PAX7 and MyoD Proteins Expression in Response to Eccentric and Concentric Resistance Exercise in Active Young Men

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Abstract

**Objective:** Satellite cells play an important role in muscle regeneration, which this process can be affected by different genes including PAX7 and MyoD. Exercise training known as an important strategy for mediating the satellite cell's function. Therefore, the main purpose of the present study is to investigate the changes in PAX7 and MyoD protein expression in response to eccentric and concentric resistance exercise in healthy young men.

**Materials and Methods:** In this semi-experimental and cross-sectional study, 10 healthy men (age range 18-30 years old) participated. They were randomly divided into two equal groups (n=5) to perform one of two high-intensity eccentric or concentric knee extensions muscle contraction protocols. The contractions included a maximum of 12 sets of 10 repetitions, with a 30 second rest time interval between sets. PAX7 and MyoD protein expression was assessed using Immunohistochemistry analysis from the Vastus Lateralis muscle needle biopsy samples that have been taken 24 hours before and 3 to 4 hours after the end of the exercise protocol.

**Results:** We observed that the PAX7 protein expression level increased significantly after eccentric (47.75%) and concentric (39.21%) (P=0.01) intervention. While, the MyoD protein expression level reduced (38.14%) significantly following acute eccentric resistance exercise (P=0.01).

**Conclusion:** It seems that eccentric or concentric muscular contraction modulates the expression of PAX7 and MyoD protein expression in the skeletal muscle, with further effects observed in eccentric resistance exercise.

**Keywords:** Concentric Contraction, Hypertrophy, MyoD, PAX7, Resistance Exercise

Introduction

Skeletal muscle, as one of the most adaptable tissues of a living organism, responds differently to different stresses. These responses are different such as muscle mass increase and angiogenesis (1). Many of the adaptations resulting from repetitive strength training, such as increased lean mass and strength, are probably due to the high degree of skeletal muscle plasticity in the response to loading. Different strength training stimuli can manifest them in a variety of molecular responses that lead to specific adaptations of skeletal muscle to the type of strength training performed (2). The molecular mechanisms that lead to skeletal muscle adaptations are gene expression in different levels, RNA and protein (3).

Resistance training is one of the training methods that leads to increased muscle mass, muscle differentiation, and etc. (4). Resistance training may lead to the skeletal muscle hypertrophy through satellite cells.

Therefore, protein synthesis is increased and new nuclei will be added to maintain muscle area. Increased protein synthesis or satellite cells following resistance training can be stimulated by a variety of signals, such as hormonal and myogenic regulatory factors (5). Resistance training includes concentric and eccentric contractions that are performed against an external load (6). Eccentric activity produces a larger amount of force per muscle unit than concentric activity, meaning that eccentric contraction has a greater load capacity than concentric contraction (7). Therefore, at the same constant load, concentric activity is performed at a relatively higher intensity than the eccentric activity, which results in the summoning of more units and a greater increase in the level of growth factors than eccentric activity (8).

Myogenic regulatory factors (MRFs) superfamily consisting of four members: including myogenin, MRF4, myogenic differentiation factor (MyoD) and
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Myf5. Among them, MyoD is responsible for muscle hypertrophy, which this function requires satellite cells (SCs) to repair load-induced muscle damage (9). Ancestor cells activate regulatory genes (such as PAX7) that are essential for the formation of satellite cells (10). With the migration of met-C and CD34 receptors in response to muscle damage, satellite cells begin to proliferate and activate MyoD and Myf5. Subsequently, cause to activate myogenin and MRF4 and other differential genes (11). Satellite cells are regulated by the PAX7 protein expression, which known as cell cycle activators. Moreover, it is responsible for activating and regulating the pool reserve of satellite cell. A medium and low-intensity exercise increased the PAX7 protein expression level, while a high-intensity exercise decreased PAX7 protein expression level in comparison with the control groups. These results indicate that the intensity of exercise activity potentially increases the PAX7 protein expression (12). When eccentric and concentric movements are performed separately, they exhibit distinct physiological characteristics in comparison with each other (13). From all the above, it can be deduced that the signaling pathways of these two contractions will probably lead to structural, physiological, molecular and etc. differences in the skeletal muscle. Many studies have examined the hormonal responses to these two types of contraction (8, 14, 15), but there is no study that examines myogenic regulatory factors. Although, there are limited studies that focused on the effects of different types of contractions on the muscle tissue (7, 13, 14). It seems that the effects of eccentric and concentric exercise on cellular signaling pathways such as hypertrophic pathways are different and the researchers suggested that eccentric training would produce greater hypertrophy than concentric training and is the most effective for strength gain (16). However, its molecular mechanism is remarkably unknown. We hypothesized that the difference between eccentric and concentric exercise effects on the hypertrophic pathways can be attributed to changes in the expression of different genes including PAX7 and MyoD. Therefore, we compared the acute effect of eccentric and concentric exercise resistance on the PAX7 and MyoD protein expression in active young men muscle.

Materials and Methods

The study was approved by the Tehran University Human Research Ethics Committee, Tehran, Iran (IR.UT.SPORT.REC.1397.029). All the procedures were performed in accordance with the 1964 Helsinki Declaration and its later amendments. Informed consent was obtained from all participants.

Study population

In a semi-experimental and cross-sectional study, 10 healthy male students aged 18 to 30 years of the Tehran University, Tehran, Iran, who did strength training recreationally with a history of training 3 to 6 days a week to improve their general health and improve their body composition at least at 6 months prior to study recruited. Participants were randomly divided into equal groups: concentric group and eccentric group. Their average strength training was 6 years. The inclusion criteria were an experience of resistance training and normal body mass index. Exclusion criteria was a history of drug consumption or sport ergogenic supplementation during the last six-month, history of orthopedic or cardiovascular disease. Who suffered from a chronic disease such as cardiovascular, diabetes, orthopedic problem, normal body mass index, was excluded of our study. Using G*Power, v3.1.2, a sample size of this study was calculated according to the previously reported formula (17).

Study protocol

The participants attended the laboratory for two sessions. In the first session, they were familiar with the laboratory environment, isokinetic system, resistance exercise protocol and their anthropometric parameter including height and weight were assessed. In the second session, they randomly performed one of the eccentric or concentric protocols at the same time between 8 - 9 am.

Resistance exercise protocol

In the familiarization and main protocol sessions, the subjects rested in a sitting position for 10 minutes after visiting the laboratory, the research procedure and exercise were explained to them. After that, the subject sat on the Biodex dynamometer (Biodex Medical Systems 4 Pro, Inc., Shirley, NY, USA) chair and adjustments were made to prepare the device. The isokinetic contraction protocols included eccentric and concentric knee extensions.

Eccentric protocol

Each contraction was performed in 60°/s. Subjects performed 12 sets of 10 repetitions with 30 seconds of rest between each set and a total of 120 contractions. Straps restrained movement at the shoulders, hips, and thighs (exercised leg) until the knee extensors were separated during the protocols and the participant was connected to the device. The eccentric contraction was performed at more than 90% of the maximum eccentric strength of the load, and the concentric component was inactive. The researcher returns the body to the starting position.

Visual feedback of the force signal was prepared for each person. Verbal encouragement of the participants was done if the contraction level was maintained. At
the end of each set, the perceived exertion score (RPE) was determined from a 20-point scale. All participants completed the full concentric protocol.

**Concentric protocol**

The protocol was similar to the eccentric protocol, but subjects performed stimulate contractions at 90% of their maximum instead of eccentric samples. The eccentric part of the movement was passive that the researcher returned the limb to the original position. Visual feedback and RPE report of individuals between sets were provided.

**Immunohistochemistry analysis of Pax7 and MyoD protein expression**

Pre and post-test biopsy was performed by orthopedic surgeon from the vastus Lateralis muscle on each subject 24 hours before and after the training protocol. Biopsy was performed in the distal and proximal directions of the vastus Lateralis muscle. Then, the muscle tissues were cultured on sterile gelatinous slides (23-769-521, fisher, USA). Washed with phosphate-buffered saline (PBS, P4417, Sigma–Aldrich, UK) after 24 hours. They were fixed at 4°C with paraformaldehyde (30525-89-4 , Sigma–Aldrich, UK) for 20 minutes. Muscle tissue coated slides (P0425, Sigma-Aldrich, UK) were incubated at room temperature for 2 minutes after washing with PBS in HC1 (2N) (7647-01-0, Sigma-Aldrich, UK). Following of PBS washing, the slides were exposed to Triton 100-X (T8787, Sigma-Aldrich, UK) for 30 minutes. In the next step, 10% goat serum (G9023, Sigma-Aldrich, UK) was added to the slides for half an hour. The slides were incubated overnight with the primary Pax7 antibody (1:100; Biorbyt orb1093757, Biorbyt, UK) and MyoD antibody (1:100; Biorbyt orb48951, Biorbyt, UK) at 4°C temperature. Then, they were washed twice with PBS and exposed to conjugated secondary antibodies (1:200; Biorbyt orb688925, Biorbyt, UK) for 60 minutes in the dark at 37°C. After 3 times washing with PBS, DAPI (D9542, Sigma-Aldrich, UK) was used to stain the nuclei and then viewed with an Olympus IX83 microscope (IX83, Olympus, Tokyo, Japan).

**Statistical analysis**

Descriptive statistics, mean and standard deviation were used to describe data and inferential statistics were used for between-groups comparison. The Kolmogorov-Smirnov test was also used to evaluate the normality of data distribution. In order to compare the pre-test and post-test values and between groups (eccentric vs. concentric) different, mixed-design repeated measures analysis of variance (ANOVA) was used. SPSS 21 software (SPSS Inc., Chicago, IL, USA) was used to analyze the data and Excel 2013 software was used to draw the graphs. The P≤0.05 were considered as statistically significant.

**Results**

**Demographic characteristics.**

The demographic and physiological characteristics of the subjects in the concentric and eccentric groups are presented in Table 1.

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<th>Table 1: Demographic indicators of the subjects</th>
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BMI; Body mass index and SD; Standard deviation. The results of the Kolmogorov-Smirnov test indicate the normal distribution of the studied variables among both groups.

**PAX7 protein expression**

A two-way mixed ANOVA with repeated measures showed that there were no statistically significant interaction effects (group×time) for the PAX7 [F(1.8)=1.995, P=0.293]. However, the main effect of time showed a statistically significant increase in the PAX7 from pre-test to post-test in both concentric and eccentric resistance exercise groups (P≤0.05). Results showed no significant difference in the PAX7 protein expression in the concentric and eccentric resistance exercise groups (P>0.05, Figs.1, 2). The percentage of their changes for concentric and eccentric were 47.75 and 39.21%, respectively.

**MyoD gene expression**

There was no statistically significant interaction effect (group×time) of the MyoD [F (1.8)=2.08, P=0.11]. However, the main effect of time showed a statistically significant change for the MyoD from pre-test to post-test
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in the eccentric group (P<0.05) but the concentric group wasn’t significant (P>0.05, Figs.3, 4). The percentage of their changes for concentric and eccentric groups was 47.27 and 38.14%, respectively.

**Fig.1:** Immunofluorescence PAX7 staining. Positive satellite cell population (green), myonuclear (blue) and merged image of both cell markers in pre and post time point of concentric (C) and eccentric (E) resistance exercise (scale bar: 50 µm).

**Fig.2:** Relative within group comparison of PAX7 protein expression following resistance exercise. RE; Resistance exercise and *; Significant differences compared with pretest.
Fig. 3: Immunofluorescence MyoD staining. Positive satellite cell population (green), myonuclei (blue) and merged image of both cell markers in pre and post time points of concentric (C) and eccentric (E) resistance exercise (scale bar: 50 µm).

Fig. 4: Relative within group comparison of MyoD protein expression following resistance exercise. RE: Resistance exercise and *; Significant differences compared with pretest.

Discussion

The main finding of the present study is a significant increase in the PAX7 after both eccentric and concentric resistance exercise interventions. Also, these changes were significant in the eccentric group rather than the pre-test values, whereas the MyoD level decreased follow of eccentric resistance exercise. Similar to our study results, Pugh et al. (18) demonstrated that a resistance exercise increases the PAX7 expression in the eight sedentary subjects, whereas the MyoD remained unchanged. Abou
Sawan et al. (19) showed that acute resistance exercise did not change the PAX7+satellite cells, whereas resistance training induces a significant increase of the SC, independent of muscle fiber type and participants’ sex. The PAX7 gene is a transcription factor in satellite cells adjacent to the nucleus and is essential for the myogenic process and satellite cell regeneration. Shefer et al. (20) showed that satellite cells will be increased per muscle fiber after moderate-intensity training. In another study, the researchers observed no differences in the levels of PAX7, MyoD and SC after resistance training (21). In the present study, it was shown that both eccentric and concentric exercises increase the PAX7 expression level of the vastus lateralis muscle.

Many adaptations, such as increased strength and lean mass, result from repetitive resistance training, due to the high degree of skeletal muscle adaptability in response to training pressure. Different training stimuli of resistance exercise can elicit different molecular responses in relation to specific skeletal muscle adaptations based on the type of resistance training, intensity, volume and the time under tension. However, previous human studies have reported that eccentric contractions stimulate protein synthesis more than concentric contractions (22, 23). It could be justified by a different pattern and the extent of muscle fiber recruitment variation between concentric and eccentric contractions.

The migratory capacity of satellite cells depends on the integrity of the cell membrane. After severe rupture of the basement membrane due to the muscle damage, satellite cells migrate to adjacent damaged myofibrils using tissue connections, but if the tissue damage is limited and rupture of the basal lamina has not occurred, the satellite cells will move from the beginning of the healthy myofibrillar membrane to the affected area to participate in muscle tissue repair. By activating satellite cells, MyoD gene expression increases rapidly (24). The present study findings suggested that upregulation of MyoD protein expression following acute eccentric exercise was significant, but there was no significant change in the MyoD protein expression after acute concentric exercise. Also, there was no difference between these two groups. In order to better understand the mechanisms of muscle hypertrophy in young women, Jensky et al. (25) evaluated changes in the expression of myostatin, follistatin and MyoD mRNAs using eccentric and concentric exercise. They observed no changes in myostatin and follistatin mRNA gene expression, but significant increasing in MyoD gene expression after eccentric exercise. In addition, concentric exercise was associated with no changes in myostatin, follistatin, or MyoD mRNA gene expression, an there was no significant differences between eccentric and concentric exercise.

Skeletal muscle myosin heavy-chain (MHC) isoform can affect the response of the MyoD gene to exercise. In confirmation of this claim, the researchers reported that MyoD and myogenin mRNA expression immediately after resistance exercise and 6 hours after was the difference between type I, type IIa and type IIx muscle fibers in healthy male subjects (26). The MyoD mRNA is also affected by long-term training. For example, Liu et al. (27) showed that strength training (six weeks) in human specimens significantly increased the MyoD mRNA expression in the triceps as predominantly fast twitch muscle. Therefore, it seems that the lack of significant changes is due to the study of the MyoD response to compatibility in this study. Previously conducted studies showed that an increase in the MyoD gene expression, 100 to 400%, immediately after exercise, while no change was observed until 48 hours after exercise (28). In another study, a strength training session on the leg extensor muscle increased the expression of the MyoD gene up to 8 hours after training, although, it diminished after 20 hours (29).

In the present study, the MyoD protein expression was measured 24 hours after the eccentric and concentric resistance exercise protocol, so it seems that the lack of measurement at different times after the protocol could not distinguish between the two types of exercise. It seems that the type of exercised muscle, training modality and the subject fitness levels affect the expression of satellite cell markers. However, it should be noted that some results did not confirm the increase in MyoD gene expression due to exercise. For example, it was seen that one strength training session in human samples had no significant effect on the MyoD expression of lateral extensor muscle (30). Also, strength training for eight weeks has no significant effect on the MyoD broad-spectrum expression level in men, with normal or reduced testosterone levels (31, 32). A study by Drummond et al. (33) showed no significant changes in the MyoD expression in vastus lateralis muscle after the anabolic stimulus, resistance exercise and essential amino acids, in young and older men. Exercises that caused muscle damage, on the other hand, did not significantly affect MyoD expression. For example, in animal models, it was observed that an increasing treadmill training session with a negative slope did not have a significant effect on MyoD expression of horseshoe muscles and openers (34). In line with this study, we did not observe significant changes in the MyoD protein expression level after concentric exercise. However, regeneration of injured horseshoe muscles has been reported to be intensified by exercise, intense and voluntary activities, which has been associated with increased MyoD protein levels (35).

Generally, it’s suggested that a single session of eccentric or concentric exercise causes significant changes in the skeletal muscle strength and hypertrophy related factors, including upregulation of Pax7 expression as a transcription factor in satellite cells close to the nucleus which is essential for myogenic and
satellite cell regenerations (36). On the other hand, it is believed that activation and proliferation of satellite cells, followed by their differentiation and fusion with myotubes, are essential for skeletal muscle hypertrophy in adults (37) and the MyoD has been shown to be upregulated prior to satellite cell proliferation (38). Also, the MyoD has been suggested to be an important regulator involved in the adaptation of skeletal muscle to mechanical stress such as exercise (39). In addition to MyoD, the previous studies’ findings showed that Pax7 is critical for the normal function of satellite cells in adult skeletal muscle (40). Therefore, since the present study findings indicated that eccentric or concentric resistance exercise can affect the expression of Pax7 and MyoD, it can be concluded that exercise training positive effects on skeletal muscle tissue can partly be exerted by changing the expression of Pax7 and MyoD. However, the mechanism underlying this effectiveness should be determined in the future studies. Unfortunately, we don’t investigate the changes in the different skeletal muscle hypertrophic factors and determined the difference between the effects of these two protocols (eccentric or concentric) on genes and proteins involved in muscle hypertrophy required further researches.

### Conclusion

Overall, the present study showed that one session of eccentric and concentric resistance exercise activity leads to changes in the expression of Pax7 and MyoD proteins which are involved in skeletal muscle myogenic regulatory factors. However, these changes are generally in greater magnitude following eccentric resistance exercise modality than concentric resistance exercise intervention.

### Acknowledgements

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### Author’s Contributions

S.K.M., B.B; Conceptualization, Methodology, Exercise Program. M.Gh., B.B.; Data curation, Writing- Original draft preparation, and Supervision. M.Gh.; Writing- Reviewing and Editing. All authors read and approved the final manuscript.

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