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# RESISTANCE TRAINING INDUCES MUSCLE-SPECIFIC CHANGES IN MUSCLE MASS AND FUNCTION IN RAT.

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## ABSTRACT

RESISTANCE TRAINING INDUCES MUSCLE-SPECIFIC CHANGES IN MUSCLE MASS AND FUNCTION IN RAT. **Sukho Lee, Roger P. Farrar**. **JEP**online. 2003;6(2):80-87. Resistance exercise is frequently used to induce hypertrophy in animal model. This study examined the effects of 8 weeks of resistance training followed by 4 to 8 weeks of detraining on muscle mass and function. The resistance training consisted of climbing (5 reps/3 sets) a ladder carrying a load suspended from the tail. The weights were increased gradually throughout the 8 weeks of training, with the average weight at the end of training equal to 950 g; 332% body wt. Following 8 weeks of training, we observed a 17.5% increase in absolute muscle mass and 23% increase in peak tetanic tension (Po) in the flexor hallucis longus (FHL). Surprisingly, the more commonly used plantar flexor muscles including soleus (1%), plantaris (2.9%) and gastrocnemius (0%) did not appear to be affected by resistance training. Muscle masses of FHL were still significantly higher after detraining compared to control level (14.6% in 4 weeks and 9.7% in 8 weeks). Therefore, additional length of detraining would be required to return muscle mass and strength to pre-training level. The changes in Po values were proportional to changes in mass, maintaining specific tetanic tension (SPo) from 28.2±0.4 to 30.7±1.0 N/cm$^2$ across groups. We believe that this model provides is highly suitable for future studies that investigate mechanism of hypertrophy in response to resistance training.

Key Words: Ladder climbing, Flexor hallucis longus, Detraining.

## INTRODUCTION

Skeletal muscle is a highly plastic tissue, undergoing various physiological and biochemical changes in response to stimuli. An increase in muscle mass, or hypertrophy, is an important adaptation induced by various stimuli. It has become a major concern for athletes involved in power and strength-related events, as well as bodybuilding. A decrease in muscle mass, atrophy, may occur due to a number of perturbations of the neuromuscular system including reduction of stimulus induced by inactivity, aging, injury and muscular disease. Therefore, maintaining proper muscle mass and strength has been a critical factor for the elderly as well as in persons with musculo-skeletal injury, muscular diseases, or during exposure to microgravity.

Various electrical, pharmacological and physiological interventions have been developed and tested to find the most effective method for inducing hypertrophy. Among those interventions, progressive resistance training has consistently been demonstrated to increase skeletal muscle mass and strength. Several animal models resembling human resistance exercise have been used to study the effect of resistance training on skeletal muscle mass and function (10,14,17). These studies, however, used electrical stimulation or food reward to conduct resistance exercise. Thus, it is not clear how these manipulations affect the observed hypertrophic response in those studies. Also, other resistance training studies used muscle weight / body weight ratio as an indication of muscular hypertrophy (9,12,14). The average final body weights of resistance-trained animal are less than control animals in those studies. Therefore, it is hard to determine if the results are due to the changes in muscle mass or are result of a reduction of body weights.

Recently, our laboratory developed a resistance training protocol for the mouse model and tested the effect of different types of training protocols on muscle mass and strength. Mice are the animals of choice for genetic manipulations and thus establishing parameters of physiological adaptation in mice is of significant importance. However, pilot work in our laboratory had established that there was little response of the muscles of the lower leg from resistance training in the mouse model. Therefore, we were interested in applying the same resistance training protocol to the rat model to see if it induces significant hypertrophy in lower muscle groups.

We are also interested in cataloging changes in muscle characteristics during different length of detraining protocols. Detraining is the partial or complete loss of training-induced adaptations upon removal of the training stimulus. The effects of detraining may reflect the specificity of training adaptations, the length of the training protocol, as well as the duration of detraining. There are only a few animal studies that have investigated the effects of detraining on resistance training-induced adaptations (6). Based on the half-life of myofibrillar proteins (9-12 days), once the stimulus of hypertrophy is removed the muscle mass should return to pre-training levels by 8 weeks.

Therefore, the primary aim of this study was to examine the effects of 8 weeks of resistance training, which produces an absolute increase in muscle mass and strength. Then, we evaluated muscle mass and function after the detraining period for 4 and 8 weeks to determine whether 8 weeks of detraining was enough to return the muscle mass to its pre-training level.

## METHODS

### Animal care and experimental design

Twenty female Sprague-Dawley rats at the age of five months were obtained from the Animal Resource Center of our university. All animals were randomly assigned into two groups: (1) control (N=5), (2) resistance training (N=15). After completion of 8 weeks of resistance training, animals in resistance trained group were divided into one of the three groups (N=5 per group): (1) Resistance training (RT), (2) 4 weeks of detraining (DT4) and (3) 8 weeks of detraining (DT8). The rats were housed in pairs and kept on a standard 12:12-h dark-light cycle. Training was conducted during the day. All animals were provided water ad libitum and weighed once every week. Food intake in sedentary control and detraining animals was moderately restricted to balance with their resistance-trained counterparts.

### Resistance Training

Climbing of a 1m ladder with 2 cm grid ladder inclined at 85 degrees with weights attached to their tails was

used as resistance exercise. Rats were familiarized with the exercise for three days. Three days after familiarization, the resistance training was begun using cylinders containing weights that were attached to the base of tail with foam tape (3M Conan), a Velcro strap and a hook. Briefly, the cylinders were fastened to the tail by wrapping the upper portion of the tail (2-3 cm from the proximal end) with Velcro on top of foam tape (Figure 1). Then, the appropriate weights were inserted into the cylinders. The rats were positioned at the bottom of the climbing apparatus and motivated to climb the ladder by grooming action to the tail. The initial weight attached was 50% of their body weight and increased gradually throughout the 8 weeks of training period. The resistance training consisted of 3 sets of 5 repetitions with a 1min rest interval between the reps and 2 minutes between the sets for 8 weeks. When the rats reached the top of the ladder, they were allowed to recover in the resting area. This procedure was repeated until either the rat finished all three sets of training or failed to climb the entire length of the ladder. Electrical shock (0.2-0.3 m Amp) was used to motivate the rat to climb when necessary. The training was stopped when any rat neglected to climb up the ladder following three successive shocks to the tail. The rats in the training group were trained twice a day (9:00 a.m. and 2:00 p.m.) every third day for 8 weeks.

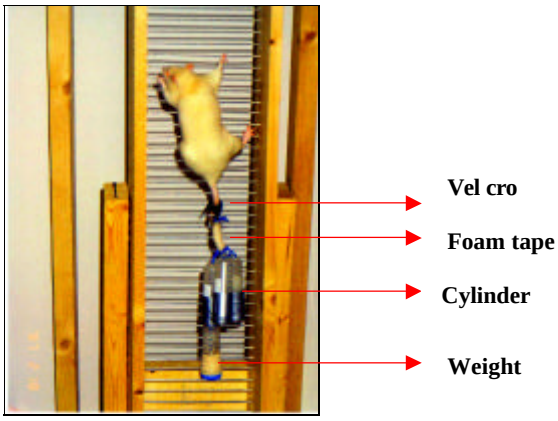


**Figure 1. A photograph of the ladder and weight suspension on the tail of a rat.**

**Detraining**

The rats were kept in cages throughout the detraining period either four or eight weeks following 8 weeks of resistance training. No exercise was performed during the period.

**Muscle cross sectional area**

The total muscle cross sectional area (CSA) of the flexor hallucis longus (FHL) was calculated using the formula: CSA ($mm^2$)=muscle mass (mg) x (1.06 ($mg/mm^3$) x muscle fiber length (mm)). The fiber length of FHL was determined by a nitric acid digestion technique (2).

***In situ* contractile properties of FHL**

The rats were anesthetized with sodium pentobarbital (100mg/kg) and the FHL was exposed for in situ evaluation of the contractile properties. The digital tendon of FHL was isolated and connected to the lever of a dual-mode servo galvanometer (Model 3005 B, Cambridge Technologies). The contractions of FHL were induced by stimulation of the sciatic nerve through a silver wire electrode with a 330ms, 5-10 volts, 0.5 sec duration by a Grass 8S stimulator. The muscle was kept wet in mineral oil and maintained between 36.5°C and 37.5°C with a radiant heat lamp. The muscle length was adjusted to optimal muscle length with a micrometer, while determining peak twitch tension (Pt). The muscle was stimulated at 0.5 Hz, 5 volts for the peak twitch tension and 100 Hz to 175 Hz, 10 volts for peak tetanic tension (Po). The contraction time (CT) and half relaxation time (1/2RT) were recorded during each contraction. The galvanometer was interfaced with a computer (Macintosh Quadra 840 AV) equipped with a National Instruments A/D board. The data was stored and analyzed using Labview software (Version 3.0). Each contraction was followed by 1-min rest, and resting muscle length (Mlo) was measured at the end of contractile measurement.

**Statistical Analyses**

All data were expressed as means±SD. A one-way analysis of variance (ANOVA) was used to determine significant overall main effect. Fischer's least significant difference was used to test for group differences. A significance level of $p<0.05$ was used for all comparisons

## RESULTS

**Body and muscle weights**

No significant differences in the final body weights among the groups were observed (285.9±3 g). Also, there were no significant differences in the weights of adrenal gland among the groups. Resistance training significantly increased muscle weights of FHL by 17.5% over the CON group (Figure 2) ($p<0.05$).

Surprisingly, other plantar flexor muscle groups (soleus, plantaris and gastrocnemius) measured in this study were not changed by eight weeks of resistance training (Table 1). Four and eight weeks of detraining resulted in 2.5% and 6.6% decrease in the FHL weights from the trained status, respectively. However, there was not a significant difference in muscle mass of FHL among these groups (RT vs DT4 vs DT8). Also, weights of FHL in both DT4 and DT8 group were still significantly higher than that in control group ($p<0.05$).

**Table 1. Body weights and muscle masses.**

| *Variable* | *CON* | *R* | *DT4* | *DT8* |
|---|---|---|---|---|
| ***BW (g)*** | 286.5±1.8 | 285.6±1.1 | 285.8±0.8 | 285.7±1.5 |
| ***FHL (mg)*** | 463.4±10.5 | 544.4±18.6* | 530.9±34.0* | 508.2±52.9* |
| ***Sol (mg)*** | 121.1±8.1 | 122.3±12.9 | 128.5±13.9 | 131.2±15.1 |
| ***Plan (mg)*** | 344.8±16.2 | 355.1±7.9 | 355.7±17.9 | 348.4±22.4 |
| ***Gast (mg)*** | 1706±22.1 | 1698±19.0 | 1695±86.1 | 1739±65.5 |
| ***Adre )mg)*** | 45.1±1.2 | 45.3±1.8 | 46.4±5.7 | 47.8±3.1 |

Data represent mean ±SD. BW, body weight; FHL, flexor hallucis longus, Sol, soleus; Plan, plantaris; Gast, gastrocnemius; Adre, adrenal gland. * Significant difference from control group ($p<0.05$).

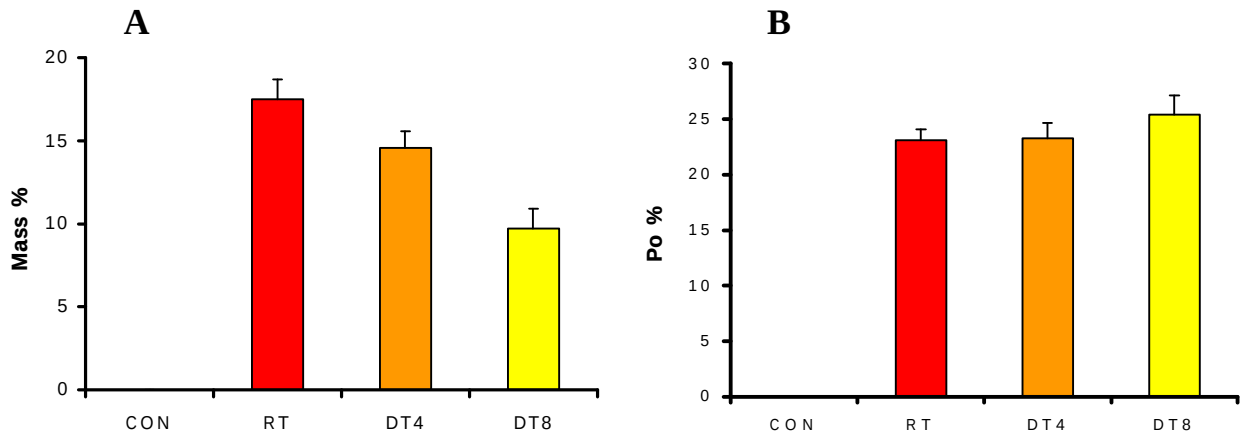


**Figure 2. A) Muscle mass of FHL increased 17.5% in RT. B) Peak tetanic tension (Po) of FHL increased 23% in RT and was maintained during the detraining period. =Significant difference from Control group ($p<0.05$). Values are expressed as mean±SD. CON=Control; RT=Resistance training; DT4=4 weeks of detraining; DT=8 weeks of detraining.**

### Training response

The initial weights attached were about 50% body weight. Therefore, about 150 g of weight was used for the first training session. Then it was increased gradually throughout the eight weeks of training period. The average weights carried by the end of each week were recorded (Figure 3). The average final weight attached to the tails of rats at the end of training was 950 g equivalent to 332% of their body weight.

### Muscle cross sectional area

Muscle cross sectional area (CSA) was calculated using an equation described in methods section. The mean fiber length of the FHL was 25±3% of the muscle length at resting muscle length, as determined from nitric acid-digested samples. The muscle CSA of FHL showed a 17.4% increase in the RT group over the

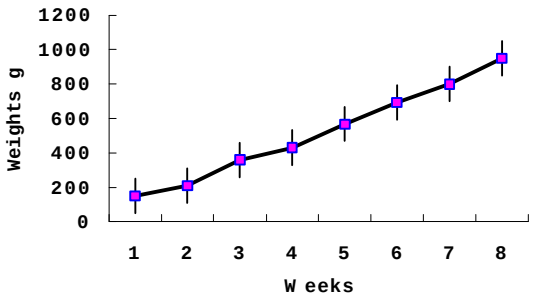


**Figure 3. The average weights carried each week.**

CON group ($p<0.05$). Detraining resulted in a decrease in CSA from the trained status both in DT4 group and DT8 group by 0.4% and 7.4%, respectively. However, the CSA of both DT groups were significantly larger than that in CON group ($p<0.05$).

**Contractile properties of FHL**

There was no significant difference in muscle length (MLo) and half relaxation time (HRT) among the groups (Table 2). However, contraction time (CT) was decreased in RT group and returned to pre-training value after detraining. Neither resistance training nor detraining alone changed in peak twitch tension (Pt). Peak tetanic tenson (Po) was increased 23.1% in RT group compared to control level ($p<0.05$). Po was well maintained in both detraining groups (DT4 and DT8) and no significant difference was observed among the groups (RT, DT4 and DT8) (Figure 2B, Table 2). Therefore, there was no significant difference in specific tension (SPo) normalized peak tetanic tension divided by CSA among the groups (Table 2).

**Table 2. Contractile properties of FHL.**

| | *CON* | *RT* | *DT4* | *DT8* |
|---|---|---|---|---|
| ***CSA ($mm^2$)*** | 48.2±0.9 | 56.5±3.0* | 56.3±3.0* | 52.3±5.1 |
| ***Mlo (mm)*** | 36.7±0.6 | 36.5±0.6 | 36.2±0.8 | 36.7±1.1 |
| ***CT (ms)*** | 36.8±0.8 | 34.6±0.5* | 36.7±1.2$^{+}$ | 36.0±0.9$^{+}$ |
| ***HRT (ms)*** | 26.8±0.8 | 22.0±2.4 | 23.6±4.4 | 25.8±6.0 |
| ***Pt (mN)*** | 2785±73 | 2782±251 | 2828±115 | 2907±143 |
| ***Po (mN)*** | 13564±399 | 16696±1203* | 16717±707* | 16534±1236* |
| ***SPt ($N/cm^2$)*** | 5.8±0.2 | 4.9±0.5 | 5.0±0.4 | 5.0±0.4 |
| ***Spo ($N/cm^2$)*** | 28.2±1.0 | 29.6±2.4 | 29.8±2.7 | 30.72±2.3 |

Data represent mean±SD. CSA, cross sectional area; Mlo, resting muscle length; CT, contraction time; HRT, half relaxation time; Pt, peak twitch tension; Po, Peak tetanic tension; SPt, specific peak twitch tension; SPo, specific peak tetanic tension. * Significant difference from CON, $^{+}$ Significant difference from RT group ($p<0.05$).

## DISCUSSION

The training protocol used in this study was based on the progressive overload principle of resistance exercise. The number of sets, rest periods and training frequency used in this study were similar to human resistance training protocols. In this model, the FHL was highly responsive to the training stimulus resulting in 17.5% increase in muscle mass and 23% increase in Po of FHL over the control level. Surprisingly, the more commonly used plantar flexor muscles including soleus, plantaris and gastrocnemius did not appear to be affected by the training. We believe that the selective hypertrophy of FHL is due to the recruitment patterns during the climbing exercise. We observed that rats place their toes on the ladder and the initial phase is a lengthening of the FHL. The rat then goes through plantar flexion and knee extension in concentric contractions. The FHL thus goes through an eccentric phase and then a concentric phase. The

eccentric exercise has been shown to be more effective than concentric exercise for improving muscle mass and strength (1, 8, 18). However, several studies using a similar resistance training protocol reported hypertrophy of plantar flexor muscles (4, 10). The reason for this discrepancy might be explained by the training duration. The rats in Duncan's model were trained for 26 weeks and the rats in Klitgaard's protocol were trained for 36 weeks. Thus, it might be possible that we could induce hypertrophy for other plantar flexor muscles if we increase the length of training protocol from 8 weeks.

In our knowledge, there was only one study that reported hypertrophy of FHL muscle in response to resistance training (12). However, the target muscle of that study was an adductor longus muscle not FHL. Therefore, it might be also possible that there might be a hypertrophy of FHL in other resistance training studies but they simply did not measure it.

The hypertrophy observed in present study is greater than the values reported from other resistance training models (9, 12, 17). Only a few studies have demonstrated a comparable level of exercise-induced hypertrophy to the present study (10, 14). However, other studies trained the rat for a longer period of time (12 to 36 weeks) and with a greater frequency of training compared to present training protocol. Also, the hypertrophy was only significant when expressed relative to body weight (9,12,14). We observed an increase in absolute muscle mass and peak tetanic tension only after eight weeks of resistance training in present study. Therefore, the protocol used in present study would be an excellent model to study muscular response to resistance exercise.

The most significant alteration in contractile properties resulting from resistance training is an increased peak tetanic tension (Po) (16). We observed a 23% increase in Po from FHL in present study. Peak tetanic tension is closely related to muscle mass and CSA. Therefore, an increased Po in this study may be explained by an increased muscle mass (17.5%) and CSA (17.4 %). Despite a large increase in Po with resistance training, Pt was unaffected in this study. However, Klitgaard (1988) reported increases in Pt that were approximately proportional to the increase in Po. The reason for this discrepancy might be explained by the training duration. The rats in Klitgaard's protocol were trained for 36 weeks. Therefore, it may be possible that longitudinal differences exist in the time course of Pt and Po adaptations.

Studies investigating the effect of resistance training on contraction time (CT) and half relaxation time (HRT) have produced conflicting results. Some investigators have demonstrated a decrease (10) while others have observed an increase (6) or no change (5) in CT and HRT in response to resistance training. In present study, CT was decreased after resistance training and returned to pre-training level during the detraining period despite of no changes in HRT. These findings indicate possible alterations of muscle fiber type and/or myosin heavy chain isoform from slow to fast type in response to resistance training even though we did not measure those parameters in this study. The reason for discrepancy between CT and HRT in present study is not clear.

Most detraining studies have investigated the reversal of endurance training-induced adaptations using either human (3, 11) or animal (13, 15). There are only few animal studies that have investigated the effects of

detraining on resistance training-induced adaptations. When male hamsters were subjected to weight training, they exhibited hypertrophy in the biceps brachii (25%), soleus (18%) and extensor digitorum longus (EDL) (20%). The muscle masses of the soleus and EDL returned to control level after five weeks of detraining. The biceps brachii, however, returned to control level after 15 weeks of detraining (6). In present study, the muscle mass of FHL did not return to pre-training level even after eight weeks of detraining. Therefore, additional length of detraining would be required to return muscle mass and strength to pre-training level.

**Conclusion**

The present study demonstrated absolute increases in muscle mass and strength in FHL after eight weeks of resistance training. The magnitude of hypertrophic response in the present study was remarkable given the short period of time compared to previous studies. Thus, this model may provide an excellent method for future studies that investigate mechanism of hypertrophy in response to resistance training. The increased muscle mass and strength of FHL were maintained above the control level even after eight weeks of detraining period. Therefore, a longer detraining period would be required to return muscle mass and strength to pre-training level.

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