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Original Article

The effect of 6-week high intensity interval training on the VEGF/COL-18 ratio and some echocardiographic indices in rats with myocardial infarction

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Abstract

Introduction: Myocardial infarction (MI) is the irreversible cell death caused by ischemia in parts of myocardium. The molecular process of increased capillary density in response to activity and its appropriate intensity is not clear yet. Therefore, this research aimed to evaluate the effect of 6-week high intensity interval training on the VEGF/COL-18 ratio and echocardiographic indices in rats with MI.

Methods: Twelve Wistar male rats of 10 weeks old and mean weight 250-300gr were allocated to two groups of experimental (60 minutes of interval treadmill running for four minutes with the intensity of 85-90 and two minutes of active rest at 50-60 percent of VO2max for four days a week for 6 weeks) and control group (without any training). Real-time PCR was used to assess the expression of VEGF and COL-18 genes after inducing MI, and shortening fractional and ejection fraction were investigated as echocardiographic indices. Data were analyzed in SPSS18 using independent t test ($\alpha \leq 0.05$).

Results: The findings showed that there was no significant increase in the VEGF/COL-18 ratio in the HIIT group (1.856 mg/ml) as compared with the control group (1.245 mg/ml) ($p=0.263$). A significant increase was observed in the HIIT group for SF (77.461±7.022%) and EF (41.625±6.847%) as compared with the control group (64.483±3.695%) and (31.320±3.460%), respectively ($p=0.001$).

Conclusion: In general, 6 weeks of high intensity interval training can effectively increase angiogenesis factors and improve myocardial function in male Wistar rats after MI.

Introduction

Cardiovascular diseases are the number one cause of death in the world, and more and more people die every day from these diseases. Myocardial infarction (MI) occurs when the blood supply to the heart cells is stopped temporarily and 40% to 50% of vessels are blocked. Depending on the vessels involved, the extent and severity of the disease vary from person to person (1). The ischemia caused by MI can cause abnormal heart function and arrhythmias. The left ventricle enlarges, resulting in reduced capillary density. Reduced capillary density leads to higher risk of apoptosis of heart muscle cells (2). A variety of factors including hypoxia, hemodynamic forces, metabolites, vasodilators, muscle contractions, some cytokines, and stretch, are affect on angiogenesis in the heart tissue. Thus, revascularization in the heart tissue, increases in ejection fraction (EF), increases in left ventricular muscle fibers shortening, or left ventricular shortening fractional (SF) are adaptive advantages that improve patients' cardiovascular function. Consequently, the assessment of the interaction between angiogenesis stimulating and inhibiting factors in a variety of conditions can contribute to finding an effective way to increase angiogenesis and ultimately improve the life quality in patients with MI (3).

Vascular endothelial growth factor (VEGF) - the most powerful and the most important factor affecting angiogenesis - increases migration and proliferation of endothelial cells and vascular network formation, and is necessary for the differentiation of endothelial cells to sprout new capillaries from previous vessels (angiogenesis) during the development of capillary network (4).

Collagen 18 (C0L-18) is the most important extracellular matrix protein that is a strong endostatin precursor. Endostatin - the most powerful angiogenesis inhibitor - decreases proliferation and increases apoptosis in endothelial cells. Endostatin binds to angiogenic factor receptors of VEGF and inhibits proliferation and migration of endothelial cells and ultimately the growth of capillary network (5, 6).

Angiogenesis is now regarded as an adaptive mechanism that is aggravated or suppressed by various factors. The role of regular physical activity in health is well documented, but most people do not participate in regular physical activities, which appears to be due to

the lack of time. High-intensity interval training (HIIT) in a short time has recently been proposed to overcome the problem of lack of time to participate in training and thus increasing physical activity and the health of such people. This training is a strong stimulant for cardiovascular and muscular adaptations and leads to increased maximal oxygen consumption (Vo2max), metabolism, increased athletic performance, reduced carbohydrate consumption and reliance on fat, improved insulin function, reduced blood pressure and cardiovascular fitness improvement in patients with heart diseases and hypertension. It can also initiate coronary angiogenesis. Several studies have been conducted on the effect of endurance and resistance training on angiogenesis, most of which indicated the positive effect of endurance activities on angiogenesis. The results of resistance training effects are more inconsistent (7, 8).

HIIT and its effect on angiogenesis have received less attention and it appears that Only one study examined the effect of HIIT on angiogenesis and angiostatic factors. However, there is no study in which the inhibiting factors and effective receptors in the simultaneous process and also the probable mechanism of high intensity interval training on angiogenesis have been considered (9).

Hypoxia generated during HIIT is reported to cause increased levels of myoglobin (10, 11). A significant positive relationship was also observed between HIIT and stretching force (12). Burgomaster suggested that HIIT can lead to muscle adaptations and especially create phosphate degradation and glycogen increase (13). Rodas also reported an improvement in the glycolytic enzyme function after HIIT (14). HIIT can improve muscle buffering capacity and ionic regulation (13, 15). HIIT also increases nitric oxide (NO) – a strong vasodilator – in cardiac muscles of patients with heart diseases (16). Such exercises can increase anabolic hormones such as growth hormone (GH) and Insulin-Like Growth Factor-1 (IGF-1) in a short time (17). A study on muscle metabolic adaptations to HIIT mentioned an increase in the amount of adenosine (18). Fibrinogen, a risk factor for cardiovascular diseases, is also affected by HIIT (19). Mechanism of action of stretching in angiogenesis is related to the role of Matrix Metallo Proteinase (MMPs). There are contradictory results in this regard. Nazari reported that during HIIT, the muscle length gets longer than the rest time and this stretch can increase MMP levels (20). On the contrary, Danzig reported no change in MMPs in response to HIIT (21).

According to what was said regarding factors affecting vascularization of skeletal and cardiac muscles during exercise including hypoxia, hemodynamic forces, metabolites, vasodilators, cytokines and different stretches, and the results of previous studies indicating the positive and significant relationship between HIIT and these factors; it becomes clear that in many cases, HIIT cause positive transformations in physiological variables, each of which can somehow affect human health. Whether HIIT can induce main changes affecting angiogenesis such as increased VEGF and decreased COL-18, is a question that the present study aimed to answer; that is, whether HIIT leads to angiogenesis in patients with MI, can increase VEGF and inhibit COL-18 as an endostatin precursor, and improve heart function by increasing ejection fraction and left ventricular shortening fraction.

Materials and Methods

In this developmental study, 12 ten-week-old male Wistar rats were randomly divided into two groups of six: control and intervention. The rats were housed in separate cages with free access to water and food according to the principles of laboratory animal care[1] under the 12-hour sleep-wake cycle. Then rats underwent surgery and their left anterior descending (LAD) artery was blocked to create severe MI (22). The rats were anesthetized and underwent Doppler echocardiography with an echocardiographer (GE Healthcare™, the U.S.) to ensure MI incidence. The left ventricle end-diastolic diameter (LVDd), left ventricular end-systolic diameter (LVDs), end-diastolic volume (EDV) and end-systolic volume (ESV) were measured during this process. Left ventricular SF and left ventricular EF were also relatively calculated according to the following formula (22):

EF =(LVDd2 -LVDs2)/LVDd2

SF =((LVDd – LVDs)/LVDd)×100

Rats with SF ≤ 35% were considered rats with MI and selected for this study (22). Then rats spent two weeks in post open-heart surgery recovery. In the third and fourth weeks, rats were familiarized with the treadmill (Danesh Salar Iranian™, Iran) by walking slowly at a speed of 5 meters per minute for five minutes per day, four days a week. At this point, all rats were able to carry out activities and there were no losses. At the end of the fourth week, VO2max of rats was obtained by maximal exercise testing in accordance with the formula and tables set forth in the studies by Morten et al. (2007) and Wisloff et al. (2000) to estimate the initial speed of running in rats (24 and 23).

The running speed of each rat on a treadmill was calculated individually according to its VO2max. Rats then rested for two days. Finally, the surviving rats with MI were randomly assigned into two groups of HIIT and control (CTRL) and the training protocol was conducted (23, 24).

The rats in the HIIT group (as the present popular training in the world whose effects on the variables of interest received less attention), worked on the treadmill (intermittent running) for six weeks, four days a week, and 60 minutes in each session. Each working period consisted of four minutes running at 85-90% VO2max intensity and two minutes of recovery at 50-60% VO2max intensity (25). The rats warmed up before the start of the main phase of training by walking for 8 minutes at a speed of 5 meters per minute on a treadmill. In contrast, the control group rats (with MI) did not have any training (25).

After six weeks training, and after two days resting, the rats were anesthetized for echocardiography and cardiac muscle tissue samples were obtained in the MI affected area in order to measure RNA values of VEGF

[1] NIH-publication

and COL-18 genes as strong endostatin precursors after freezing by Real-time PCR method in the following steps in the genetics laboratory.

1- Obtaining VEGF and COL-18 samples
2- Extracting RNA from samples
3- Examining samples' optical density with a spectrophotometer
4- Synthesizing cDNA from RNA
5- Real-time PCR
6- The study of VEGF and COL-18 gene expression in the intervention and control groups (Biooneer™ lab kits, Korea, Step One ABI™ Real-time PCR device, the U.S., and Memmert™ Primer device, Germany).

The studied primer sequence for VEGF was:
VEGF forward: 5´-TGAGACCCTGGTGGACATCTT-3´
VEGF reverse: 5´-CACACAGGACGGCTTGAAGA-3´
VEGF probe: 5´-CCCCGATGAGATAGAGTAT-3´

The studied primer sequence for COL-18 was:
COL-18 forward: 5_GGCCGGGACCTGACA-3´
COL-18 reverse: 5_GCTGTGGTGGTGAAGCTGTAG-3´
COL-18 probe: 5_ACTACCTCATGAAGATCC-3´

After the reaction, the raw data were extracted from the device as Δct values and then gene expression graphs were drawn using Graph Pad software. The qualitative statistical data collected by Real-time PCR were analyzed using the SPSS18 statistical software. Kolmogorov-Smirnov test was used to determine data normality, and in the event of normal data distribution, independent t-test was used for data analysis at a significance level of 0.05.

Results

Table 1 shows the changes in EF and SF values (Mean ± SD) ten weeks after surgery (four weeks of recovery after surgery and six weeks of physical activity) as well as independent t-test results of these factors in the control and HIIT groups (Table 1). The descriptive statistics and independent t-test results of the participants are presented in Table 2 (Table 2).

The independent t-test results showed that EF was significantly more in the HIIT group than in the control group (p=0.001) and the increase in SF was significant in the HIIT group compared to the control group (p=0.001) (Table 1). The independent t-test results also showed that although values of COL-18 were higher in the HIIT group than in the control group, this difference was not statistically significant (p=0.340), while there was a significant difference between the two groups in VEGF values (p≤0.001) and the VEGF values were higher in the HIIT group than in the control group. The increase in VEGF/COL-18 ratio was not significant (Table 2).

Table 1. A comparison of changes in EF and SF (Mean±SD) and the independent t-test results in the intervention and control groups

Variables and Groups	Ejection Fraction (%)	Shortening Fraction (%)
The intervention group (HIIT)	77.461±7.022	41.625±6.847
Control group	54.483±3.695	31.320±3.460
(P-value)	0.001	0.001

P≤0.05*

Table 2. A comparison of (independent t-test) the mean and standard deviation of the intervention and control groups at COL-18 and VEGF indices (mg/ml)

Indices	group	Number	Minimum	Maximum	Mean	SD	Pvalue
COL-18 (mg/ml)	Control	6	0.55	2.52	1.265	0.977	0.340
	Intervention (HIIT)	6	1.23	2.36	1.724	0.518	
VEGF (mg/ml)	Control	6	0.91	1.07	1.002	0.073	0.001
	Intervention (HIIT)	6	5.06	7.91	6.397	1.280	
VEGF/COL18 rato (mg/ml)	Control	6	0.36	1.96	1.245	0.727	0.001
	Intervention (HIIT)	6	0.73	0.03	1.856	1.029	

Discussion and Conclusions

The results indicate that although the increase in VEGF/COL-18 ratio in the HIIT group was not significant compared to the control group, six weeks of HIIT led to an increase in this ratio as well as a significant increase in VEGF compared to the control group and improved heart function in rats with MI with a significant increase in SF and EF.

The results of this study are inconsistent with the results of the only study that was found on the effect of HIIT on angiogenesis by Holloway et al., comparing the effects of HIIT and endurance training on the symptoms of heart failure and changes in the structure of the heart muscles in rats. That study examined VEGF and eNOS and the results showed no significant effect of HIIT on the angiogenesis process (9). The inconsistency in results might be due to differences in training protocols and that they did not assess inhibitory and stimulating factors together.

It appears that HIIT induced factors affecting VEGF gene expression and thereby stimulated angiogenesis. HIIT activated angiogenesis by creating hypoxia. A hypoxia-inducible factor was not hydroxylated in hypoxic conditions, remained stable, migrated to the nucleus, and induced factors influencing angiogenesis (10). The hypoxia induced by HIIT (17) releases cytokines that enter angiogenesis process by entering endothelial cells through an endothelium-derived relaxing factor (EDRF) called nitric oxide (NO) which is stimulated by fibroblast growth factor2 (FGF-2) upregulation. Furthermore, the immediate increase in stretch force from HIIT leads to vasodilator secretion, particularly nitric oxide, through the activation of ion channels, especially potassium channels (26) which upregulates VEGF and VEGFR-2 to stretch force and NO release. HIIT increase muscle adaptation and especially reduce creatine phosphate degradation. It increases gluconeogenesis, anabolic and adenosine

hormones that stimulate the VEGF gene expression (27). However, adenosine is produced due to HIIT and dephosphorylation of AMP By ecto-5'-nucleotidase from hypoxic tissue in the extracellular space adjacent to parenchymal cells, which has an important role in angiogenesis (28). Extracellular adenosine produced by HIIT activates adenosine receptors and then VEGF is released from parenchymal cells (29). Adenosine can also stimulate proliferation of vascular endothelial cells or vasodilation in the development and regeneration of new vessels by regulating pro- and anti-angiogenic growth factors. Evidence suggests that in some circumstances, adenosine might be necessary as a mediator of 50% to 70% of hypoxia-induced angiogenesis (29). The stretch makes the muscles longer during HIIT than during rest, and this stretch can increase MMP levels (20). Metalloproteases are secreted from the endothelial cells and break down basement membranes in that area through the entrance of calcium into the cell, its depolarization, activation of voltage-gated potassium channels, calcium entry into the cell and hyperpolarization; endothelial cells then start to migrate and proliferate (30).

Finally, all factors activate messages on endothelial cells with increasing VEGF and binding it to specific receptors, resulting in proliferation and migration of endothelial cells and increased vascular permeability (5). VEGF synthesizes DNA through upregulation of anti-apoptotic elements, and by breaking down the basement membrane, phosphorylation of intracellular endothelial adhesion components, and firm attachments lead to the survival, proliferation, migration and permeability of endothelial cells (6).

Structural and performance compatibility of the left ventricular structure due to training was more than other parts of the heart (31). Regarding the echocardiographic indices, the increased left ventricular EF and SF are organized responses to stroke volume which results from adaptation to exercises**,** application of Overload on the heart, and thickening of the ventricular walls. In other words, the increase in left ventricular muscle fibers shortening percentage and left ventricular ejection fraction indicated the superiority of left ventricular function after exercise (32).

The results of this study indicated that the exercise protocol used in this study - 60 minutes of high-intensity interval running on a treadmill at 85-90% VO2max intensity, four days a week for six weeks - was able to increase angiogenesis stimulants in the heart and improve heart function.

Due to the lack of information and studies related to the effects of HIIT on the process of angiogenesis, further studies in this regard are recommended, so that this training method can be used more confidently to improve the lives and performance of the cardiovascular system in patients with myocardial infarction.

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