	EX	8	11.2 ± 1.18	
TA	CON	8	4.24 ± 0.26	0.751
	EX	8	4.18 ± 0.45	
EDL	CON	8	0.97 ± 0.12	0.75
	EX	8	0.1 ± 0.18	
FHL	CON	8	0.76 ± 0.6	0.065
	EX	6	1.27 ± 0.5	
PLN	CON	8	2.14 ± 0.17	0.11
	EX	8	2.33 ± 0.26	

5. Skeletal Muscle Cross Sectional Area

In the analysis, more than 600 numbers of fibers in each group were counted and measured in the skeletal muscle cross sectional areas of EDL muscle. As a result of the analysis of EDL muscle, Ex group had a bigger average fiber size (1738μm²) than Con Group (1396μm²). In TA muscle, more than 500 numbers of fibers in each group were counted and measured. In TA, Ex group had a bigger average fiber size (2233μm²) than Con Group (1137μm²) (Figure 13. and Table 9)

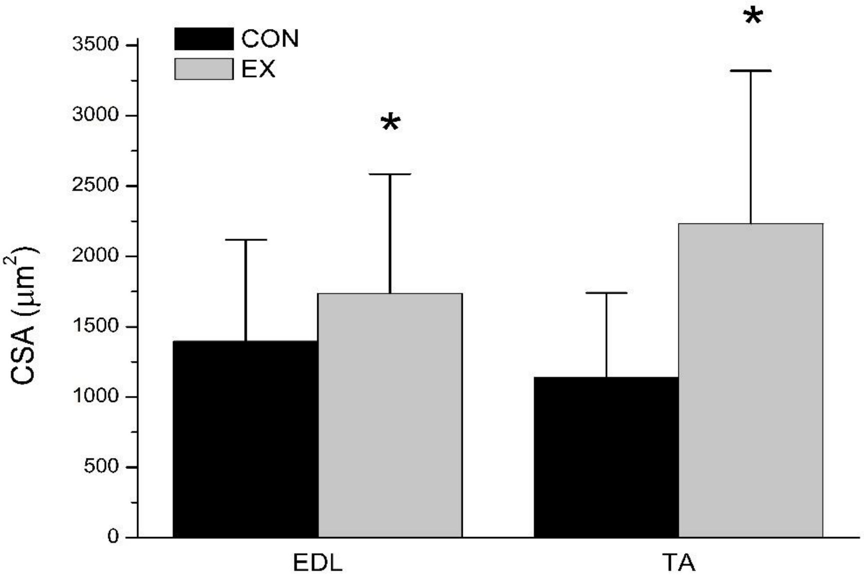


Figure 13. Skeletal muscle CSA

Values are mean ± S.D.; * significantly different from Con group ($p<0.001$).

Table 9. Skeletal CSA analysis

Variable	EDL		TA	
	CON (n = 7)	EX (n = 8)	CON (n = 7)	EX (n = 8)
	623 fibers	612 fibers	854 fibers	513 fibers
	Mean ± SD		Mean ± SD	
CSA (μm^2)	1396 ± 722	1738 ± 847	1137 ± 604	2233 ± 1087

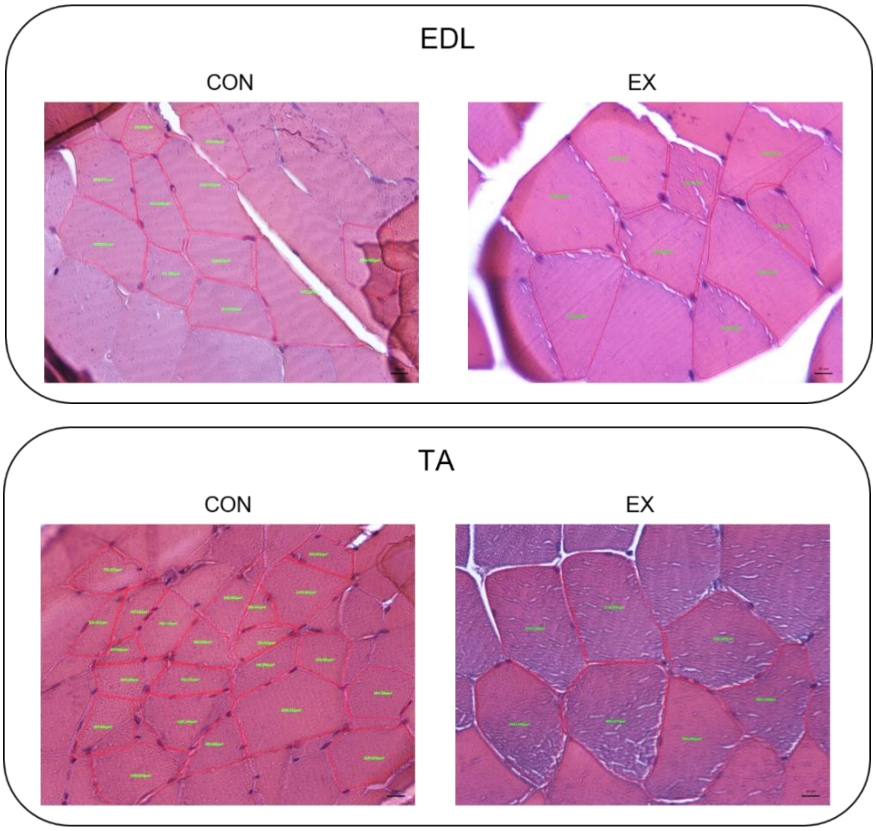


Figure 14. Images of skeletal muscle CSA

6. Change of Grip Strength

Grip strength tests were conducted at the beginning of exercise training, at the end of 4th week of exercise, at the end of 8th week of exercise and after the last session of exercise period. The values of grip strength were described in Table 10. To minimize the effect of animal’s body weight, variables of grip strength were divided by each of their body weight. As a result, Con group showed relatively higher grip strength than Ex group ($p=0.072$) at the first test. However, at the third and last value showed Ex group had higher grip strength than Con group ($p<0.05$, each).

Table 10. Grip strength record

Variable	Group	N	Mean ± SD (g)	*P* value
First (before exercise training)	CON	8	1642 ± 191	0.072
	EX	8	1424 ± 249	
Second (after 4th weeks of EX)	CON	8	2221 ± 141	0.089
	EX	8	2040 ± 235	
Third (after 8th weeks of EX)	CON	8	2214 ± 299	0.024
	EX	8	2552 ± 228	
Last (after 12th weeks of EX)	CON	8	2210 ± 137	0.003
	EX	8	2610 ± 263	

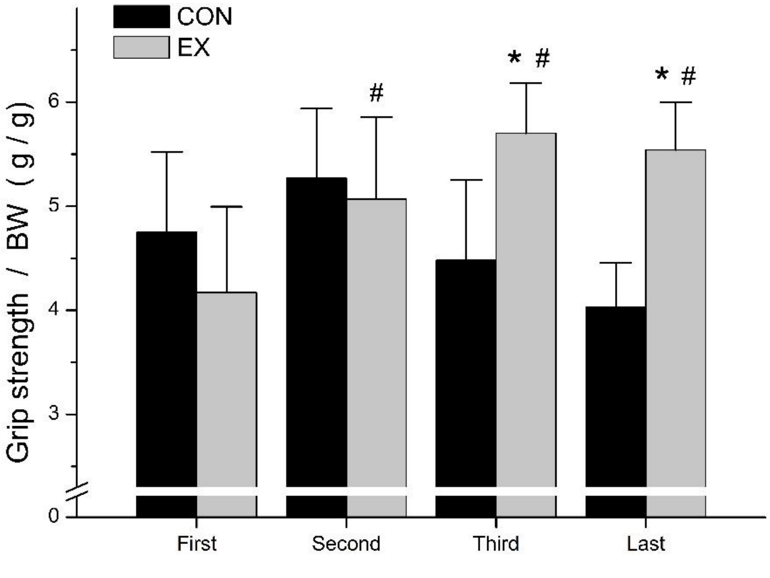


Figure 15. Change of grip strength

Values are mean ± SD; n=8/group; * significantly different from Con group (p<0.05); # significantly different from Ex group first test.(p<0.05).

7. Muscle Quality

The MQ is a force per unit muscle mass and it was used to represent one of the resistance exercise effects. The grip strength was measured by grasping of two forepaws, and the interpretations of the result were presented in two ways. First, BIC was used as a denominator and relative grip strength value was used as a numerator (Figure 16). Second, skeletal muscles that were dissected from seven regions were summed as a denominator and variables of adjusted grip strength were used as a numerator (Figure 16). As a result, Ex group had higher MQ (7255±684) than Con group (4676±591) when BIC muscle was used ($p<0.001$) (Figure 17. A). Moreover, the MQ of 7 regions of skeletal muscles, Ex group had higher MQ (532±75) than Con group (352±42) ($p<0.001$) (Figure 17. B).

$$\text{Muscle quality} : \frac{\text{Grip strength / Body weight (g/g)}}{\begin{array}{c}\Sigma\ \text{Skeletal muscle wet weight (mg)} \\ \text{(BIC or 7 regions)}\end{array}}$$

Figure 16. Formula of muscle quality

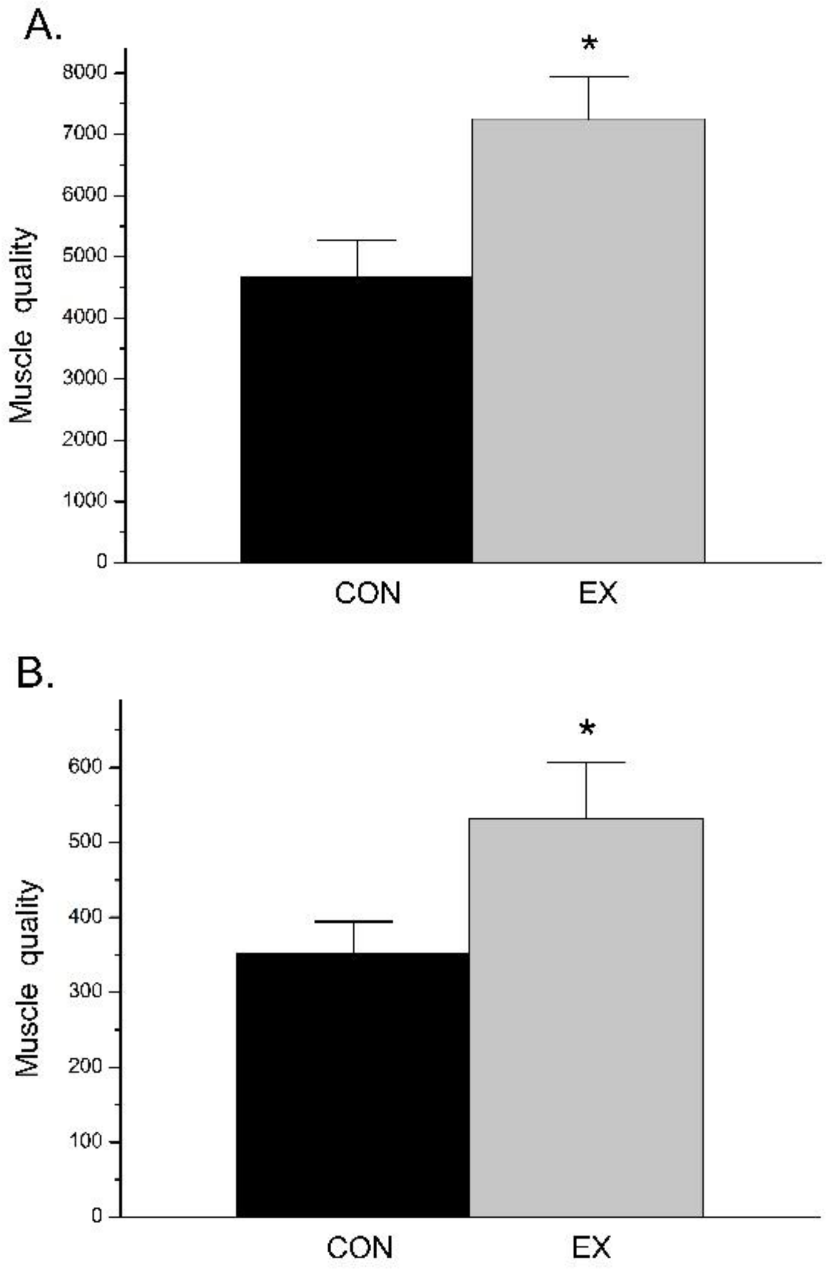


Figure 17. Muscle quality index

Values are mean ± SD.; n=6~8/group; * significantly different from Con group (p<0.001).

IV. DISCUSSION

This study was originally designed to represent previous studies to confirm ladder climbing exercise as an effective resistance exercise for skeletal muscle hypertrophy study. There were various resistance exercise types based on which apparatus was used, such as squatting movement in fixed posture, jumping movement in water with weighted belt on animal, and ladder climbing with weight attached. These three kinds of resistance exercise types were generally accepted to researchers who were interested in animal models of resistance exercise. In this study, however, the ladder climbing exercise model was chosen because of the motivation factor of exercise performance was considered. The squat-motion based apparatus was considered to be the ideal to mimic human weight-lift motion, but it was decided to be excluded as the performance of animal originates from electrical shock which is non-voluntary and as it may induce stressful reactions of animals. The other apparatus, jump-motion with weighted belt on rat, also had a disadvantage since the source of motivation came from an animal's fear of drowning as rat's jumping is being held in the water. These two kinds of resistance exercise were based on extrinsic motivation, whereas climbing-motion based exercise used intrinsic motivation. Unlike other resistance exercise model, ladder climbing exercise used natural habits of rats. The rats had an instinct to climb up when they were placed on the ladder.

The results showed that 12 weeks of progressive resistance ladder climbing exercise led to significant 1) differences in body weight between control group and exercise group from the 7th week to the last consecutive exercise training period, 2) increase in relative skeletal muscle weight of FHL, 3) growth of cross sectional areas in EDL and TA, 4) improvement of grip strength, and 5) increment in muscle quality.

The major differences from other previous studies were following two points. First, this study modified the exercise protocol in a way that the intensity of exercise to be much higher than any other study that had been reported. Second, this study focused on various regions of skeletal muscle where two parts (biceps and triceps) of fore-limb and six parts (gastrocnemius, soleus, flexor hallucis longus, plantaris, tibialis anterior and extensor digitorum longus) of hind-limb to reveal which skeletal muscle regions were responsible to progressive resistance ladder climbing exercise.

There were two possible reasons which might be responsible for body weight difference between control group and exercise group. First, the rats' body weight gain could have been down regulated as the ladder climbing exercise was executed during growth phase of rats. According to the study (Sengupta, P. 2012), normal Sprague-Dawley rats weighed normally about under 500 g at 22-week-old and leveled off at about 550 g in their lifespan. However, control group of this study showed relatively higher body weight (mean; 537 g) at 22-week-old than normal Sprague-Dawley rat. Moreover, the body weight of control group in this study reached its normal maximum level faster than exercise group. Second, the relatively high exercise intensity could have down regulated the animals' body weight. The previous study reported electrical shock might be the possible reason of body weight decrease (Tamaki et al. 1992). Although the record (Figure 8. And Table 2) showed a constant elevation of weight loads, which could be interpreted as the intensity was fairly appropriate for rats ability to perform, the heavy weight load could have possibly acted as a stressful factor that made body weight gain to be reduced.

The result showed that control group had heavier skeletal muscle wet weight (Figure 11). However, control group had significantly heavier body weight than control group at the end of the experiment, wet skeletal muscle should be adjusted to its body weight. Although there was no significant difference (p=0.065) between groups, exercise group showed heavier FHL muscle than control group (Figure 12). The pervious study (Hornberger Jr, T. A. and R. P. Farrar. 2004) suggested that FHL was highly responsible to the progressive resistance exercise stimulus. Although previous study reported there was no body weight difference between trained group and control group, the present study showed a similar aspect on skeletal muscle hypertrophy in FHL muscle.

The analysis of skeletal muscle cross sectional areas was used as a critical evidence of resistance exercise effects in various studies (Yarasheski, KE. et al. 1990; Adams G. et al. 1993; Duncan et al. 1998). In the Yarasheski's study in 1990, the fibers of rectus femoris (RF) were analyzed and they analyzed RF in three different muscle types (type Ⅰ, Ⅱa and Ⅱb) and showed increased CSA in type Ⅱb of exercise group. The muscle type Ⅱb, known as fast twitch muscle fiber, was the most sensitive fibers to resistance training (Duncan et al. 1998). According to the Duncan's study in 1998, fibers of EDL and SOL muscles were increased by 26 weeks of ladder climbing exercise. They reported approximately 20% greater fiber size in EDL muscle of exercise group. In addition, in SOL muscle, exercise group had approximately 40% bigger fiber size than control group. In present study, it showed a similar tendency of growth in skeletal muscle fibers and this confirmed the presence of skeletal muscle hypertrophy effect (Figure 13 and Table 9).

The grip strength test was used to evaluate rats fore limb strength. This assessment apparatus was used broadly in research fields that investigated muscular functions (Brooks, S. P. and S. B. Dunnett. 2009). In addition, studies which used grip strength meter generally focused either on two major fields: neuromuscular functions (Butchbach, M. E., et al. 2007; Corti, S., et al. 2008; Passini, M. A., et al. 2010) or drug effect (Spurney, C. F., et al. 2009;

Nieoczym, D., et al. 2012). The present study referred antecedent studies to minimize researcher bias and errors from handling animals. All tests were conducted by the same examiner. The tail was pulled by an examiner at horizontal angle to the bar in the same speed as possible each time. In addition, unlike other studies that reported grip strength values from hind-limb, it was unable to accomplish measuring grip strength as the animals were used in present study could not grasp the bar by its hind-limb paws. The animals simply put their paws on the bar rather than trying to grab the bar in response to the examiner's pulling, which was expected.

The concept of MQ was broadly used in various studies which focused on either exercise-related intervention effect or correlation between age related function. In human skeletal muscle research, MQ was generally held responsible for force generation from quadriceps muscle area (Goodpaster, B. H., et al. 2006; Newman, A. B., et al. 2006; Delmonico, M. J., et al. 2009). However, in present study, the values attained from BIC (fore-limb) and grip strength from two front paws were used for presenting MQ index (Figure 17 A). Considering the grip strength test could only be done in the front paws, this attempt of analysis presentation was considered to be suitable in this particular study.

In conclusion, this study focused on the effect of resistance exercise and demonstrated that 12 weeks of progressive resistance ladder climbing exercise induced skeletal muscle hypertrophy. Furthermore, this study confirmed that ladder climbing exercise model could be an acceptable apparatus for a skeletal muscle hypertrophy research model.

REFERENCES

Adams, G. R., et al. (1993). "Skeletal muscle myosin heavy chain composition and resistance training." Journal of Applied Physiology 74: 911-911.

Alway, S. E., et al. (2005). "Muscle hypertrophy models: applications for research on aging." Canadian journal of applied physiology 30(5): 591-624.

Archer, Z. A., et al. (2003). "Normal Distribution of Body Weight Gain in Male Sprague-Dawley Rats Fed a High-Energy Diet." Obesity research 11(11): 1376-1383.

Brooks, S. P. and S. B. Dunnett (2009). "Tests to assess motor phenotype in mice: a user's guide." Nature Reviews Neuroscience 10(7): 519-529.

Butchbach, M. E., et al. (2007). "Abnormal motor phenotype in the SMNΔ7 mouse model of spinal muscular atrophy." Neurobiology of disease 27(2): 207-219.

Cholewa, J., et al. (2013). "Basic Models Modeling Resistance Training: An Update for Basic Scientists Interested in Study Skeletal Muscle Hypertrophy." Journal of cellular physiology.

Corti, S., et al. (2008). "Neural stem cell transplantation can ameliorate the phenotype of a mouse model of spinal muscular atrophy." The Journal of clinical investigation 118(10): 3316-3330.

Delmonico, M. J., et al. (2009). "Longitudinal study of muscle strength, quality, and adipose tissue infiltration." The American journal of clinical nutrition 90(6): 1579-1585.

Duncan, N. D., et al. (1998). "Adaptations in rat skeletal muscle following long-term resistance exercise training." European journal of applied physiology and occupational physiology 77(4): 372-378.

Goodpaster, B. H., et al. (2006). "The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study." The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 61(10): 1059-1064.

Heinemeier, K. M., et al. (2007). "Short-term strength training and the expression of myostatin and IGF-I isoforms in rat muscle and tendon: differential effects of specific contraction types." Journal of Applied Physiology 102(2): 573-581.

Hornberger Jr, T. A. and R. P. Farrar (2004). "Physiological hypertrophy of the FHL muscle following 8 weeks of progressive resistance exercise in the rat." Canadian journal of applied physiology 29(1): 16-31.

Lee, S., et al. (2004). "Viral expression of insulin-like growth factor-I enhances muscle hypertrophy in resistance-trained rats." Journal of Applied Physiology 96(3): 1097-1104.

Lowe, D. A. and S. E. Alway (2002). "Animal models for inducing muscle hypertrophy: are they relevant for clinical applications in humans?" Journal of Orthopaedic & Sports Physical Therapy 32(2): 36-43.

Meijer, H., et al. (2008). "Effects of firing frequency on length-dependent myofascial force transmission between antagonistic and synergistic muscle groups." European journal of applied physiology 104(3): 501-513.

Mikesky, A. E., et al. (1991). "Changes in muscle fiber size and composition in response to heavy-resistance exercise." Medicine and science in Sports and Exercise 23(9): 1042-1049.

Newman, A. B., et al. (2006). "Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort." The Journals of

Gerontology Series A: Biological Sciences and Medical Sciences 61(1): 72-77.

Nieoczym, D., et al. (2012). "Influence of sildenafil on the anticonvulsant action of selected antiepileptic drugs against pentylenetetrazole-induced clonic seizures in mice." Journal of Neural Transmission 119(8): 923-931.

Norenberg, K. M. and R. H. Fitts (2004). "Contractile responses of the rat gastrocnemius and soleus muscles to isotonic resistance exercise." Journal of Applied Physiology 97(6): 2322-2332.

Ogasawara, R., et al. (2013). "mTOR signaling response to resistance exercise is altered by chronic resistance training and detraining in skeletal muscle." J Appl Physiol (1985) 114(7): 934-940.

Passini, M. A., et al. (2010). "CNS-targeted gene therapy improves survival and motor function in a mouse model of spinal muscular atrophy." The Journal of clinical investigation 120(4): 1253-1264.

Pette, D. and R. S. Staron (1997). "Mammalian skeletal muscle fiber type transitions." International review of cytology 170: 143-223.

Scheffer, D. L., et al. (2012). "Impact of different resistance training protocols on muscular oxidative stress parameters." Applied Physiology, Nutrition, and Metabolism 37(6): 1239-1246.

Scott, W., et al. (2001). "Human skeletal muscle fiber type classifications." Physical therapy 81(11): 1810-1816.

Sengupta, P. (2012). "A Scientific Review of Age Determination for a Laboratory Rat: How Old is it in Comparison with Human Age?" Biomedicine International 2(2).

Seo, D. Y., et al. (2014). "Humanized animal exercise model for clinical implication." Pflügers Archiv-European Journal of Physiology: 1-15.

Shoepe, T. C., et al. (2003). "Functional adaptability of muscle fibers to long-term resistance exercise." Medicine and science in Sports and Exercise 35(6): 944-951.

Spurney, C. F., et al. (2009). "Preclinical drug trials in the mdx mouse: assessment of reliable and sensitive outcome measures." Muscle & nerve 39(5): 591-602.

Stein, J. M. and H. A. Padykula (1962). "Histochemical classification of individual skeletal muscle fibers of the rat." American Journal of Anatomy 110(2): 103-123.

Tamaki, T., et al. (1992). "A weight-lifting exercise model for inducing hypertrophy in the hindlimb muscles of rats." Medicine and science in Sports and Exercise 24(8): 881-886.

Volek, J. S., et al. (1999). "Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training." Medicine and science in Sports and Exercise 31: 1147-1156.

Yarasheski, K., et al. (1990). "Effect of heavy-resistance exercise training on muscle fiber composition in young rats." Journal of applied physiology (Bethesda, Md.: 1985) 69(2): 434.

국문 초록

쥐의 저항성 사다리 운동이 골격근 비대에 미치는 영향

김 장 회
서울대학교 대학원
체육교육과

골격근은 신체의 40~50%를 차지하는 중요한 기관 중 하나이며, 저항성 운동은 골격근의 질량을 유지하거나 증가시키는 효과적인 운동방법으로 알려져 왔다. 다양한 연구에서 저항성 운동을 통한 골격근 내 생리학적 변화가 보고되고 있으며, 이 중 실험동물을 사용하여 골격근의 비대현상을 보다 자세히 기술하고 있다. 한편, 저항성 운동종류와 이에 따른 운동강도, 운동빈도 그리고 총 운동기간에 따른 다양한 연구결과들이 소개되고 있다. 본 연구는, 12주간의 사다리를 이용한 점진적 저항성 운동이 Sprague-Dawley rat의 골격근 비대에 미치는 영향에 대하여 관찰하였고, 이를 통하여 사다리를 사용한 운동에 대한 효용성 평가에 있다.

본 연구에 사용된 Sprague-Dawley rat은 운동군과 통제군, 각각 8마리로 분류되었으며, 운동군은 경사각이 85도로 설정된 100cm 길이의 사다리를 꼬리에 추를 달고 올라가는 방법으로 실시되었다. 총 12주 동안 운동이 실시되었으며, 매 주차마다 운동부하를 점진적으로 올려 시행하였다. 변인으로서 체중, 근력, 골격근의 무게와 골격근의 단면적이 사용되었으며, 체중은 매주 측정하였고, 근력은 운동의 시작 전, 운동 시작 4주후, 8주후 그리고 운동이 끝난 다음 측정하였다. 골격근의 무게는 쥐의 앞발에서

이두박근과 뒷발에서 가자미근, 비복근, 전경골근, 장지신근, 장무지굴근 그리고 족저근을 적출하여 무게를 측정하였고, 적출한 골격근 중 전경골근과 장지신근에서 단면적 분석이 이루어졌다.

그 결과, 12주간의 점진적 저항성 사다리운동이 Sprague-Dawley rat의 근력을 향상시켰으며, 장무지굴근의 골격근 비대현상과 함께 전경골근과 장지신근에서의 근섬유 단면적의 증가를 관찰 할 수 있었다.

따라서, 동물모델에 있어 사다리를 올라가는 점진적 저항성 운동방법이 효과적인 저항성운동 모델 중 하나로서 제시될 수 있을 것으로 사료된다.

주요어 : 점진적 저항성 운동, 골격근, 골격근 비대현상, 근섬유 단면적
학 번 : 2011-21606