Effect of High-Intensity Interval Training (HIIT) on Vascular Endothelial Growth Factor (VEGF) in Heart Muscle in Healthy Rats

Parvaneh Nazarali, Zahra Kohestani Sini and Mohamadreza Kordi

ABSTRACT

The effect of exercise on angiogenic factors is a subject that has interested many researches. Vascular endothelial growth factor (VEGF) is a secretory protein with a molecular size of 25 to 45 kDa. It is a signal protein generated by cells that stimulate angiogenesis. The aim of this study was to investigate the effect of 1, 3 and 5 sessions of high-intensity interval training on VEGF in the heart muscle of healthy rats. Thirty two healthy male rats, aged 3-4 weeks and weighed 250±20 g, were subdivided into four groups randomly: control group (n=8), 1 session (n=8), 3 sessions (n=8), and 5 sessions (n=8) of high-intensity interval training. The exercise training included 1 hour of high-intensity interval training. Sampling from heart muscle was done twenty-four hours after the activity. Then, the heart tissue levels of VEGF were examined by using ELISA method. Independent t-test and one-way ANOVA were run to analyze the data. The results showed no significant change in VEGF in the 1, 3 and 5-session groups in comparison with the control group. It is likely that 5 sessions HIIT could not increase VEGF protein levels and promote angiogenesis, or that they increased the level of VEGF gene or other angiogenic expression but did not contribute to protein synthesis or increase capillary density.

KEY WORDS: Vascular endothelial growth factor, High-intensity interval training, Heart muscle, Heart disease

INTRODUCTION

Cardiovascular diseases account for the majority of fatality rate across the world and incur immense treatment costs. Medical researchers tend to focus on the prevention of cardiovascular diseases rather than their treatment [Marron et al., 2000]. Since the function of tissues is directly associated with the vascular network inside them, blood vessels need to develop concurrently with the development of new tissues. Thus, sprouting new blood vessels from the pre-existing vascular network has been brought into attention over the last years as a prevention method. New vessels are typically feeble and fragile; however, the same vessels could decrease the fatality rate associated with coronary artery diseases [Richardson et al., 2000]. Thus, every method to reduce cardiovascular diseases, particularly the low-cost ones, is considered as an important prevention method. This has been addressed by medical researchers over the last two decades [Toya and Malik, 2012]. Since sports and exercise training have been considered as one of the key factors in preventing cardiovascular diseases, it is highly important to identify appropriate training methods in this regard. Attentions have increasingly turned toward the impact of exercise training on gene expression and production of VEGF that is the most important factor contributing to angiogenesis. VEGF is one of the most important specialized regulators of angiogenesis. It is a secretory protein with a molecular size of 25 to 45 kDa that is mainly produced by endothelial cells, smooth muscle, tendon, platelets, thymus and skeletal muscles [Ranjbar et al., 2012]. VEGF stimulates endothelial cell proliferation and differentiation, increases vascular permeability, prevents endothelial apoptosis and regulates vasodilatation [Nourshahi M et al., 2012]. VEGF provides for the survival, proliferation, migration and permeability of vascular endothelial cells through incremental adjustment of anti-apoptotic factors [Zachary 2001], DNA synthesis, destruction of the basement membrane and phosphorylation of endothelial intercellular adhesion components and tight junctions, respectively. VEGF is typically introduced as VEGF-A to be distinguished from two other types of VEGF (B-D) that are mostly involved in lymph angiogenesis [Rosalinda, ...
In this study, VEGF denotes VEGF-A. VEGF is a signal protein generated by cells that stimulate angiogenesis. It is part of the system that stores extra oxygen to be used at times when the blood circulation is not sufficient. VEGF is typically involved in angiogenesis during fetal development, after injuries, in muscles during exercise training and at the event of atherosclerosis. It can be produced in a cell that has failed to receive sufficient oxygen [Shweiki, 1992]. When a cell suffers hypoxia, hypoxia-inducible factor 1 alpha (HIF-1α) is produced that is a copying factor [Jensen, 2004]. HIF contributes to VEGF release through changing the amount of erythropoietin. The circulating VEGF then adheres to VEGF receptors on endothelial cells, which induce the secretion of tyrosine kinase and movement toward angiogenesis paths [Prior, 2004]. Studying angiogenesis and VEGF production, Koch et al. (2001) compared the rats with low and high aerobic capacity for running on treadmill. The results showed that the rats with higher aerobic capacity were in a better condition in terms of VO₂max. Considering the normality of the data, one-way ANOVA was used to examine the variation among the four groups. Independent t test was used to compare the effects of different training sessions separately. The level of significance was set at P≤0.05. Data analysis was conducted using SPSS 19.

Methodology:

The present study is a basic experimental research conducted to investigate the effect of high-intensity interval training on VEGF concentration in heart muscle in healthy male Wistar rats. In this regard, a number of 32 male Wistar rats weighed 250±20 g and aged 3-4 weeks were purchased from Pasteur Institute of Iran. The rats were kept at the light-dark cycle of 12 hours light and 12 hours dark, 50% moisture and 22±3°C temperature. They had free access to food and water. The rats were randomly assigned into 4 groups: 1 session (n=8), 3 sessions (n=8) and 5 sessions (n=8) of high-intensity interval training and control (n=8). The control group did not do any exercise training; however, the control rats were placed on an immobile treadmill for 10-15 minutes per session in order to create similar situations. The rats in the training groups practiced the training program on the treadmill for a week to be familiarized with the training protocol. The training program was developed based on the research conducted by Harman et al. (2009) and Hoydal et al. (2007) [Haram et al., 2009; Hoydal, 2007]. The protocol was also used by Esposito and colleagues (2011) [Esposito et al., 2011]. The main training program consisted of high-intensity interval running for 1 hour, which was performed in three stages: 1. Warm-up: running on the treadmill with no incline for 6 minutes with a speed of 15-20 meter per minute and an intensity of 50-60% VO₂max. 2. Main training: running with variable speeds at 7 intervals (4 minutes of high-intensity running with a speed of 25-36 meter per minute at an intensity of 70-90% VO₂max) with 5-20° incline followed by 3 minutes of low-intensity training with a speed of 25-36 meter per minute (an intensity of 50-60% VO₂max with 5-20° incline). 3. Cool-down: running on the treadmill with a speed of 15-20 meter per minute, an intensity of 50-60% VO₂max, and no incline. Twenty-four hours into the completion of the training program, the rats were anesthetized via Ketamine (50 mg per kg of body weight) and xylazine (30 mg per kg of body weight) gases using intraperitoneal injection. Myocardial biopsy was carried out after sedation of the rats. The extracted tissues were transferred into micro tubes and kept in liquid nitrogen until they were taken to the laboratory. Then they were kept in a fridge at -80°C. The data were analyzed using VEGF kit (made in China) with an accuracy of 5.01 Ng per liter and the enzyme-linked immunosorbent assay (ELISA) method. In this regard, the samples were homogenized and centrifuged at a speed of 2000-3000 RPM for 20 minutes and analyzed using Hyperion Elisa Reader.

Data analysis:

Descriptive statistics including mean and standard deviation were used to describe the data. Kolmogorov-Smirnov test was run to examine the normality of the data. Considering the normality of the data, one-way ANOVA was used to examine the significance of VEGF variations among the four groups. Independent t test was run to compare the effects of different training sessions separately. The level of significance was set at P≤0.05. Data analysis was conducted using SPSS 19.

Results:

Table 1 illustrates VEGF concentration in the heart muscles of healthy male Wistar rats.
Results of independent t test revealed no significant difference in VEGF variations between 1-session high-intensity interval training and control groups (P=0.90). The results also showed no significant difference in VEGF variations between 3-session high-intensity interval training and control groups (P=0.28). No significant difference was also observed in VEGF variations between 5-session high-intensity interval training and control groups (P=0.85). The results of one-way ANOVA showed no significant difference in VEGF variations between the control group and 1, 2 and 5-session high-intensity interval training groups (P=0.51).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Men</th>
<th>SD</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>0.1125</td>
<td>0.054</td>
</tr>
<tr>
<td>1 session</td>
<td>8</td>
<td>0.1125</td>
<td>0.026</td>
</tr>
<tr>
<td>3 session</td>
<td>8</td>
<td>0.1357</td>
<td>0.027</td>
</tr>
<tr>
<td>5 session</td>
<td>8</td>
<td>0.1138</td>
<td>0.022</td>
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Discussion:
Statistical analysis suggested that 1, 2 and 3 sessions of high-intensity interval training did not exert a significant effect on VEGF concentration in the heart muscle of male Wistar rats. This is partly inconsistent with some studies. For example, Wu et al. (2009) studied the subjects with cardiovascular diseases. They showed that three days of high-intensity training for an hour per day induced small changes in VEGF concentration though the change was not statistically significant. The interesting point was that VEGF concentration showed a greater increase in the heart muscle of the rats with cardiovascular diseases comparing with the healthy rats following high-intensity training [Wu et al., 2007].

These results were not in agreement with the findings of [Jian et al., 2004]. But the results of this study were in agreement with Danzig [Danzig et al., 2010]. Danzig showed that VEGF levels did not change, following an acute session of exercise. The mechanism behind VEGF decreases in response to acute exercise is not clear well [Jian et al., 2004]. Jian et al showed that the reduction of serum VEGF after acute exercise does not mean the decrease in the amount of VEGF production in muscle. However, possible explanations might be as follows: 1) increased VEGF binding-affinity to its receptors at the endothelium, which would stimulate angiogenesis in the local tissues such as heart and skeletal muscles, 2) a substantial increase in circulating VEGF binding proteins such as heparin sulfate and Endothelial Progenitor Cell (EPC) [Jian et al., 2004; Suhr et al., 2007; Rullman], which would protect the vascular system from a deleterious increase in VEGF-induced hyper-permeability [Jian et al., 2004].

Which reported reducing serum VEGF following acute activity

Despite the present findings, Tamada (2012) and Tang (2011) reported that high-intensity training had a significant effect on VEGF concentration in healthy rats. Healthy rats were examined in the present study while Tamada (2012) and Tang (2011) studied the rats with cardiovascular diseases [Tomada et al., 2012; Tang et al., 2011]. Asma and colleagues (2012) reported that short-term high-intensity training had a significant effect on VEGF concentration. This is inconsistent with the present findings because the subjects took steroid in combination with training. However, consistent with the present findings, they observed no VEGF variations in the subjects that only did short-term high-intensity training [Asmaa and Manal, 2013]. The present findings are also inconsistent with the findings of Van Craenenbroeck et al. (2012) who concluded that the contribution of high-intensity training was due to greater blood circulation rather than the training intensity [Van et al., 2010]. The inconsistency between the present findings and the findings of Van Craenenbroeck and colleagues could be due to the differences in blood supply, which could be considered as one of the limitations of the study. Thus, it seems that high-intensity training has a significant impact on VEGF concentration only when other factors intervene such as cardiovascular diseases, cardiovascular risk factors, exercise-induced hypoxia and exercise-induced increase in tissue perfusion.

Analysis of the existing mechanisms show that since there is perfect control and coordination in keeping balance between the factors increasing and inhibiting angiogenesis (i.e. angiogenic and angiostatic factors) at rest position, the balance should be disturbed in order to induce angiogenesis. Thus, it seems that a maximum of five training sessions was not sufficient in the present study to disturb the balance. Therefore, although the training intensity was high, the number of sessions was not enough to contribute to VEGF concentration. Endostatin, adiponectin and natriuretic peptides are the key inhibitors of VEGF production. Jian et al. (2004) contended that lack of increased VEGF concentration following exercise training does not mean that training does not contribute to VEGF production. Lack of increased VEGF concentration following exercise training may relate to the connection to other proteins such as Heparan sulfate and endothelial progenitor cells (EPC) that increase after heavy physical activity. The majority of studies have shown that endostatin increases in response to physical activity. Endostatin is one of the key inhibitors of VEGF. It has also been shown that adiponectin is one of the main inhibitors of VEGF that increases in response to high-intensity activity [Jian-Wei et al., 2004]. Research has shown that atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) play an important role in regulating serum VEGF. ANP and CNP are made up of 32 and 53 amino
acids, respectively. While ANP is typically produced in the heart, CNP is produced by endothelial cells. There is evidence that ANP and CNP prevent the production of VEGF in endothelial cells [Hoffner et al, 2013]. Besides, it is shown that natriuretic peptides increase in response to high-intensity activity. It is likely that increased VEGF inhibitors (i.e. ANP and CNP) account for the lack of change in VEGF concentration. This, however, needs further study [Rullman et al, 2007]. Somatostatin may be another reason for a lack of VEGF increase following exercise training. Somatostatin is a hormone that prevents cell growth and angiogenesis. It has two active forms of 14 and 28. It has five receptors that exist on the majority of body tissues. One of these receptors is sst2-R that exists on endothelial cells as well. Research has shown that the attachment of somatostatin to sst2-R receptor prevents the production of VEGF in endothelial cells. On the other hand, somatostatin secretion increases in response to high-intensity activity. Thus, it is also likely that exercise-induced somatostatin increase accounts for the reduced VEGF concentration [Domenico et al, 2007].

Conclusion:
Considering the present findings and review of the literature, it seems that a maximum of 5 training sessions was not sufficient to increase VEGF protein. Alternatively, the training could have increased gene expression but failed to result in the production of proteins due to insufficient training duration. Moreover, training intensity and duration may not be enough to come up with a resolution as to the contribution of high-intensity training to increasing VEGF levels in cardiac tissue. Thus, it seems that 1, 3 and 5 sessions of high-intensity interval training does not contribute to incremental changes in VEGF in the heart muscle of male Wistar rats. It seems that high-intensity training has a significant impact on VEGF concentration only when other factors intervene such as cardiovascular diseases, cardiovascular risk factors, exercise-induced hypoxia and exercise-induced increase in tissue perfusion.

REFERENCES


