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Original Article

The Effect of Eight Weeks of High-Intensity Interval Training on Follistatin Gene Expression in the Fast and Slow Twitch Muscles of Rats with Myocardial Infarction

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Abstract

Background: Myocardial infarction causes mitochondrial atrophy and loss of function by reducing mitochondrial volume. Therefore, researchers are interested in finding a way to reduce the injuries and treat them. The study aims to evaluate the effect of 8 weeks of high-intensity interval training on follistatin (FST) gene expression in the fast and slow twitch muscles of rats with myocardial infarction.

Methods: The study was conducted in 2020 at the Cardiac Research Center, Shahid Rajaei University of Medical Sciences (Tehran, Iran). For this purpose, 12 male Wistar rats with myocardial infarction were assigned to the experimental group high-intensity interval training (3 days a week for 30 min, each interval consisting of 4 min of running with 85-90% VO_{2max} intensity and 2 min of active recovery with intensity of 50-60% VO_{2max} for 8 weeks) and a control group. Then, the expression of follistatin in fast and slow twitch muscle contraction genes was investigated as triggers and inhibitors of muscle atrophy. Statistical data were analyzed with SPSS18 ($\alpha \geq 0.05$). To determine the normality of the data, the Kolmogorov-Smirnov test was used, and in the case of normality of the data distribution, the independent samples *t* test was used.

Results: Independent *t* test results showed that FST gene expression in the slow twitch (ST) muscle contraction group was significantly decreased compared with the control group ($P<0.001$). Moreover, the expression of the FST gene in fast twitch muscles was significantly increased in the severe exercise group compared with the control group ($P<0.001$).

Conclusion: Overall, 8 weeks of intense intermittent exercise decreased FST gene expression in slow and fast twitch muscles in rats with myocardial infarction.

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Keywords ● Myocardial infarction ● Muscular atrophy ● Exercise ● Gene expression

What's Known

- No study has been conducted to investigate the impact of 8 weeks of high-intensity interval training on the atrophy of fast and slow twitch muscles in patients with myocardial infarction.

What's New

- This research supports the role of high-intensity interval training on the increase of follistatin after cardiac infarction, which results in the reduction of atrophy in the fast and slow twitch muscles.

Introduction

One of the most important tissues of the body is the muscle mass, which plays an important role in maintaining the body structure and ultimately improving the performance of the cardiovascular system and especially the respiratory system.[1]

Skeletal muscle is strengthened in response to various physiological stimuli, including sports and exercises with different intensities and durations, which ultimately increases the functional capacity of people.[2] The structure and function of muscle tissue can be affected by various diseases such as myocardial infarction (MI) or heart attack.

Blockage of blood vessels and dysfunction of blood supply and oxygen delivery, as a result of heart attack, with a decrease in the volume of mitochondria, causes a change in the phenotype of slow-twitch muscles to fast-twitch, as well as atrophy of muscle fibers and loss of people's performance,[3] and affects their daily life. Therefore, finding a way to minimize the complications caused by this condition and even its partial treatment has always been the focus of researchers.

Follistatin (FST) protein is a negative regulator of muscle growth and size, which increases about 10 min after myocardial infarction.[4] Reducing the activity of this protein can prevent muscle atrophy.[5] On the other hand, myostatin, as the strongest inhibitor of FST, can bind to the FST receptor (activin IIb),[6] and inhibit its activity.

Deletion of the *FST* gene in muscle causes a decrease in muscle mass, while its excessive expression causes excessive muscle growth.[7] FST protein gene expression is affected by various physiological and pathological conditions, including muscle atrophy, heart attack, biogenesis, and sports activity.[8]

Regular physical activity has an established role in health. High-intensity interval training (HIIT) by creating hypoxia[9] is a strong stimulus in the direction of cell adaptation and is recommended for people's health. This training method is a strong stimulus for cardiovascular and muscular adaptations and can increase VO_{2max}, metabolism and exercise performance, reduce the use of carbohydrates and reliance on fat, improve insulin performance, reduce blood pressure, and in cardiac and hypertensive patients, cause improved cardiovascular fitness.[10]

Considering the effect of this training method on the increase of skeletal muscle mass and muscle hypertrophy,[11] and the information presented in connection with the important role of FST protein as an inhibitory factor in the atrophy process, this training method can hopefully be effective in reducing muscle atrophy in patients with myocardial infarction.

There has been little research regarding the effect of high-intensity interval training on the factors affecting muscle atrophy in fast-twitch fibers, specifically in patients with myocardial infarction. However, a large number of studies have been conducted about the effect of strength activities on muscle atrophy in subjects without myocardial infarction.[11, 12]

Most of the previous researches indicate the positive effect of strength activities on the reduction of FST as a trigger for muscle atrophy,[13-15] and fewer studies have investigated the role of FST protein.[5, 16]

Rostaei and others suggested that after 8 weeks of HIIT in male Wistar rats, the levels of follistatin gene expression in the soleus muscle and extensor digitorum longus in the training group were significantly reduced compared with the control group. Moreover, after the implementation of this protocol, the expression of FST was more suppressed in fast-twitch muscles than in slow-twitch muscles.[17] Biglari also investigated the effect of 8 weeks of HIIT on the levels of follistatin (*FST*) gene expression in healthy male rats. In its study, the reduction of follistatin (*FST*) was not significant.[18]

Considering the previous studies and the lack of information about the effects of high-intensity interval training on muscle atrophy and specifically FST of the slow and fast twitch muscles of patients with myocardial infarction, the need to conduct such a study becomes clear. Therefore, this study aims to investigate the effect of 8 weeks of high-intensity interval training (as the independent variable) on the level of *FST* gene expression in the fast and slow twitch muscle (as the dependent variable) in rats suffering from myocardial infarction.

Materials and Methods

The present experimental study was conducted in 2020 at the Cardiac Hospital, Shahid Rajaei University of Medical Sciences (Tehran, Iran). The study and the animal care protocol for research were approved by the Ethics Committee of Marvdasht Islamic Azad University, Fars, Iran (IR.IAU.M.REC.1399.041).

The current research was a fundamental study with an experimental method. In the present study, 12 male Wistar rats aged 10 weeks were purchased from the Pastor Institute of Iran as the statistical sample. Rats were kept in separate cages with free access to water and food in a 12-hour dark-light cycle following the principles of laboratory animal care (NIH- publications).[19]

To perform this study, 18 Wistar rats were purchased from the Pasteur Institute of Iran. The samples were selected by convenience sampling method. The sample consisted of six rats in the experimental group and six rats in the control group.

To induce myocardial infarction in rats in the present study, the direct intervention method was used. In this method, the left anterior descending artery (LAD) of rats was blocked by silk suture 6-0.[20] The rats were first placed in the laboratory environment for 1 week (figure 1).

Then the rats were anesthetized with ketamine (150 mg/Kg) (Medistar, Ascheberg, Germany) and xylazine (15 mg/Kg) (Riemser, Greifswald, Germany), and their chest hair was completely removed and intubated under artificial ventilation. Next, a 4-5 cm horizontal incision was made from the left side of the chest with a scalpel so that the heart muscle could be fully seen after the chest was moved aside. At this stage, the LAD was fully exposed and then completely blocked by silk suture (Supa, Iran).

After LAD blockage, the chest, muscles, and skin were sutured, respectively. The operated rats remained under artificial respiration until they regained consciousness and started breathing naturally. Finally, the rats were placed in a separate cage to be subjected to echocardiography after 1 week. It should be noted that all surgeries were performed by a veterinary specialist.

After blockage of the left anterior descending artery (LAD) through the surgery and being induced by myocardial infarction, they went through a 4-week post-surgery recovery period. In the third and fourth weeks of the recovery period, the rats were introduced to the treadmill (Daneshsalar, Iran) by walking slowly on it (at a speed of 5 meters per min for 5 min a day and 4 days a week). At this stage, all rats were able to perform activities and had no casualties.

At the end of week 4, based on the formula presented in Morten and Wisloff's study, rats' VO_{2max} was measured using the maximum sports activity test to estimate their initial running speed.[21, 22]

The running speed of each rat on the treadmill was calculated individually according to its VO_{2max}. After that, the rats rested for 2 days. Then, to ensure rats became induced by myocardial infarction, all of them underwent Doppler echocardiography to measure hemodynamic parameters. In this process, indicators such as left ventricular diameter at end-diastole (LVDd), left ventricular diameter at end-systole (LVDs), end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF), and left ventricular fractional shortening (FS) were measured.

The rate of myocardial infarction in the rats was 35≥FS, upon which they were selected for this study. Ejection fraction (EF) and fractional shortening (FS) were calculated according to the following formulas.[20]

EF=(LVDd2 -LVDs2)/LVDd2,

FS=((LVDd–LVDs)/LVDd)*100

LVDd: Left ventricular diameter at the end of diastole;

LVDs: Left ventricular diameter at the end of systole.

Finally, the surviving rats with myocardial infarction were randomly divided into two groups of high-intensity interval training (HIIT) and control group (CTRL), and after 2 days the training protocol was implemented.

The training protocol consisted of 30 min of interval running on the treadmill, each interval including 4 min of running with an intensity of 85-90% of VO_{2max} and 2 min of active recovery with an intensity of 50-60% of VO_{2max}. The training was performed 3 days a week for 8 weeks,[23] in the same way, and the rats warmed up for 5 min with an intensity of 40-50% VO_{2max} before starting the main phase of the training.

Running speed gradually increased every 2 weeks by 0.02 m/s.

Eventually, after 8 weeks of training protocols, following 2 days of rest, the remaining rats were again anesthetized for echocardiography, and samples were taken of extensor digitorum longus (fast twitch) muscle to measure mRNA level follistatin genes (FST) following freezing at -70 °C by qRT-PCR (quantitative real-time polymerase chain reaction) method in molecular genetic laboratory.[24, 25]

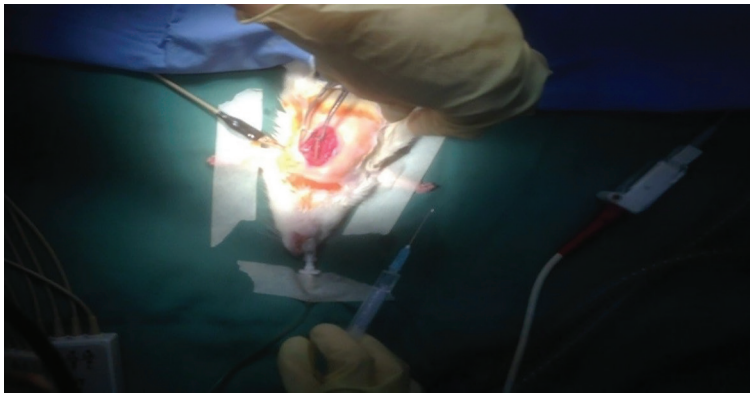

Figure 1: Rat's affliction with myocardial infarction performed by open heart surgery.

In this study, follistatin gene expression levels in Wistar rats afflicted with myocardial infarction were evaluated by real-time polymerase chain reaction (RT-PCR).

Steps of Procedures

Our first step was to prepare the samples for follistatin, followed by extracting their RNA, and then their optical absorption was verified by an optical absorption spectrometer. An RT-PCR reaction was carried out. Then, the mRNA expression levels of the follistatin gene were examined in the samples of both experimental and control groups.

Internal Control Gene

The internal control gene (also, known as housekeeping, reference, or standard gene) is a gene that has permanent and stable expression.[26] To measure the decrease or increase in the expression of the target gene, its expression is compared with the expression of the internal control gene. In this study, glyceraldehyde phosphate dehydrogenase (*GAPDH*) was used as the internal control gene. Since the *GAPDH* gene is stably expressed in most cells and tissues, it has been suggested as a housekeeping gene and is used by biological researchers in reactions such as RT-PCR.[27]

Specific Primers of RT-PCR

The sequence of *FST* and *GAPDH* gene sequence, which was used as the internal control gene, was obtained from the NCBI site. Then, the specific primers were designed using the Primer Express program. Finally, to check their accuracy and characteristics, the designed primers were blasted by the NCBI and the Gene Runner program.

Reaction of RT-PCR

To measure gene expression, gene replication was performed using RT-PCR reaction according to the relative standard method.

Relative quantification of RT-PCR was done by measuring the increase in fluorescence radiation as a result of the binding of CyberGreen dye using the ABI-7500 device. The components of the RT-PCR reaction in the final volume of 20 µL and the final concentration of the materials are shown in table 1.

The collected statistical data were analyzed using SPSS 18 (IBM, USA). To determine the normality of the data, the Kolmogorov-Smirnov test was used, and in the case of normality of the data distribution, the independent samples *t* test was used to analyze the data at a significance level of ($\alpha \geq 0.05$).

Results

In table 2, the results of the descriptive statistics and independent samples *t* test related to the FST index of fast and slow twitch muscles in the experimental and control groups are presented.

The results of the Kolmogorov-Smirnov test showed that the data distribution of both HIIT and control groups in FST of the fast and slow twitch muscles is normal. Therefore, the precondition for using parametric tests was established.

The results of the independent samples *t* test showed that there is no significant difference between the two control and HIIT groups in the FST index of the slow-twitch muscle, and according to table 3, the levels of FST index of the slow-twitch muscle in the control group are significantly lower than the HIIT group ($P<0.001$).

Table 1: The materials used for Real-time Polymerase Chain Reaction (RT-PCR)

Reactant	Volume (µL) 10^{-6}
SYBR TaqMan (2X) Master Mix)	10
Forward Primer (10 µM)	0.5
Reverse Primer (10 µM)	0.5
Reverse Transcription reaction solution (cDNA)	2
dH2O (distilled water)	7

Table 2: The Sequence of the primers examined in the follistatin gene

Forward: 5′-GGCGTACTGCTTGAAGTGAA-3′
Reverse: 5′-GGGAAGCTGTAGTCCTGGTC-3′

Table 3: Follistatin index of slow and fast twitch muscles in the experimental and control groups

Muscle fiber type	Group (n=6)	Minimum-maximum	Mean±standard deviation	P value *
Slow twitch follistatin	Control	3.78-5.88	5.21±0.88	<0.001
	HIIT	8.03-9.42	8.92±0.57	
Fast twitch follistatin	Control	6.37-7.65	7.03±0.51	<0.001
	HIIT	8.95-10.43	9.82±0.55	

*Significance at P=0.001; HIIT: High intensity interval training; Independent t-test

Table 4: Changes in the ejection fraction and fractional shortening of the control and experimental groups

Variables / Group	Time of echocardiography	Ejection fraction (%)	Fractional shortening (%)	P value *
HIIT	1 week after surgery	59.56±5.09	27.42±3.12	<0.001
	12 weeks after surgery	7.46±7.022	41.62±6.84	<0.001
Control	1 week after surgery	55.85±13.75	25.64±7.96	<0.001
	12 weeks after surgery	64.48±3.69	31.32±3.46	<0.001

*Significance at P=0.001; HIIT: High intensity interval training; Independent *t* test

According to the study, there is a significant difference between the control and HIIT groups in the FST index of fast twitch muscle (P<0.001). As per table 3, the values of the FST index of fast twitch muscle in the control group are significantly lower than the HIIT group (P<0.001).

Echocardiography Indicators

In table 4, changes in ejection fraction (EF) and fractional shortening (FS) values, 1 week and 12 weeks after surgery (including 4 weeks of post-surgery recovery and 8 weeks of sports activity intervention) in the control and experimental groups (HIIT) are presented.

Discussion

The results of this research revealed the effect of 8 weeks of high-intensity interval training on the significant increase of FST in the fast and slow twitch muscles.

Although no research has been found to directly examine the effect of 8 weeks of high-intensity interval training on atrophy in the fast and slow twitch muscles of patients with myocardial infarction, the results of this research are in line with the results of the studies of Rostaei and others who stated that 8 weeks of HIIT decreased the levels of *FST* gene expression in the extensor digitorum longus fast twitch muscle.[17]

Probably, one of the reasons for the increase in FST after adapting to 8 weeks of interval training can be the disturbed balance of muscle growth regulators in favor of positive regulators.

In normal conditions, there is a balance between important positive regulators such as Insulin-like Growth Factor-1 (IGF-1) and FST and negative ones such as FST, but this balance shifts towards negative regulators when the muscle is damaged and shifts to positive regulators when a load is applied to the muscle, as in the sports activities. It seems that this relationship is established through a very complex negative feedback loop. Thus, one of the possible reasons for the increase in FST after adapting to 8 weeks of training can be the disturbed balance of muscle growth regulators towards positive regulators.

The increase of each of the factors in the positive or negative feedback loop through some related factors such as PI3K and GSK3, MuRF-1, and atrogen-1, affects the expression and secretion of myostatin from muscle cells. As a result of adaptation to training, this dual activity of myostatin is suppressed, which ultimately causes a decrease in the amount of myostatin, followed by an increase in FST and a decrease in atrophy and necrosis of muscle cells.[20-22]

On the other hand, it seems that in the current study, adapting to 8 weeks of interval training has improved mitochondrial function and has been effective in improving their performance by increasing the size of mitochondria in the fast and slow twitch muscles. The positive regulation of Peroxisome proliferator-activated receptor alpha (*PPARalpha*) and Peroxisome proliferator-activated receptor gamma (*PPARgamma*) following the increase in Peroxisome proliferator-activated receptor-γ coactivator 1-α (*PGC-1alpha*) gene expression and its signaling are also effective factors in increasing mitochondrial DNA replication.[28-30] Still again, 8 weeks of high-intensity interval training increased PI3K levels and activated protein kinase B (AKT) by increasing IGF-1. AKT is effective in increasing FST by activating the mammalian target of rapamycin (mTOR) on one hand, and by affecting FOXO on the other hand.

As a result of adaptation caused by 8 weeks of high-intensity interval training, the expression of important mitochondrial biogenesis factors such as PGC-1 alpha and Nuclear respiratory factor 1 (NRF-1), as well as the mitochondrial transcription factor A (Tfam) augmented, which increased follistatin, being probably effective in inhibiting myostatin from the IGF-1/AKT/pathway. Inhibition of myostatin causes the inactivation of SMAD2 and SMAD3 and prevents skeletal muscle atrophy by inactivating E3 ligases.[28, 31]

The limitations of this study were the lack of measurement of other factors related to skeletal muscle growth, such as IGF-I, MGF, FoxO, Smads, and GASPs, and the lack of examination of the body composition and cross-sectional area of the muscle fibers to ensure the occurrence of hypertrophy.

Conclusion

Overall, the results of this research support the role of high-intensity interval training on the increase of FST after cardiac infarction, which results in the reduction of atrophy in the fast and slow twitch muscles.

Therefore, it is suggested that the researchers interested in this field investigate the effect of other upstream stimulatory and inhibitory factors affecting muscle atrophy in the slow and fast twitch fibers. It is also suggested that in the following studies, in addition to the effect of training intensity on the stimulating and inhibiting factors of atrophy, the total volume and cross-sectional area of the muscles should also be investigated.

Acknowledgment

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Authors' Contribution

E.R: Study concept and design, data acquisition, reviewing the manuscript; M.Gh: Study concept and design, drafting and reviewing the manuscript, H.Gh: Statistical analysis, Data analysis and interpretation, drafting and reviewing the manuscript; All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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