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Research Article

Effect of Eight Weeks of Resistance Training on PAX7 and MyoD in Fast and Slow-Twitch Skeletal Muscles of Old Rats

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Abstract

Background: Box 7 protein (PAX7) and myogenic differentiation (MyoD) proteins are the essential proteins to regulate the satellite cell and muscle hypertrophy.

Objectives: We aimed to investigate the effect of resistance training (RT) on PAX7 and MyoD in soleus and extensor digitorum longus (EDL) muscles of old rats.

Methods: In this experimental study, 12 old female rats were randomly divided into two groups including RT and control groups. The RT program was three days a week for eight weeks, in which the rats climbed a one-meter vertical ladder with 26 steps. The carried weights varied from 50% of body weight in the first week to 100% of body weight in the 8th week. Kolmogorov- Smirnov test and independent sample *t* test was used for statistical analysis ($P \leq 0.05$).

Results: MyoD protein in RT group significantly increased in soleus and EDL muscles compared with the control group ($P = 0.001$). Also, Pax7 significantly increased in the soleus muscle in the RT group compared with control group ($P = 0.002$); nevertheless, there was no significant difference in Pax7 protein in the EDL muscle between the RT and control groups ($P = 0.10$).

Conclusion: RT can lead to proliferation and renewal of satellite cells in the soleus and EDL muscles by increasing PAX7 and MyoD proteins.

Keywords: Aging, Box 7 protein, Myogenic differentiation proteins, Exercise, Muscle

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Background

Sarcopenia is the gradual loss of muscle mass and strength/performance in old age, which is increasingly identified as an important factor in the occurrence of negative health consequences towards the end of life (1). The diagnostic criteria for this disease are the changes in muscle strength and volume, cellular and molecular changes, and consequently the changes in muscle quality (2). Recent evidence suggests that in the elderly, a large proportion of satellite cells change from a silent state to an aging state, which prevents the proliferation and regeneration of the satellite cell reservoir. To determine the myogenic status of satellite cells, the expression of the transcription factor of the paired box 7 protein (Pax7), myogenic regulatory factors, and the myogenic differentiation (MyoD) protein, are important for identifying the silent satellite cells, proliferation, and differentiating satellite cells, respectively. The first step in this muscle repair process is to activate the silent satellite cells by the Pax7 protein, which leads to rearranging MyoD (3). The results indicate that skeletal muscle satellite cells require Pax7 expression, or Pax7 expression is responsible for the survival of satellite cells (4). It has been reported that in animal models, MyoD is lower in fast and slow-twitch muscles of elderly rats, while this amount is lower in the soleus muscle at the lowest value and also the response of the MyoD gene of the soleus muscle to the plantaris (plantar muscle) (5). Under the damaged muscle (pharmacology, pathology, or exercise), the expression of the MyoD gene increases, indicating the proliferation of satellite cells in order to repair the damage (6). In this regard, resistance training (RT) is an efficient strategy to increase skeletal muscle mass and strength in the elderly (7,8). Sports activities activate the adaptation processes and muscle structure repair. RTs are very effective for compensating sarcopenia, which has many clear effects on this phenomenon. Moreover, long-term RT can restore the contents of muscle fiber satellite cells to the level of healthy untrained adults (9). RT would lead to muscle hypertrophy in the elderly. Here, some possible mechanisms that lead to RT-induced muscle growth resulting in the elderly may be increased muscle protein synthesis, activation and proliferation of satellite cells, production of anabolic hormones (testosterone), and decreased cytokine activity. Furthermore, it has been shown that satellite cells increase in both type I and type II muscle fibers in response to a period of RT in healthy young men. In addition, increasing age can disrupt the activation and proliferation of the satellite cells in elderly men (10). Snijders and colleagues reported that the content of satellite cells and MyoD protein content increased significantly after 12 weeks of RT (5). D'Souza et al found that 9 weeks of exercise significantly increased Pax7 protein in gastrocnemius and soleus muscles (11). Despite the many benefits of exercise in the physiological health of skeletal muscles in the elderly, very little research has been done on the effect of exercise on the amount of protein involved in the sarcopenia process in these people. Currently, a certain conclusion has not been reached regarding sports exercises because of the differences in the studied muscles, as well as different participant preparation, age, and sex.

Objectives

It has been found that aging does not affect all types of fibers in the same way, and type II fibers are more affected. Thus, in addition to a slow-twitch muscle, we also considered a fast-twitch muscle, so that in addition to measuring the content of proteins, we can also compare these types of muscles with each other. Therefore, we aimed to investigate the effect of RT on Pax7 and MyoD proteins in soleus and extensor digitorum longus (EDL) muscles of old rats.

Methods

In this experimental study 12 female 20-month-old Sprague-Dawley rats with a mean ± standard deviation (SD) weight of 250 ± 30 g were purchased and kept under standard conditions to adapt with the new environment for one week. Then all rats were randomly divided into two groups (RT and control) of six rats. The RT groups underwent eight weeks of RT, three sessions per week, climbing a one-meter vertical ladder with 26 steps, with two centimeters of space between each step with a slope (85 degrees). Each session consisted of three sets via five repetitions, with one minute of rest between each repetition, and two minutes of rest between each set. In the first week, the carried weights increased by 50% of body weight, and gradually increased by 10% per week, and finally reached 100% of their body weight by the eighth week. In order to warm up and cool down, climbing the weightless ladder twice was considered before and after each training session (12). 24 hours after the last training session, the rats were anesthetized by ketamine (30-50 mg/kg) and xylazine (3-5 mg/kg), then soleus and EDL muscles were removed, rinsed in physiological saline, and then immediately placed in a frozen nitrogen tank (for transferring to the freezer) for further measurements at a temperature of -80 °C in the freezer. PAX7 and MyoD were measured by Western blot method.

Western Blot Method

To extract the muscle tissue proteins from the buffer, RIPA containing 0.05 mM Tris buffer (pH=8), 150 mM sodium chloride, 0.01% EGTA, 1% sodium dodecyl sulfate (SDS) with 0.1% of anti-protease cocktail (Sigma, USA) were used. Proteins were measured by a luminescence chemical reaction (ECL) by densitometry analysis with ImageJ software (version 1/8/1122). Pax7 antibody (serial number: SC-81648, Santa Cruz Company, USA), MyoD antibody (serial number: ab64159, Abcam Company, USA), and beta-actin antibody (serial number: SC-47778, Santa Cruz Company, USA) were used (13).

Statistical Analysis

Kolmogorov-Smirnov test and independent sample *t* test were used for statistical analysis ($P \leq 0.05$).

Results

Mean and SD of PAX7 and MyoD proteins are shown in Figures 1 and 2. The results of independent sample *t* test showed that 8 weeks of RT significantly increased MyoD in the soleus and EDL muscles ($P = 0.001$, Figures 1A & B). Besides, 8 weeks of RT led to a significant increase in Pax7 in the soleus muscle ($P = 0.002$, Figures 2A & B). In contrast, eight weeks of RT did not significantly change Pax7 levels in the EDL muscle ($P = 0.10$, Figures 2A & B).

Discussion

The results revealed that eight weeks of RT significantly increased MyoD levels in the soleus (about 65%) and EDL (about 200%) muscles and significantly increased Pax7 in the soleus muscle (about 31%). Nevertheless, this increase in Pax7 protein content in the EDL muscle (about 23%) was not significant. In other studies, it has been shown that increasing age could disrupt the activation and proliferation of satellite cells in elderly men (14). One study investigated the content of Pax7 and MyoD proteins induced by RT following the strengthening of the satellite cells in the skeletal muscle of men and women. In men, the Pax7 protein content did not change significantly after 12 hours, and then decreased within 24 hours after exercise. On the other hand, the Pax7 protein content in women decreased at 12 and 24 hours after RT. In men, the content of MyoD protein increased at 12 and 24 hours after RT. In women, it decreased 12 hours after RT, and no significant change was observed 24 hours after RT (3). The achieved results of the current research on the soleus muscle are inconsistent with the results of the mentioned study on the Pax7 protein content.

We observed an increase in the content of Pax7 protein in the soleus muscle. However, in Luk and colleagues' study (3), after 12 and 24 hours, insignificant and reduction can be observed, respectively. One of the most important factors in these results is the time of measuring protein content. Measuring the content or levels of

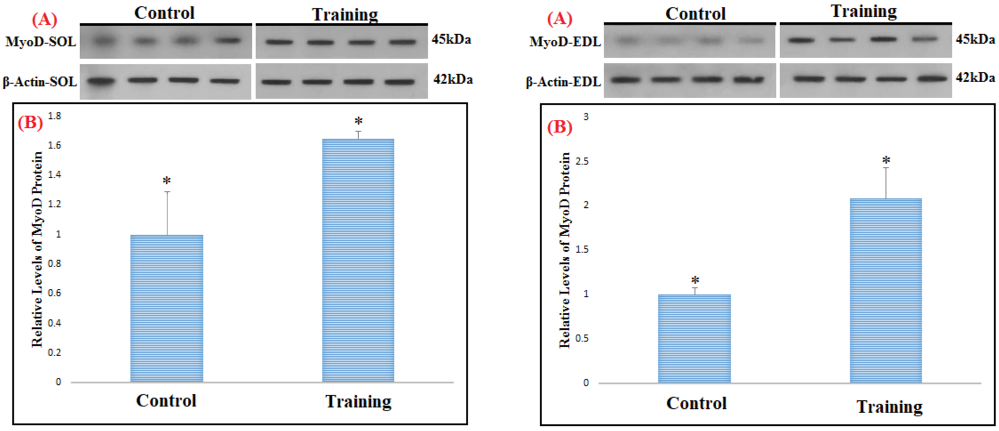

Figure 1. MyoD Protein Levels in the Studied Groups. (A) Western blot images of MyoD protein and beta-actin (β-actin) as the control loading in the soleus muscle tissue (SOL) and ED. (B) Bar chart (mean and standard deviation) shows the quantified values of MyoD protein bands. * Shows a significant difference at the level of $P \leq 0.05$.

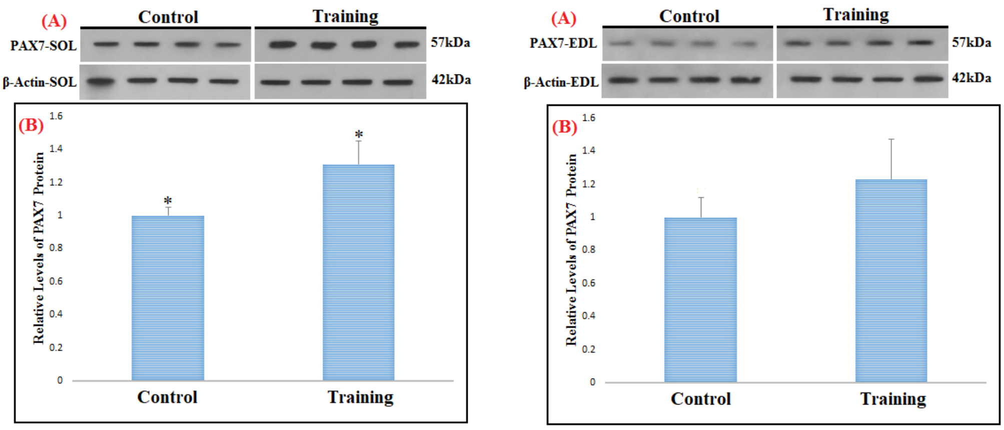

Figure 2. Pax7 Protein Levels in the Studied Groups. (A) Western blot images of Pax7 protein and beta-actin (β-actin) as the control loading in the soleus muscle tissue (SOL) and EDL. (B) Bar chart (mean and standard deviation) showing the quantified values of Pax7 protein bands.* Shows a significant difference at the level of $P \leq 0.05$

proteins in the laboratory can have very different results. However, it is worthwhile to mention that the Pax7 protein content in the EDL muscle, despite the increase, was insignificant, which was consistent with the results of the Pax7 protein content after 12 in the mentioned study and not with other times. Regarding the results of MyoD protein content, we found an increase that was consistent with the results of Luk and colleagues' study (3) in men, but was contradictory in women. Despite the differences in the results of both studies, the type of exercise used was RT. As such, other factors such as intensity, repetition, and recovery period between sets must be considered. The type of subjects could also be another essential factor in our study. All these factors, in turn, can affect the results of studies. In another study on the effect of RT on Pax7 and MyoD protein content in elderly men (i.e. 67 years). The group performed RT of the leg press and leg extensions at approximately 95% of their 10-RM, with a 2-minute rest between each set. The vastus lateralis muscle was biopsied. In this regard, Pax7 protein content was not significant in the RT group. After RT, MyoD protein content increased significantly (15). The results of the mentioned study were inconsistent with the results of our study in terms of the Pax7 protein content in the soleus muscle, but were consistent in terms of the EDL muscle. The results of both studies are consistent in terms of MyoD protein content. The most important factor that can be mentioned is the sex of the subjects; we studied female rats while the mentioned study was done on men. Muscle type is another important factor. The vastus lateralis muscle in human subjects is an intermediate muscle, whereas the soleus muscle in rats is a very fast muscle. It seems that the composition of the fiber type affects the response of these proteins, especially the MyoD protein.

The RT in the vastus lateralis muscle in human subjects increased the expression of the MyoD gene immediately and six hours after the training session, which is in consistent with the results of the current research (16). Muscle biopsies in different parts of the muscles and at different times after a period of exercise indicate acute changes in the satellite cell content and activation status during or after exercise. The satellite cells have also been shown to increase in both type I and type II muscle fibers in response to a period of RT in healthy young men, which may occur in different ways or may not occur at all (15,17). The satellite cells move to the damaged area to participate in the repair of muscle tissue from the intact part of the myofibril under the membrane. By activating the satellite cells, six hours after muscle damage, the expression of the MyoD gene increased rapidly. For this reason, in adult skeletal muscle, this transcription factor is considered as an indicator of the satellite cell activation and proliferation (18).

Conclusion

It appears that RT by increasing PAX7 and MyoD, can lead to proliferation and renewal of satellite cells in the soleus and EDL muscles. It also seems that the study of PAX7 changes in EDL muscle depends on factors such as intensity, and type and duration of training, and more studies should be done in this area.

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Authors' Contribution

Laboratory studies and tests: H E; Study and review: J N AND F D; Analysis and interpretation of data: H E and M H.

Conflict of Interests

The authors declare no competing interests.

Ethical Approval

The researcher received introduction letters from Shiraz School of Rehabilitation Sciences with Approval ID: IR.SUMS.REHAB.REC.1399.038.

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