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The Effect of Eight Weeks of High Intensity Interval Training on Genes Expression of eNOS, HIF-1 and VEGF in Myocardial Infarction Rats

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Abstract

Introduction: Myocardial infarction is cell death in part of the myocardial during an ischemia. Cell death process in response to activity and appropriate intensity is not clear yet. Therefore, the aim of this study was to evaluate the effect of eight weeks of high intensity interval training on endothelial constitutive nitric oxide synthase, hypoxia-induced factor-1 and vascular endothelial growth factor in rats with myocardial infarction.

Methods: 12 male Wistar rats weighing 250 to 300 grams were assigned into two groups: the experimental group (60 minutes running on a treadmill on an interval basis, each interval four minutes with intensity of 85-90 and two minutes of active recovery with 50- 60 % VO_{2max}, Four days a week for eight weeks) and the control group (without training intervention). Genes expression were investigated by the PCR technique. Data were analyzed using SPSS (version 18) with Independent sample t-test ($p \leq 0.05$).

Results: The results showed that endothelial constitutive nitric oxide synthase in high intensity interval training group (4.755) was significantly higher than the control group (3.615) (p= 0.012), hypoxia-induced factor in high intensity interval training group (9.015) was significantly higher than control group (1.49) (p= 0.001) and vascular endothelial growth factor in high intensity interval training group (6.855) was significantly higher than control group (1.425) (p= 0.001).

Conclusion: Generally, eight weeks of high intensity interval training with increasing endothelial constitutive nitric oxide synthase and hypoxia-induced factor- 1 increased vascular endothelial growth factor and eventually increased angiogenesis and improved cardiac function in male rats after myocardial infarction.

Keywords: Angiogenesis, Myocardial Infarction, High Intensity Interval Training

Introduction

The balance between heart muscle growth and increased coronary artery is one of the determinants of cardiac function (1). Disturbance in this balance converts the physiological function of the heart into pathologic hypertrophy and is an important factor in the development of heart ischemia and heart disease, such as myocardial infarction (MI) (2). MI is permanent and irreversible destruction and death of a part of the heart muscle due to the loss of blood flow and the occurrence of severe ischemia in that part of the heart (3). Ischemia caused by MI causes abnormal cardiac function and arrhythmias. The left ventricle is enlarged, resulting in less capillary density. Increasing hypertrophy without increasing angiogenesis results in hypoxia, and long-term hypoxia leads to p53 accumulation and, as a result, deactivates and decreases hypoxia-induced factor-1 (HIF- 1) (angiogenesis stimulus), resulting in an increased risk of a heart attack (4). Reducing capillary density encounters

heart muscle cells at risk of apoptosis (5). Various factors including hypoxia, hemodynamic forces, metabolites, vasodilators, muscle contraction, some cytokines and stretch types, affect the possibility of developing new vessels in a process called angiogenesis, which is growth and evolution of new blood vessels through germination of existing endothelial cells in the heart tissue. Angiogenesis ensures the life and growth of a new thickened myocardium, so recurrent angiogenesis in the heart tissue to improve cardiovascular function of patients is a compromise advantage. Therefore, measuring angiogenesis triggers can help find an effective way to increase the angiogenesis process and ultimately improve the quality of patients with MI (6). The vascular endothelial growth factor (VEGF) as the strongest and most important factor affecting angiogenesis increases the migration and proliferation of endothelial cells and formation of vascular networks. The VEGF is the main hemodymer glycoprotein coupled to heparin, with a molecular weight of 4500 Daltons, which is essential for the differentiation of endothelial cells and for the germination of new capillaries from the previous arteries (angiogenesis) during the growth and development of the capillary network (7). When VEGF binds to its specific receptors on the endothelial cell, activates messages that promote the proliferation and migration of endothelial cells and increase vascular permeability (8). Further, VEGF synthesizes DNA by increasing the amount of anti-apoptotic elements. The destruction of the base membrane and the phosphorylation of intercellular endothelial adhesive components and tight joints lead to the survival, proliferation, migration and permeability of the endothelial cell, respectively. In the early stages of angiogenesis, the incremental regulation of VEGF depends on the release of nitric oxide (NO) (9). NO is locally secreted by vascular muscle endothelium and muscle fibers during contraction and in response to high blood flow or shear stress (10). NO from L-Arginine is synthesized by different types of cells. The main source of its production in endothelial cells of the arteries is endothelial constitutive nitric oxide synthase (eNOS), which is activated during exercise and shear stress. Hypoxia activates eNOS and ultimately produces NO. In the early stages of angiogenesis, the incremental regulation of VEGF and VEGFR-2 depends on shear stress and NO release (10). eNOS is naturally occurring in vascular endothelium and decreases with endothelial loss, which causes a lot of serious illnesses such as high blood pressure and diabetes (11). Hypoxia-induced factor (HIF-1) is also one of the most important factors regulating the VEGF transcription process, which consists of two sub-units HIF-1α and HIF-1β. Due to hypoxia, calcium free increases. Due to the increase in calcium, PI3K is activated and the AKT and mTOR pathways increase significantly in the HIF-1 protein of the mRNA. HIF-1 activation during hypoxia creates adaptations such as increasing the expression of the VEGF gene to reduce the negative effects of hypoxia (4). Although the physiological mechanisms of cellular response to the hypoxia are found only at the molecular level, the role of HIF1 in activating the transcription of the VEGF gene in hypoxia cells has been well documented (2). Today, in patients with MI, angiogenesis has been considered as an adaptive mechanism that is affected by various factors of escalation or suppression. The role of regular physical activity in health has been well documented, recently, high intense interval training (HIIT) has been considered by researchers. This training is a powerful stimulant for cardiovascular and muscle adaptation, which increases the maximum oxygen uptake, metabolism, increased exercise performance, reduced carbohydrate intake and fat-based retention, improves insulin function, decreases blood pressure, affects cardiac patients and hypertension, and improves cardiovascular fitness. Recently, a practice of developing

cardiac angiogenesis has also been taken into consideration. In relation to HIIT it has been reported that HIIT is induced and hypoxia may also increase myoglobin levels (12). Truijens also reported in his research hypoxia formation in HIIT (13). Concerning the effect of endurance activities, the results of previous studies indicate that training is effective in vascular regeneration and angiogenesis in patients with MI (14, 15). Concerning the effect of resistance training, the results are more controversial (16, 17). In the meantime, HIIT and its effect on the angiogenesis process, and in particular its upstream effective factors, have not been sufficiently considered. Semenza *et al.* (2000) stated that HIF1α protein content increased in response to HIIT (2). Kassia *et al.* (18) stated that HIIT increases NO in the heart muscle of cardiac patients (18). Holloway *et al.* compared HIIT and endurance training on symptoms of cardiac failure and cardiac muscle changes in rats. In this study, VEGF and also eNOS were investigated and the results indicated no significant effect of HIIT on angiogenesis process (19). Karbalaeifar *et al.* also examined the effect of six weeks of HIIT on selected angiogenesis agents. They stated that HIIT can increase VEGF (20). According to the presentations regarding the factors affecting the vascularization of skeletal and cardiac muscles during exercise, which included: hypoxia, metabolites, and vascular dilatators, and the results of previous studies that there was a positive and significant relationship between HIIT and these factors, it turns out that HIIT in many cases causes a positive change in physiological variables, each of which can in some way be effective in human health. But the fact that the use of HIIT causes major changes to angiogenesis is a question that the research seeks to answer, i.e., whether the HIIT with an increase in eNOS causes angiogenesis in patients with MI and can increase VEGF, HIF-1 and eNOS.

Methods

In this developmental study, 12 ten-week old male Wistar rats with MI were randomly divided into experimental and control groups of six. The rats were kept in separate cages with free access to water and food packs, based on the principles of animal care in the laboratory (NIH-publication), and according to the 12-hour sleep and waking cycle. The rats were then subjected to surgery and their left artery descending (LAD) was blocked, and thus the rats were infected with severe MI (21). To diagnose MI, rats, being anesthesized, were echocardiographically dopplered with an echocardiographic device (GE Healtcare Brand, USA). Fractional shortening (FS) in relative terms was calculated according to the following formula (21):

$$FS = (LVDd - LVDs) / LVDd) * 10$$

Rats with FS≤35 percent were selected as MI rats for this study (21). The rats then ran for two weeks of recovery after open heart surgery. In the third and fourth weeks, the rats got to know to walk gently on treadmill (Danesh salar-e- Iranian, Iran) at a speed of five m / min, five minutes a day and four days a week. At this stage, all the rats were able to perform activities and did not have any casualties. At the end of the fourth week, the maximum oxygen uptake (VO_{2max}) of rats were measured by Maximum Sport Exercise Test, according to the formula and table set out in Morten *et al.* (22), and Wisloff *et al.* (2000), to estimate the initial speed of running the rats (23). The speed of each rat on the treadmill was calculated based on the VO_{2max} individually. The rats then took rest for two days. Ultimately, surviving rats with MI were randomly divided into two groups of HIIT and control, and the exercise protocol was performed. Experimental group rats in the HIIT (as a practice of the day, the effect of which on the variables considered in this research is less studied), did interval running on the treadmill four days a week, for eight weeks and each session for 60 minutes. Each interval included four minutes of running with

an intensity of 85- 90 % of VO_{2max} and two minutes of active recovery at a rate of 50- 60 % of VO_{2max} (24). The rats acted in a warm-up program for eight minutes at a running speed of 5 m / min on the treadmill before the start of the main training phase. In contrast, control rats (with MI) did not perform any training (24). After eight weeks, and lastly, after two days of rest, the remaining rats were again anesthesized for echocardiography; and imaging of the muscle tissue of the MI region was carried out to measure the RNA values of the eNOS, HIF- 1 and VEGF genes. Samples were transferred to the genetic laboratory after freezing, and the mentioned factors were measured using the Real Time-PCR method (by a laboratory kit Biooneer, Korea; the Real Time-RCR machine Step one ABI, USA; and primer Memmert, Germany). After the reaction, raw data was extracted from machine as ΔΔct and then graph pad software was used to plot the gene expression. Quantitative data were analyzed by Real Time PCR using SPSS 18 software. The Kolmogorov-Smirnov test was used to determine the normality of the data, and in the case of normal distribution of data, independent sample t-test was used at a significant level of 0.05 for analyzing the data.

Results

Descriptive statistics and the results of independent sample t- test for rats in the eNOS, HIF-1 and VEGF indices are presented in Table 1. The results of independent sample t-test showed that there was a significant difference between the two groups of control and HIIT groups in eNOS index (p= 0.01). According to Table 1, the eNOS index values in the HIIT group (4.755) were higher than the control group (3.615) (Table 1). There was a significant difference between the two groups of control and HIIT in the VEGF index (p= 0.001) and the values of the VEGF index in HIIT group (6.855) were greater than the control group (1.425) (Table 1). There was a significant difference between control and HIIT groups in the HIF-1 index (p= 0.001), and the values of HIF-1 index in the HIIT group (9.015%) were higher than the control group (1.49) (Table 1).

Table 1. the results of independent sample t- test for compare the eNOS, HIF- 1, and VEGF in HIIT and control groups

Variable	Group	Minimum	Maximum	Mean	Standard Deviation	Independent t Test
eNOS (mg/ml)	Control	1.82	5.41	3.61	1.61	0.01 *
	HIIT	2.59	6.92	4.75	2.06	
VEGF (mg/ml)	Control	0.84	1.17	1.42	0.15	0.001 *
	HIIT	4.21	5.29	6.85	0.47	
HIF-1 (mg/ml)	Control	0.90	1.18	1.49	0.07	0.001 *
	HIIT	5.06	7.91	9.01	1.28	

* significant level in p≤ 0.05

Discussion

In general, according to the results, eight weeks of high intense interval training can increase the expression of eNOS, HIF-1 and VEGF genes in the rats with MI. The results of this study were contradictory with the results of the research by Holloway *et al.* who compared HIIT and endurance training on symptoms of heart failure and cardiac muscle changes in rats. In this study, VEGF as well as eNOS were investigated and the results indicated no significant effect of HIIT on the angiogenesis process. However, the results were consistent with the results of Karbalaeifar *et al.* (2016), who stated that six weeks of HIIT increased VEGF, as well as Kassia *et al.* (2014) who stated that HIIT was associated with an increase in NO in the heart

muscle of cardial patients. Concerning HIF-1, the results of this study coincided with the results of Semenza (2000). The reason for the contradiction in the results could be the difference in the protocol of the training.

HIIT seems to induce effective factors in the expression of VEGF genes, thereby stimulating angiogenesis. HIIT by activating hypoxia have enabled generating veins. The hypoxia-induced factor is not hydroxylated in hypoxic condition, rather it persists and migrates to the nucleus and induces effective factors in angiogenesis (12). Also, hypoxia caused by HIIT (25) releases cytokines, which by entering endothelial cells enters the angiogenetic chains through the endothelial-derived relaxing factor (EDRF) called nitric oxide, which is stimulated by over-regulation fibroblastic growth factor (FGF- 2). On the other hand, the immediate increase in stretching force due to very high intense interval trainings is greater, through activation of ionic channels, especially potassium channels, secreting vascular dilators, especially nitric oxide (10), which regulates the incremental increase of VEGF and VEGFR- 2 in stretching and release of NO. Due to very by HIIT, increased muscle adaptation, in particular, reduced creatine phosphate degradation and increased glycogenogenesis occur; also, anabolic and adenosine hormones increase, which stimulate the expression of the VEGF gene (8). On the other hand, due to very HIIT and AMP dephosphorylation by octo-5 nucleotidease, the adrenalin parenchymal adrenal cell is produced from hypoxic tissues adjacent to the extracellular space which plays an important role in the process of angiogenesis (26). Extracellular adenosine induced by HIIT adenosine receptors and subsequently liberates VEGF from parenchymal cells (27). Hypoxia caused by a very HIIT, by inhibiting hydroxylation, halts HIF-1α decomposition. Thus, HIF-1α is accumulated and the increased density provides the binding of HIF-1α to HIF-1β and the formation of the HIF- 1 complex (18). Finally, the response between the HIF-1 and the target gene initiates the transcription of the VEGF gene (18). Finally, all factors, by increasing the VEGF and binding it to its specific receptors on the endothelial cell, have triggered messages that promote the proliferation and migration of endothelial cells and increase vascular permeability (28). VEGF synthesizes DNA by increasing the anti-apoptotic elements, and by degrading the base membrane and phosphorylated intercellular endothelial adhesive components and tight joints results in the survival, proliferation, migration and permeability of the endothelial cell (29).

Conclusion

In general, the results of this study indicated that the training protocol used in this study - 60 minutes of high intense interval jogging on a treadmill with an intensity of 85- 90 % VO_{2max} and four days a week for six weeks - has been able to be effective in increasing the factors to stimulate cardiac angiogenesis and improve cardiac function. Given the lack of information and research on the effects of HIIT on the angiogenesis process, further studies are recommended in this area so that this practice can be considered more reliable in order to improve the quality of life and improve the function of the cardiovascular system of patients with MI.

Ethical issues

In this research rats were kept in separate cages with free access to water and food packs in accordance with NIH-publication in a 12-hour sleep and wake cycle.

Authors' contributions

All authors equally contributed to the writing and revision of this manuscript**.**

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