Golestan University of Medical Sciences

Jorjani Biomedicine Journal

Online ISSN: 2645-3509

The Effect of Eight Weeks of Moderate-Intensity Endurance Training on Myocardial Capillary Density, Ejection Fraction and Left Ventricular Shortening Fraction in Male Rats with Myocardial Infarction

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Article Type:

Short Communication

Article History:

Received: 1 Oct 2020
Revised: 10 Oct 2020
Accepted: 14 Oct 2020

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DOI: 10.29252/jorjanibiomedj.8.4.34

Abstract

***Background and Objective*:** Vascular obstruction and impaired blood flow and oxygen delivery cause a myocardial infarction and limit a person's function. Therefore, finding a way to create new arteries and replace blocked arteries has always been of interest to researchers, including exercise physiologists. Therefore, this study was conducted to evaluate the effect of eight weeks of moderate-intensity endurance training on the capillary density of the myocardium and ejection fraction and left ventricular shortening fraction in male rats with myocardial infarction.

Material and Methods: 16 ten-week-old male wistar rats with mean weight of 250-300gr were allocated to two groups of experimental (60 minutes of interval treadmill running for four minutes at an intensity of 65-70% VO2max and two minutes of active rest at 50-60 percent of VO2max for 5 days a week for 8 weeks) and control group (without any training). Immunohistochemistry was used to measure the capillary density of the myocardium. Ejection fraction and left ventricular shortening fraction were measured by Doppler echocardiography. Data were analyzed in SPSS18 using independent samples t test ($\alpha \leq 0.05$).

Results: The findings showed that there was a significant increase in the capillary density of the myocardium ($p=0.000$), ejection fraction ($P = 0.001$) and left ventricular shortening fraction ($P = 0.001$) in the experimental group as compared with the control group.

Conclusion: In general, eight weeks of moderate-intensity endurance training can effectively increase angiogenesis in male Wistar rats after MI.

Keywords: Capillary density, Myocardial Infarction, Endurance Training

Introduction

Cardiovascular diseases are one of the most important causes of death in the world and the number of patients with this disease is increasing day by day. Myocardial infarction (MI) occurs when the blood supply the to heart cells is disrupted in the short term and an average of 40 to 50 percent of the arteries is blocked. The extent and severity of the disease varies from person to person, and will vary depending on the involvement of the arteries. In general, blockage of arteries and impaired blood flow and oxygen delivery cause heart attacks and limit the function of individuals (1). Therefore, finding a way to create new arteries and replace blocked arteries, as well as increase the percentage of blood transfused from the heart or ejection fraction (EF) and increase the percentage of shortening of left ventricular muscle fibers and left ventricular shortening fraction (FS) to improve cardiovascular function of patients is a compromising advantage and has always been considered by researchers, including sports physiologists.

Angiogenesis is the growth and development of new blood vessels through the germination of existing vascular endothelial cells, which depends on the coordination of several independent processes. The natural formation of new blood vessels is associated with a controlled order that involves complex interactions between endothelial cells and matrix proteins and soluble factors, causing endothelial cell proliferation and migration to form new vessels (2).

According to the results of recent research on the possibility of creating new blood vessels in a process called angiogenesis in the heart tissue, under the influence of various factors (hypoxia, hemodynamic forces, metabolites, vasodilators, muscles contraction, some cytokines and types of traction), regeneration of blood vessels in the heart tissue to improve the cardiovascular function of patients is a compromised advantage. This process is controlled by the balance between angiogenic factors (vessel regenerating factors) and angiostatic factors (inhibitory factors). Normally, there is a balance between angiogenic and angiostatic factors, but in physiological and pathological situations, this balance is always disturbed, one of which is sports activity (3). Therefore, it is expected that evaluating the effect of exercise on the process of angiogenesis can help to find an effective way to increase the process of angiogenesis and ultimately improve the quality of patients with myocardial infarction.

The role of regular physical activity in health has been well established, and in the meantime, endurance training (moderate intensity) has always been considered by researchers. These exercises are a powerful stimulant for cardiovascular and muscular adaptations , increase maximal oxygen consumption (VO2max), metabolism, and athletic performance, reduce carbohydrate and fat dependence, improve insulin function and lower blood pressure, improve cardiovascular fitness in patients with cardiovascular disease and hypertension (4).

Different training methods have been considered as one of the effective factors in causing cardiac angiogenesis and ejection fraction and left ventricular shortening fraction (5, 6). In the meantime, there is no study examining the effect of eight weeks of moderate-intensity endurance training on changes in the capillary density of the heart muscle and ejection fraction and left ventricular shortening fraction of rats, especially in patients with MI.

Considering the presented materials related to the factors affecting vascularity of skeletal and cardiac muscles during exercise and the results of previous research on the positive and significant relationship between endurance training and these factors (7), we can hope that this training method be effective on cardiac angiogenesis.

Therefore, in the present study, we sought to answer the following question:

Does eight weeks of moderate-intensity endurance training as an independent variable affect capillary density and myocardial angiogenesis, as well as the left ventricular ejection fraction and shortening fraction as dependent variables in male rats with myocardial infarction?

Materials and Methods

In this developmental study, 16 ten-week-old male Wistar rats with myocardial infarction were randomly divided into two groups of control and experimental (n = 8). Rats were kept in separate cages with free access to water and food packages according the to principles of laboratory animal care (NIH-publication) and 12-hour dark-light cycle. The rats then underwent surgery and their left artery descending (LAD) was blocked, causing the rats to develop myocardial infarction (8).

Being anesthetized, the rats underwent Doppler echocardiography (J E Health Care Brand, USA) to ensure that they had MI.

During this process, left ventricular diameter at the end of diastole (LVDd), left ventricular diameter at the end of systole (LVDs), end-diastolic volume (EDV), and end systolic volume (ESV) were examined.

The left ventricular shortening fraction (FS) and the left ventricular ejection fraction (FE) were also calculated relatively according to the following formulas (8):

EF = (LVDd2 -LVDs2) / LVDd2

FS = ((LVDd - LVDs) / LVDd) * 100

Rats with FS≤ 35% were selected as rats with MI in this study (8).

Afterwards, the rats underwent a two-week recovery period after open heart surgery. In the third and fourth weeks, the rats got acquainted with the treadmill (Daneshsalar Iranian brand, made in Iran) by walking slowly at a speed of 5 meters per minute for 5 minutes a day and 4 days a week. At this stage, all rats were able to function and suffered no casualties. At the end of the fourth week, VO2max of rats was measured by maximal exercise activity test, according to the formula and table in Morten and Wisloff research and to estimate the initial running speed of rats (9).

To measure VO2max, rats first warmed up for 10 minutes, then the VO2max test was started at a rate of 0.1 m / s, and the speed of the treadmill was added every two minutes at a rate of 0.1 m / s, until the rats were completely exhausted (in the exhaustion, the rats fell backwards and did not respond to automatic stimulation of the treadmill), and the rate of exhaustion of the rats with MI was converted to VO2max by y = 1.14x + 9 (9, 10) formula, where:

x = rate of exhaustion in meters per minute.

y = VO2max in milliliters per kilogram of body weight per minute.

The running speed of each rat on the treadmill was calculated individually according to its VO2max. The rats then rested for two days. To ensure that they had MI, the anesthetized rats underwent Doppler echocardiography, using an echocardiogram (GE Healthcare,

USA). During this process, the left ventricular shortening fraction (FS) was measured relatively. Rats with FS≤ 35% were selected as rats with MI for this study (8). Finally, surviving rats with myocardial infarction were randomly divided into two groups of moderate-intensity and control (CTRL) endurance training and a training protocol was performed.

Moderate intensity endurance training protocol

Rats in the experimental group performed endurance training five days a week for eight weeks. The rats ran on the treadmill alternately for 60 minutes per session. Each work alternation consisted of 4 minutes of running at an intensity of 70-65% VO2max and 2 minutes of active recovery at an intensity of 50-60% VO2max (11). Before starting the main phase of training, the rats performed warm-up for 8 minutes at a speed of 5 meters per minute on the treadmill in form of walking. In contrast, rats (with myocardial infarction) in the control group did not exercise during eight weeks. After eight weeks and eventually, following two days of rest, the surviving rats were anesthetized for re-echocardiography and cardiac muscle tissue sampling was performed in the MI area to measure capillary density. The samples were transferred to a genetic laboratory after freezing. Immunohistochemistry (alkaline phosphatase activity) was used to measure the capillary density of the heart muscle. For this purpose, the slides were washed in poly-L-lysine solution for three minutes and completely moved in distilled water.

After removing the water from the slides inside the rack, it took 70 hours for the slides to dry. After removing, the slides were set in the box and at this time the slides were charged. The frozen muscle, which was slightly defrozen, was glued to the mold with a special glue and was placed in a freezing section with a temperature of minus 30˚ C and sliced. The samples were then fixed in methanol solution for five minutes and then washed in saline buffer solution for 15 minutes. In the next step, after drying around, the samples were immersed in oxygenated water and distilled water for five minutes, respectively. Superblock was poured on it for five minutes and NCL-31 antibody was added. Alkaline phosphatase solution was also added to the slide for two hours, then rinsed in PBS solution for five minutes and was placed in post-primer solution (solution added after the first antigen and antibody) for two hours in a circular motion to the shaker. The solution was washed again with phosphate buffer saline (PBS) for 10 minutes. In the next step, the secondary polymer solution was incubated on the sample for two hours. One drop of non-chromosomal DNA was added to 19 drops of the substrate. Then of the above 20 drops, five drops of the solution was added to the slide and washed with distilled water for 10 minutes. In the final stages, the samples were stained with background hematoxylin for 30 seconds and the slides were dried and mounted in the open air with Intlan glue. At this stage, the slides were ready for counting and examination.

Capillary density was reported as a number in the microscopic field. It should be noted that all steps of measuring capillary density were performed in the laboratory. The collected statistical data were analyzed using SPSS18 statistical software. The Shapiro-Wilk test was used to determine the normality of the data and if the data distribution was normal, independent samples t-test at the significance level of 0.05 was used to analyze the data and test the hypotheses.

Result

Table 1 presents the descriptive statistics and the results of independent samples t-test for the experimental and control groups in the myocardial capillary density index. As can be seen in Table 1, the mean capillary density of the heart muscle in the experimental group (184.875). was higher than the control group (105.000). Also, the ejection fraction in the experimental group (78.7) is higher than the control group (65.3) and the left ventricular shortening fraction in the experimental group (42.6) is higher than the control group (32.3).

The results of Shapiro-Wilk test showed that the distribution of data in both groups of training and control of myocardial capillary density is normal. Therefore, the precondition for using parametric tests was established.

The results of independent samples t-test showed that there was a significant difference between the control and training groups in the capillary density of the heart muscle (P = 0.001) and according to Table 1, the capillary density of the heart muscle in the training group was more significant than control group.

Also, the results of independent samples t-test show that the ejection fraction in the training group (78.45) is significantly higher than the control group (65.47) (P = 0.001) and the increase in shortening fraction is also significantly higher in the training group (42.45) than the control group (32.11) (P = 0.001) (Table 1).

Table 1: Descriptive statistics and results of independent samples t-test related to the experimental and control groups in myocardial capillary density (number in microscopic field), ejection fraction and left ventricular shortening fraction

Index	Group	Number	Mean±SD	Level of Significance
Capillary Density	Control	8	105.48± 13	0.001*
	Training	8	184.14±87.03	
Ejection Fraction	Control	8	65.3±47.70	0.001*
	Training	8	78.7±45.13	
Shortening Fraction	Control	8	32.3±11.51	0.001*
	Training	8	45.72±42.6	

05 ≤ ٠/ * P-value

Discussion

The results of this study show that as a result of eight weeks of endurance training with moderate intensity (65 to 70%) VO2max capillary density, ejection fraction and left ventricular shortening fraction increased significantly. In general, according to the results, it can be stated that eight weeks of moderate-intensity interval training increases the process of angiogenesis in rats with myocardial infarction.

Regarding the effect of endurance training and specifically on myocardial angiogenesis, results of this study are consistent with the results of Gu et al. (2006) who showed an increase in capillary density in the myocardial and skeletal muscle of 12-week-old male rats (6). The results are also consistent with Frisbee et al. (2006) who studied the effect of 10 weeks of moderate-intensity endurance training on capillary density in six- to seven-week-old male rats and stated that the

increase in NO produced in response to exercise increased the amount of VEGF secreted by endothelial cells, and this eventually led to an increase in capillary density (12).

Probably in the present study, due to adaptation to eight weeks of moderate intensity interval endurance training, HIF-1 levels increased and finally with increasing expression of VEGF gene from signaling pathways angiogenesis increased. In other words, eight weeks of moderate-intensity endurance interval training on the one hand created ROS, which increased AMPK and P38γ MAPK, and on the other hand increased CaMKK and CaMKII by increasing free Ca. CaMKK directly increased AMPK, which increased PGC-1 alpha. CaMKII also directly increased PGC-1 alpha by increasing P38γ MAPK. PGC-1 alpha increased estrogen-related receptor-α (ERR-α), which was involved in VEGF gene expression. On the other hand, it seems that intracellular oxygen levels decreased during training sessions and the activity of PHDs enzyme also decreased, which led to protein stabilization and nuclear transfer of HIF-1α, and this HIF-1α started transcription of various hypoxia-compatible genes such as angiogenesis-related genes, among which vascular endothelial growth factor (VEGF) is the strongest endothelial-specific mitogen (13) after the formation of HIF-1β3-activated heterodimer.

In general, it can be said that increasing oxygen consumption during training reduces oxygen to such an extent that it inhibits prolyl hydroxylase and leads to the accumulation of HIF-1α protein and its transfer to the nucleus. Finally, in the nucleus, HIF-1α and ARNT (HIF-1β) activate target genes such as VEGF. As a result of all the mentioned changes, VEGF is released as a result of eight weeks of moderate-intensity interval endurance training, and by binding to its receptor at the endothelial cell surface by phosphorylation, it activates the PI3K / P38MAPK / RAF pathway, which, in turn, provides proliferation, migration and permeability of vascular endothelial cells and their survival, and ultimately leads to the formation of new capillaries, and by strengthening the interaction of cells and the precise construction of the matrix, it stabilizes the new vessel and increases capillary density (14).

It can be said that eight weeks of moderate-intensity interval endurance training by impacting factors affecting the stimulation of angiogenesis (shear stress, metabolites, vasodilators, traction and hypoxia) has caused vascular regeneration in infarct tissue.

In connection with echocardiographic indices, increasing the left ventricular ejection fraction and shortening fraction is an organized response to increase the stroke volume, which is caused by adaptation to exercise and the application of volumetric load on the heart and thickening of the ventricular walls. In other words, increasing the percentage of shortening of left ventricular muscle fibers and the percentage of left ventricular ejection fraction show the superiority of left ventricular systolic function after exercise (15).

Conclusion

In general, it can be said that eight weeks of moderate-intensity interval endurance training by impacting factors affecting the stimulation of angiogenesis (shear stress, metabolites, vasodilators, traction and hypoxia) has caused vascular regeneration in infarct tissue and also by increasing ejection fraction and left ventricular shortening fraction has improved heart function.

Acknowledgments

This article has been taken from the doctoral dissertation of Ms. Shirin Heidarpour in the field of cardiovascular exercise physiology, Islamic Azad University, Borujerd Branch, which was done at the student's personal expense and with the support of the university's vice chancellor for research and has been approved by the ethics committee of the Islamic Azad University of Borujerd with the code IR.IAU.B.REC.1399.049 The authors would like to thank all the people who contributed to this research.

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How to cite:

Heidarpour SH, Ghahramani M, Hosseinpour Delavar S. The effect of eight weeks of moderate-intensity endurance training on myocardial capillary density, ejection fraction and left ventricular shortening fraction in male rats with myocardial infarction. Jorjani Biomedicine Journal. 2020; 8(4): 34-41.