Original Article

The effect of eight weeks resistance and aerobic training on myostatin and follistatin expression in cardiac muscle of rats

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Abstract

Introduction: The clinical studies have shown that the myostatin gene expression and its serum density occur more frequently in heart patients than in healthy individuals. The purpose of this study is to investigate the influence of 8-week resistance and aerobic exercise on the myostatin and follistatin gene expression of myocardium muscle of healthy male Wistar rats.

Methods: In this experimental study, 20 five-week-old adult Wistar rats (250 ± 26.5 g) were divided into three groups: healthy control group (n = 6), resistance exercise group (n = 7), and aerobic exercise group (n = 7). The resistance and aerobic exercise plan consisted of 8 weeks and 3 sessions per week. The resistance exercise group performed climbing a one-meter 26-stair ladder with a slope of 85 degrees for 3 sets of 5 repetitions per session. The aerobic exercise group performed running at a speed of 12 meters per minute for 30 minutes during the first sessions gradually increasing up to a speed of 30 meters per minute for 60 minutes during the final sessions (equivalent to 70% to 80% of maximum oxygen consumption). The differences between the groups were evaluated using a one-way analysis of variance (ANOVA) test. When appropriate, LSD post-hoc test was used. The significance level for the study was less than 0.05.

Results: The results of this study shows that after 8 weeks of exercise, there is no significant difference between myostatin mRNA gene expression levels of the heart muscle among the three groups of control, resistance exercise, and aerobic exercise (P = 0.172, F = 1.953). However, the mean differences between follistatin mRNA levels of the heart muscle among the three groups of control, resistance exercise, and aerobic exercise are statistically significant (F = 38.022, P = 0.001). Furthermore, the ratio of follistatin to myostatin mRNA gene expression of the heart muscle (P = 0.001, F = 10.288) shows significant difference among the three groups.

Conclusion: Our results indicate that the resistance and aerobic exercise could cause a decrease in myostatin and an increase in follistatin levels, thus preventing many muscular physiological disorders such as arthritis and muscle weakness.

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Introduction

The clinical studies have shown that the myostatin gene expression and its serum density occur more frequently in heart patients as compared with healthy individuals. Myostatin gene expression increases within the periods of skeletal muscle inactivity and/or the prevention of serum myostatin leads to the building of strength and muscle mass. Myostatin is a protein produced by skeletal muscle cells which penetrates into the blood of living cells and inhibits the muscle growth. The growth and differentiation myostatin/factor 8 has been introduced as a factor causing muscle weakness. The myostatin gene is located in the centromeric region of chromosome 2 and contains three exons and two interferons in the lower area of the gene. The myostatin gene acts as mediator of the gene expression in relation to the control of the fiber muscle formation, and basically inhibits the muscle growth through the prevention of myoblast proliferation. This activity of myoblast is mainly related to the growth of prenatal muscles during the myoblast proliferation and differentiation period. In this sense, the activity of myostatin can be influenced by other interactive factors such as follistatin, the pseudo gene of follistatin, the serum protein associated with the growth and differentiation factor, and the myostatin receptor (activin IIb). The most significant role of follistatin, a
glycoprotein nearly expressed in all tissues of mammals, is to neutralize the activity of TGF-β family proteins such as myostatin. In the presence of follistatin, myostatin is not able to connect to its own receptor and thus can prevent the muscular dystrophy caused by myostatin. Myostatin expressed during periods of inactivity increases skeletal muscle or the inhibition of serum myostatin increases strength and muscle mass. Therefore, it seems that the resistance training leads to decreased expression of myostatin. According to the findings, the myostatin gene expression in the heart muscle can change following the physical activity or the myocardial infarction. These changes are so important and influential that can also affect the skeletal muscles, causing them to atrophy. However, considering the importance of physical activity in the prevention and treatment of many diseases, specialists suggest the exercise and nutritional counseling to treat cardiovascular diseases prior to drug therapy. In addition, exercise performance causes more satisfaction and pleasure as compared with therapeutic and drug regimens. In this sense, on one hand, the study seems to be important because the results of this study maybe used to help in the treatment and non-drug rehabilitation of some diseases such as heart failure and abnormal thickening of the heart muscle. On the other hand, determining the role of myostatin in the mechanism of cardiac adaptations following the resistance and aerobic exercises can offer a new position for myostatin in exercise sciences. Now, given the fact that the simultaneous effect of resistance and aerobic exercises is not emphasized as just aerobic exercises on the myostatin and follistatin gene expression levels, which are considered as one of cardiovascular risk factors, and that there is still some uncertainty in the limited studies carried out regarding the intervention of resistance and aerobic exercises in reducing the expression of this gene. The present study thus aimed at exploring the influence of 8-week resistance and aerobic exercise program on the myostatin and follistatin gene expression in the heart muscles of male Wistar rats.

Materials and Methods

Subjects
In this experimental study, 20 five-week-old adult Wistar rats (250 ± 26.5 g) were divided into three groups: healthy control group (n = 6), resistance exercise group (n = 7), and aerobic exercise group (n = 7). The rats were placed in an animal house under the laboratory conditions for 2 weeks (temperature between 20 and 22°C with the 12 hour light/dark cycles). The rats stayed and were kept in Plexiglas cages with perforated doors and fed on special food for rodents. Likewise, the water was provided by a special glass bottle and their cages were disinfected with 70% alcohol 3 times a week.

The training program

The familiarization phase and resistance exercise
The resistance and aerobic exercise plan consisted of 8 weeks and 3 sessions per week. After a week of familiarization with the laboratory environment, the rats were familiarized with the way of climbing a ladder with a weight equivalent to the 30% of body weight of the animal for 10 to 15 minutes through a cylinder which was attached to its tail. The resistance exercise group performed climbing a one-meter 26-stair ladder with a slope of 85 degrees for 3 sets of 5 repetitions per session. The rest interval between the sets was two minutes while it was 1 minute between the repetitions. The way of adding weight was that the amount of weight strapped to the rats in the first week equaled the 30% of their body weight which gradually increased to almost 200% of their body weight in the last 2 weeks.

Aerobic exercises
In this study, the aerobic exercise group performed running at a speed of 12 m/min for 30 minutes during the first sessions gradually increasing up to a speed of 30 m/min for 60 minutes during the final sessions (equivalent to 70% to 80% of maximum oxygen consumption).

Biopsy and variable measurement
Twenty-four hours after the last training session and 12 hours after fasting, the rats in all groups were sacrificed after transferring to the genetic laboratory and their muscle tissues were used as samples to estimate the levels of myostatin mRNA and follistatin. After anesthetizing and fixing the animals on the board of rodent surgery, the autopsy was performed. The muscle tissue samples were taken immediately after the autopsy, the samples were then taken from the left ventricle of the rats. And the 10% formalin was placed in fixative and was kept in the solution for 48 hours. After the first 24 hours, the new formalin was replaced with the previous formalin. After fixation with dewatered alcohol, it was molded with paraffin. After this process, the microtome sections with 5 micron thickness were taken through random sampling at regular intervals and then were examined. To investigate the expression of myostatin mRNA and follistatin in the heart muscle, the RT-PCR method with primer sequences was used. Myostatin primer included: Forward primer: 5'-TAA CCT TCC CAG CAG GA-3' and Reverse primer: 5'-CAC TCT CCA GAG CAG TAA TT-3' and follistatin primer included forward primer: 5'-CAG TGC AGC GCT GGA AAG AAA T-3' and reverse primer: 5'-TGC GTT GGC GTG ATT CAC TTA C-3'.

RT-PCR method
For RT-PCR reaction, the Chromo device along with the diagnostic conjugate of SYBER-Green product, a commercial product of TAKARA, was used in this study. In this sense, the necessary ingredients were added to the special tubes to make the reaction occur on the genes in question at different time scales and also in upper and lower parts of the incision. To reduce the possibility of error in pouring the materials, first a Master Mix was prepared for each gene, which contained all the above ingredients except the cDNA. After the complete dissolving of the
μ18 materials of Master Mix in each special real-time tube was poured and finally two micro-liters of cDNA related to each tube was individually poured.

**Statistical analysis**

All values are presented as mean ± standard deviation (SD). The data collected were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Data distribution normality and homogeneity of variance were examined with Shapiro-Wilk and Levene's test respectively. The differences between the groups were evaluated using a one-way analysis of variance (ANOVA) test. When appropriate, LSD post-hoc test was used. The significance level for the study was less than 0.05.

**Results**

*Heart muscle myostatin mRNA expression*

The results of this study shows that after 8 weeks of exercise, there is no significant difference between myostatin mRNA gene expression levels of the heart muscle among the three groups of control, resistance exercise, and aerobic exercise \( (P = 0.172, F = 1.953, \text{see Figure 1}) \). LSD test results showed that there is no significant difference in heart muscle myostatin mRNA expression among the control group with resistance group \( (P = 0.127) \) and aerobic group \( (P = 0.084) \), and also between the resistance exercise and aerobic exercise groups \( (P = 0.81) \).

*Heart muscle follistatin mRNA expression*

The mean differences between follistatin mRNA levels of the heart muscle among the three groups of control, resistance exercise, and aerobic exercise are statistically significant \( (F = 38.022, P = 0.001, \text{Figure 2}) \). LSD test results showed that there is significant difference in heart muscle myostatin mRNA expression among the control group and resistance exercise group \( (P = 0.001) \) and aerobic group \( (P = 0.001) \), and also between the resistance exercise group and the aerobic exercise group \( (P = 0.001) \). The ratio of follistatin to myostatin mRNA gene expression of the heart muscle \( (P = 0.001, F = 10.288, \text{Figure 3}) \) shows significant difference among the three groups.

**Discussion**

The aim of this study was to investigate the effect of 8 weeks of resistance and aerobic exercises on the expression of myostatin and follistatin genes of healthy male Wistar rats' heart muscles. According to the results of the study, the resistance and aerobic exercises led to a decrease in myostatin mRNA levels in the heart muscle male Wistar rats as compared to the control group, whereas these changes were not statistically significant. However, the relationship between the inhibition of myostatin and the exercise adaptations to the size and performance of the heart muscle has not been determined. In particular, whether the myostatin inhibition affects the physiological hypertrophy of the heart muscle which is caused by the adaptation to resistance exercise? Myostatin oblast is a negative regulator of muscle growth in which if a mutation occurs in the coding area of this gene, it changes the role of its regulators and causes the muscle strength through the increase of protein synthesis. The presence of this protein influences the hormone effective in the resistance of tendons and their flexibility, and it then leads to the weakness and decrease in the flexibility quality of tendons. The transforming growth factor-beta (TGF-β) is the most important cytokine of regulating skeletal muscle growth. As a member of this group, myostatin plays a crucial role in the control of muscle mass; in fact, the human-animal...
Resistance and aerobic training on myostatin and follistatin expression

In the context of muscle growth, myostatin and follistatin play a crucial role. Myostatin, a member of the TGF-β family, negatively regulates muscle growth by inhibiting muscle cell proliferation and differentiation. On the other hand, follistatin, an inhibitor of myostatin, plays a key role in preventing myostatin signaling and muscle catabolism. This balance is critical for maintaining muscle mass and function.

During exercise, particularly resistance and aerobic training, the expression and activity of myostatin and follistatin are modulated. Resistance training has been shown to reduce myostatin expression, allowing muscle growth to occur. In contrast, aerobic exercise leads to an increase in follistatin, which potentiates muscle growth. These opposing effects are thought to be mediated by alterations in the ratio of myostatin to follistatin.

The increase in follistatin following exercise is thought to be due to increased transcription of the follistatin gene. This is mediated by an increase in activin type II receptors, which then bind to follistatin and prevent myostatin from binding, thus inhibiting myostatin signaling. This competitive inhibition of myostatin by follistatin leads to an increase in follistatin expression and a decrease in myostatin expression.

The observed increase in follistatin is associated with increased protein synthesis and decreased protein degradation. This is mediated through the inhibition of myostatin signaling, which in turn allows for increased muscle protein synthesis and reduced muscle protein breakdown. This balance is critical for muscle growth and repair following exercise.

The regulation of myostatin and follistatin expression during exercise is complex and involves multiple signaling pathways. Changes in the expression and activity of these proteins are influenced by factors such as intensity and duration of exercise, as well as genetic predispositions. Understanding these mechanisms is crucial for developing effective training strategies to enhance muscle growth and performance.

In conclusion, the regulation of myostatin and follistatin expression during exercise provides a mechanism by which exercise can modulate muscle growth. By understanding the regulatory mechanisms involved, researchers can develop targeted interventions to optimize muscle adaptation to exercise.
and aerobic exercise can lead to a reduction in myostatin and an increase in follistatin and prevents the physiological disorders of muscle such as atrophy and muscle weakness. We suggest that adaptation and alteration in mRNA levels of follistatin and myostatin gene expression in the Wistar rats’ heart and skeletal muscle depend on duration and intensity of exercise. Due to the fact that the role of genetic factors, as an important factor in the development of cardiovascular disease, is still unknown in Iran. Therefore, due to limited research on the impact of physical activity, especially resistance and aerobic training on the expression of myostatin and follistatin gene and, the effect of these exercises on the heart and skeletal muscle more researches are requires. We suggest that adaptation and alteration in mRNA levels of follistatin and myostatin gene expression in the Wistar rats’ heart and skeletal muscle depend on duration and intensity of exercise. Due to the fact that the role of genetic factors, as an important factor in the development of cardiovascular disease, is still unknown in Iran. Therefore, due to limited research on the impact of physical activity, especially resistance and aerobic training on the expression of myostatin and follistatin gene and, the effect of these exercises on the heart and skeletal muscle more researches are requires.

Conclusion
Overall, it is clear that we need further studies to prove the actions of myostatin and follistatin, especially in the exercise sciences; therefore, the studies on the effect of resistance and aerobic exercises on mRNA expression of myostatin and follistatin are limited. Also, understanding the specificity of compatibility exercises may provide therapeutic goals in order to treat the cardiovascular diseases of heart muscles and show the most effective way to prevent or ameliorate these diseases.

Ethical approval
This study was approved by medical ethics committee of Ferdowsi University of Mashhad.

Competing interests
The authors declare no conflicts of interest.

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