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The Effect of High- and Low-Intensity Interval Training on Myostatin Gene Expression Levels in Muscles Fibers of Rats with Myocardial Infarction

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ABSTRACT

Introduction: Myocardial infarction (MI) is an essential coronary artery disease, which affects mitochondrial function and causes muscle atrophy due to vessel blockage and disruption in blood transfusion and oxygen transfer. Interval exercises reduce muscle atrophy, but the appropriate exercise intensity is still unknown. This study aimed to evaluate the effect of interval training with two for six weeks on Myostatin gene expression levels in slow (ST) and fast (FT) twitch muscles in rats with MI.

Method: Eighteen ten-week male Wistar rats with MI were randomly assigned into high- (HIIT) (90-85% VO_{2max}) and low-intensity interval training (LIIT) (50-60% VO_{2max}) with a control group (CG, without training). Myostatin gene expression of FT and ST was investigated as a stimulant of muscle atrophy. The training protocol was 30-minute intermittent jogging sessions on a treadmill. Each interval included 4 min of running (85-90% VO_2max for HIIT and 55-60% for LIIT) and 2-minute active recovery (50-60% for HIIT and 45-50% for LIIT) three days a week for six weeks.

Results: LIIT significantly decreased myostatin expression in both ST and FT while HIIT only decreased myostatin expression in ST compared to CG (P = 0.002, P = 0.016, and P=0.011, respectively). HIIT induced myostatin expression reduction was higher in FT compared to CG (P = 0.078). There was a significant difference in myostatin expression between CG (8.87) and the two training groups (HIIT [0.949] and LIIT [3.11]) in ST (P≤0.05), and between CG and LIITs and HIIT (1.22) and LIIT (0/975) in FT (P<0.05).

Conclusion: Six weeks of HIIT and LIIT reduced myostatin gene expression and decreased ST and FT atrophy in rats with MI.

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Introduction

Myocardial infarction (MI) is one of the essential coronary artery diseases, which is currently the leading cause of mortality worldwide. (1). In addition, MI affects skeletal and heart muscles (2).

Skeletal muscle atrophy because of mitochondrial dysfunction is one of the most critical complications of MI in skeletal muscle, particularly in slow-twitch muscle fibers (3). Blockage of blood vessels and disruption of blood transfusion and oxygen transfer affect tissues, including skeletal muscle, causing muscle fiber atrophy (2).

Skeletal muscle atrophy can be called muscle mass loss or damage due to injury or illness. Muscle atrophy is the loss or reduction of muscle mass affected by several stimuli and inhibitors (4). MI induces muscle atrophy by increasing the expression of the MyoD[1] gene and decreasing myogenin protein levels (4-5).

IGF-1[2] is also reduced in the case of MI, activating IRS1 and PI3K, reducing pAKT, and resulting in Gs3k beta[3]. Reducing pAkT also activates mTOR and causes atrophy in the skeletal muscle of the IGF-1/AKT/mTOR pathway (6). Therefore, any factor that can inhibit the IGF-1/AKT/mTOR[4] pathway should be effective in preventing

[1] myogenic differentiation
[2] Insulin-like growth factor 1
[3] Glycogen synthase kinase-3 beta
[4] Mammalian target of rapamycin

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muscle atrophy. Researchers are looking for ways to reduce the amount of myostatin, one of several stimulants and inhibitory factors that promote muscle atrophy.

Myostatin is a negative regulator of muscle growth and size, which increases rapidly after an MI (7). This protein is a part of the large family of TGFβ[5] proteins, activated and inactivated by a protease, which divides the terminals of NH2 and activates the COOH terminal.

Factors such as the COOH terminal stimulate the gene responsible for regulating myostatin. Recently, myostatin has been an inhibitor of AKT as a kinase that causes muscle hypertrophy at the time of activation of protein synthesis. Therefore, myostatin can prevent muscle atrophy with stimulation of the AKT protein synthesis (8).

Regular physical activity (i.e., exercise) has a proven role in health. High-intensity interval training (HIITs) and low-intensity interval training (LIITs) are two forms of exercise, which induce positive health outcomes (i.e., in part by inducing hypoxia) (9). Hypoxia is one of the influential factors in increasing the expression of PGC-1α levels (10) and inducing muscle hypertrophy (11). HIIT and LIIT exercise training is a potent stimulator for cardiovascular and muscle adaptations, which increases maximal oxygen uptake (VO_{2max}) and metabolism, exercise performance, insulin function, fat intake, and cardiovascular fitness. In addition, HIIT and LIIT exercise training reduces carbohydrate intake and blood pressure in cardiac and hypertensive patients (12).

There has been no direct research on the effects of HIIT on the factors affecting muscle atrophy, and LIIT has been less of a concern. However, extensive studies have been conducted in which participants still need to be evolved with MI regarding the effects of strength activities on muscular atrophy.

Most previous studies have shown a positive effect of strength activity on reducing myostatin as a stimulating agent of muscle atrophy. Ruth et al. (2003) indicated that the expression of myostatin mRNA decreased after nine weeks of resistance training (13). Wilugby et al. (2004) concluded that the expression of myostatin mRNA did not significantly differ after 12 weeks of resistance training, although muscle strength and mass increased (14). Saremi et al. (2010) observed that resistance training with and without creatine supplementation reduced the serum levels of myostatin (15). Contrary to these studies, Dale et al. (2010) examined the interactive effects between exercises and androgens on the concentration of myostatin and serum and muscle follistatin. Based on this study, none of the moderate-intensity endurance and resistance training exercises significantly changed myostatin and follistatin serum levels (16).

Further research is required considering previous studies and the lack of information about the effect of HIIT and LIIT on muscle atrophy, especially in patients with MI. Therefore, this study aimed to compare the impact of six weeks of HIIT and LIIT on the expression of myostatin gene expression levels in ST and FT (as dependent variables) in rats post-MI.

Methods

Eighteen male Wistar rats (10 weeks old) were obtained from the **R**azi **V**accine and **S**erum **R**esearch **I**nstitute. The rats underwent surgery, and their left descending artery was blocked, experiencing a MI. All rats were anesthetized and received echocardiography with doppler access (GE Healthcare brand, USA). The shortening fraction of the left ventricular (FS) during this process was relatively measured. Rats with FS ≤ 35(17) were selected as MI rats, and the MI rats were randomly divided into three groups; two experimental groups of HIIT (n=6) and LIIT (n=6) and a control group (CTRL, n=6).

After two weeks of recovery, two experimental groups were acquainted with the treadmill (*Danesh-salar-e Iranian*, Iran) with a gentle walk at a speed of 5m/s (5m a day) and four days a week for two weeks. The VO_{2max} of rats was measured by maximum physical activity based on the formula and table set out by Wislov et al. (2000) to estimate the initial speed of running rats (18). The speed of each rat was calculated on the treadmill based on the individual VO_{2max}. Then, the two experimental rat groups performed six weeks of training protocols.

In both experimental groups, the rats warmed up for 5m at 5m/s before starting the main training session. Then, 0.02 m/s per week accumulated the rats' speed, and the treadmill slope was zero

[5] *Transforming Growth Factor*-β

degrees throughout the training period. In contrast, the rats in the control group (with MI) performed no exercise.

HIIT Protocol

The training protocol in the experimental group of HIIT comprised a 30-minute intermittent jogging session on a treadmill; each interval included 4m of running with an intensity of 85-90% VO_{2max} and 2m of active recovery with a VO_{2max} of 50-60% intensity (Table 1). The training was performed three days a week for six weeks (Howidal et al. 2007), and the rats warmed up for 5m at 40 to 50% VO_{2max} before the start of the leading training phase. The running speed was gradually increased every second week by 0.02m/s.

Table 1. The training protocol in the experimental groups (HIIT and LIIT)

groups	weeks	Days per week	Intensity of training	Intensity of recovery	Time of exercise	each interval included	Over load
HIIT	6	3	85-90% $VO2_{max}$	40-50% $VO2_{ma}$	30 min	4 minutes of running and 2 minutes of active recovery	every second week by 0.02 m / s.
LIIT	6	3	55-60% $VO2_{max}$	40-50% VO2ma	30 min	4 minutes of running and 2 minutes of active recovery	every second week by 0.02 m / s.

LIIT Protocol

The training protocol in the experimental group of LIIT comprised a 30-minute intermittent jogging session on a treadmill; each interval of which included 4m of running with an intensity of 55-60% VO_{2max} and 2m of active recovery with a VO_{2max} of 45-50% intensity (Table 1). The training was performed three days a week for six weeks (Howidal et al. 2007), and the rats warmed up for 5m at 40 to 50% VO_{2max} before the start of the leading training phase. Running speed was gradually increased every second week by 0.02 m/s (Table 1).

Finally, the animals were sacrificed, and the samples of Soleus (ST) and Extensor digitorum longus (FT) muscle tissue were taken by a veterinarian to measure the mRNA levels of the myostatin gene by the qRT-PCR method.

The target genes were the peroxisome proliferator-activated receptor gamma coactivator1α (myostatin forward primer: 5′-CAGGAGAAGATGGGCTGAATCC -3′ and Reverse primer: 5′- AAGCCCAAAGTCTCTCCGGG -3′) was used for normalization. (2).

Statistical Analysis

The data were analyzed using SPSS software version 18. The Kolmogorov-Smirnov test was used to determine the normality of the data. When data were of normal distribution, independent sample t-test and ANOVA were used to test for the significant level at $p \leq 0.05$.

Table 2. Descriptive statistics and results of t-test and ANOVA of the myostatin gene expression of ST and FT in the control and experimental groups of the study

Type of Muscle Fiber	Group	Number	Mean±SD	(T test)	(ANOVA)
ST	CON	6	8.87±2.948	0.011	0.001
	HIIT	6	0.949±0.002		
	CON	6	8.87±2.948	0.002	
	LIIT	6	3.11±0.773		
FT	CON	6	3.91±2.035	0.078	0.0024
	HIIT	6	1.224±0.273		
	CON	6	3.91±2.035	0.016	
	LIIT	6	0.975±0.075		

Results

As shown in Table 2, the mean values of myostatin gene expression of ST in the control group (8.87) were higher than in the HIIT group (0.949) and LIIT (3.11). Further, the mean of myostatin gene expression of FT was more significant in the control group (3.91) than in the HIIT group (1.224) and LIIT (0.975).

There was a significant difference between the two groups of control and HIIT in the myostatin gene expression of ST (P = 0.11), and the myostatin gene expression of ST in control was more than the HIIT (see Table 2). In addition, the myostatin gene expression of FT in the HIIT was not significantly more than the control group (P = 0.078).

A significant difference between the control and LIIT groups was observed in the myostatin gene expression of ST (P = 0.002). The myostatin gene expression of ST contraction in the control group was greater than that of the LIIT experimental group (Table 2). There was also a significant difference between the two groups of control and LIIT in the myostatin gene expression of FT (P = 0.016). Myostatin gene expression of FT in the experimental group of LIIT was more than the control group (Table 2). A significant difference was found between the two groups of HIIT and LIIT in the myostatin gene expression of ST (P ≤0.001). The myostatin gene expression of ST was greater than that of the HIIT group in the experimental group of LIIT (Table 2).

In addition, ANOVA results showed a significant difference between the control and HIIT, the control and LIIT groups in the myostatin gene expression of ST (P≤0.05). Moreover, there was a significant difference between the control and LIIT group and the HIIT and LIIT group in the myostatin gene expression of FT (P=0.002).

Discussion and Conclusion

In this study, six weeks of HIIT and LIIT influenced the myostatin of ST and FT. Although the mitochondria in slow-twitch muscles shrank more than in fast-twitch muscles, and their performance decreased, myocardial infarction changed the phenotype of slow-twitch muscles towards glycolytic fast-twitch (2). No prior published research directly investigated the effect of the intensity of interval training on ST and FT atrophy in MI patients.

Nonetheless, the results were consistent with those of Ruth et al. (2003) and Saremi et al. (2010), which investigated the effect of resistance training on myostatin gene expressions (15-13). On the other hand, the present results differed from those of Wilugby et al. (2004) and Dale et al. (2010), expressing that neither endurance nor resistance training with moderate intensity had a significant change in serum levels of myostatin and follistatin (16-14). According to the research hypothesis, the contradiction in results resulted from different types, training protocols, and various subjects.

Based on the results, adaptability with six weeks of HIIT and LIIT improved mitochondrial function and effectively increased the size of mitochondria in muscles to promote their function (19). Positive regulation of PPAR[6] alpha and PPAR gamma, following the increase in the expression of the PGC-1 gene and its signaling, increased mitochondrial DNA replication (19-21).

Six weeks of LIIT increased the AMPK and alpha-PGC-1 by increasing ATP and AMP levels. Alpha-PGC-1 ultimately activates mTOR and suppresses myostatin by stimulating the TSC2[7] protein. On the other hand, six weeks of HIIT increased PI3K levels and activated AKT by increasing IGF-1. AKT was effective on FOXO[8] and inhibited myostatin by activating the mTOR (19).

The expression of important mitochondrial biogenetic factors such as PGC-1 alpha, NRF-1, and Tfam increased and inhibited myostatin from IGF-1/AKT/mTOR pathway due to the adaptation induced by six weeks of interval training with both intensities.

Inhibition of myostatin inactivated SMAD2 and SMAD3[9] and prevented skeletal muscle atrophy by disabling E3 ligases (19-22).

The organization should be developed for the discussion and prevent information replication that already appears in the manuscript's Introduction. Exercise of both types can be beneficial, emphasizing this point and the importance of exercising after a stroke.

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Conflict of Interest

There are no conflicts of interest.

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[6] Peroxisome proliferator-activated receptor
[7] *Tuberous Sclerosis Complex 2*
[8] Forkhead box protein O
[9] SMA ("small" worm phenotype) and MAD family ("Mothers Against Decapentaplegic")

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