Ilam University of Medical Sciences
Journal of Basic Research in Medical Sciences
Online ISSN: 2383-0972
Print ISSN: 2383-0506
Homepage: https://jbrms.medilam.ac.ir

Influence of Exercise Intensity on the Expression of Angiogenesis-Related Genes in the Hearts of Male Rats

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Article Info

Article type:
Research article

Article History:
Received: May. 05, 2022
Revised: Jul.06, 2022
Accepted: Jun. 18, 2023
Published Online: Dec. 24, 2023

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A B S T R A C T

Introduction: Angiogenesis, the formation of new capillaries from pre-existing vessels, crucially involves activation of the hypoxia-inducible factor 1 (*HIF-1*) and vascular endothelial growth factor (*VEGF*) genes. This study investigates the impact of exercise intensity on the expression of angiogenic genes in the hearts of male rats.

Material & Methods: Eighteen male Wistar rats were randomly assigned to three groups: High-Intensity Interval Training (HIIT), Continuous Training (CT), and control (C). Both HIIT and CT groups underwent 8 weeks of training with five sessions per week. Anesthesia and blood sampling occurred 48 hours post final training session. Gene levels of *HIF-1* and *VEGF* were measured in the left ventricle. Data analysis employed ANOVA and LSD post hoc tests ($P \leq 0.05$).

Results: *VEGF* gene expression significantly increased in both HIIT and CT groups compared to the control group ($P = 0.001$), with a more pronounced elevation in the HIIT group than the CT group ($P = 0.004$). Furthermore, *HIF-1* levels exhibited a significant reduction in both HIIT ($P = 0.001$) and CT ($P = 0.001$) groups compared to the control group, with the HIIT-induced decrease surpassing that of the CT group ($P = 0.049$).

Conclusion: The noteworthy elevation in *VEGF* and decrease in *HIF-1* gene expression levels in trained rats imply that exercise training enhances angiogenesis. Importantly, the extent of this enhancement is contingent upon exercise intensity, with HIIT demonstrating more pronounced positive effects on *VEGF* levels.

Keywords: Exercise Intensity, Continuous Training, High-Intensity Interval Training, Heart Tissue

➢ **How to cite this paper**
Kheradmand S, Asad MR, MirJavadi R, Kheradmand N, Fashi M. Influence of Exercise Intensity on the Expression of Angiogenesis-Related Genes in the Hearts of Male Rats. J Bas Res Med Sci. 2023; 10(4):43-53.

Publisher: Ilam University of Medical Sciences

Introduction

Physical activity and an active lifestyle contribute to maintaining and promoting health, especially preventing cardiovascular diseases (1). In this regard, in 2016, the European Cardiovascular Association issued a special guideline emphasizing that aerobic exercise improves cardiovascular endurance (2).

Exercise leads to an imbalance between oxygen demand and supply to cells, which is known as hypoxia. Although this condition occurs in skeletal muscle tissue, the heart muscle plays a special role as an involuntary muscle that is constantly contracted. In order to protect the heart muscle from different stresses, the continuous availability of oxygen and other substrates is essential (3). In this situation, angiogenesis is a crucial adaptation to physical training (4). This means the formation of a capillary from the previous ones so that muscle capillary density develops (5). It provides the basis for efficient training and also reduces the frequency of myocardial infarctions or, in other words, improves myocardial function by improving oxygen supply to skeletal muscles (6) and maximal oxygen uptake (VO2 max) (7).

Hypoxia-inducible factor 1 (*HIF-1*) is a transcription factor crucial for cellular adaptation to oxygen deficiency, exhibiting increased activity in hypoxic conditions (8). This heightened activity prompts the transcription of genes pivotal in angiogenesis, including vascular endothelial growth factor (*VEGF*) (9).

VEGF growth factor is a potent regulator of angiogenesis (10), which is secreted by endothelial cells in response to stimuli such as hypoxia and shear stress (11). *VEGF* is a family of *VEGF*-A, *VEGF*-B, *VEGF*-C, *VEGF*-D, *VEGF*-E, and *VEGF*-F secretory glycoprotein factors. These factors carry out their biological action on target cells by interacting with tyrosine kinase receptors (RTKs) present in the plasma membrane of the cell. After *VEGF* binding to RTKs, these receptors become dimerized and auto phosphorylated, then, after the onset of signaling cascade angiogenesis will occur. RTKs associated with these growth factors include *VEGF*R-1, *VEGF*R-2, *VEGF*R-3, and neuropilins (NRPs); including NRP-1 and NRP-2. Cardiac muscle angiogenesis induced by exercise training is dependent on *VEGF* availability in response to increased myocardial oxygen demand by cardiac hypertrophy, which can improve cardiac function and energy metabolism (12).

According to studies, serum *VEGF* levels elevated after 8 weeks of endurance training in inactive men under hypoxic conditions (13), after 8 weeks of continuous aerobic training and high-intensity interval exercise (HIIT) in healthy male rat soleus muscle (14) And also after a session of progressive aerobic exercise and HIIT in the serum of non-athlete men (11). On the one hand, *HIF-1*α gene expression was reduced after 3 (in interval hypoxia) (15) and 6 weeks of endurance training in skeletal muscles of athletes (16) and on the other, Aguiar Marschner & et al. (2019) observed short-term exercise activity increased *HIF-1* levels compared to the control group, without any changes in *VEGF* levels (17). These findings suggest that there is a contradiction in the existing literature (6).

Taking note of findings, levels of angiogenesis growth factors are influenced by the volume and intensity of exercise (18). Although studies have

shown that exercise can regulate serum levels of angiogenic factors, the molecular mechanisms associated with the initiation of the capillary network development process in response to the intensity of exercise training are not well understood (11). Also, due to the inconsistent and unclear results and the lack of sufficient information on how the effects of HIIT and continues training (CT) on the amount of gene expression of angiogenesis factors in cardiac muscle, the present study was performed to evaluate the response of HIIT and CT on the gene expression of *HIF-1* and *VEGF* in the left ventricular tissue of male rats' hearts seems to be essential.

Materials and methods

Experimental Design and Animal Husbandry

Eighteen male Wistar rats (8-week-old, 263 ± 12 g weight) were obtained from the Razi Institute (Tehran, Iran). Six rats were accommodated per cage under a 12:12 h light-dark cycle. Temperature and humidity were maintained at 22 ± 1.4 °C and 55 ± 4%, respectively. Ad libitum access to water and food, sourced from Pars Animal Feed Company, was provided. The animals were randomly assigned to three groups: High Intensity Interval Training (HIIT) (n = 6), Continuous Training (CT) (n = 6), and Control (C) (n = 6).

The control group remained sedentary, initially adapting to the environment while immobilized on the treadmill, and then exposed to the treadmill sound. The training groups underwent an 8-week motor-driven treadmill running program (Table 1). In the HIIT protocol, rats ran for 16, 24, 32, and 40 minutes in weeks 1, 2, 3, and weeks 4-8, respectively. High-intensity intervals were repeated 2, 4, 6, and 8 times in weeks 1, 2, 3, and weeks 4-8, respectively. CT sessions had an equal duration to HIIT.

To assess the animals' maximal oxygen consumption, the Bidford et al. (1979) standardized incremental test was employed. This test consisted of ten sets of three-minute steps, starting at 0.3 km/h in the first step and increasing by 0.3 km/h in each subsequent step to prevent the rats from overexertion. The study received approval from the University's Research and Ethics Committees.

Table 1. Eight-week High-Intensity Interval Training (HIIT) and Continuous Training (CT) Protocol

	HIIT				CT	
Warming up	W1-W2 (HI)	W3-W8 (HI)	W1-W3 (LI)	W4-W8 (LI)	W1-W8	Cooling down
5min (40-50% Vmax)	2 min (90% Vmax)	2 min (110% Vmax)	2min (40% Vmax)	2 min (30% Vmax)	70% Vmax	5min (40-50% Vmax)

HIIT: High-Intensity Interval Training, **CT:** Continuous Training, **Vmax:** Maximum rate of oxygen consumption, **HI:** High intensity, **LI:** Low intensity

Analysis of mRNA Expression of VEGF and HIF-1 by RT-PCR

Following the sacrifice of rats, total heart tissue RNA was isolated using Trizol^® reagent (Qiagen, Germany), following the manufacturer's instructions. The RNA samples underwent reverse transcription utilizing the Thermo Scientific Revert

Aid First Strand cDNA Synthesis Kit (Fermentas). In the subsequent step, the generated cDNAs served as templates for real-time PCR, employing SYBR green PCR master mix (SYBR green I), conducted on the Step One ABI system (Applied Biosystems). The primer sequences used in this study are shown in Table 2. The crossing threshold values obtained from real-time PCR were assessed for the target transcripts and normalized to the results for *GAPDH* mRNA, serving as the housekeeping gene.

Table 2. Primer Sequences for Real-Time PCR Amplification

Gene	Primer sequence (5' to 3')
VEGF	F- AATCCCTGCATAGAGGTACTTCCTAAT R- CTCAGATCTAGGTTCTTGGTTGAATAAG
HIF-1	F- AACACTGCCGAGCTCAAGAT R- CATCGGCTTGAGAAAAGGAG
GAPDH	F- GACATGCCGCCTGGAGAAAC R- AGCCCAGGATGCCCTTTAGT

VEGF: Vascular endothelial growth factor, ***HIF-1***: Hypoxia-inducible factor 1, ***GAPDH***: Housekeeping gene

Real-Time PCR and Gene Expression Analysis

All experiments were replicated twice within each group. The threshold cycle (Ct) for each specific gene, the corresponding housekeeping gene (*GAPDH*), and their differences (ΔCt) were determined. Subsequently, gene expression changes were evaluated using the ΔΔCT formula.

Results

Table 3 presents the means and standard deviations of the measured factors. One-way ANOVA analysis indicates a significant increase in *VEGF* levels ($P = 0.001$) in the trained groups (HIIT and CT) compared to their controls (C). Notably, this increase was more pronounced in the HIIT group than in the CT group ($P = 0.004$).

Additionally, One-way ANOVA analysis reveals a significant decrease in HIF levels ($P = 0.001$) in the trained groups (HIIT and CT) when compared to their controls (C). Remarkably, this decrease was more substantial in the HIIT group than in the CT group ($P = 0.049$) (Table 4).

Table 3. Mean ± Std. Error of *VEGF* and *HIF-1*.

Variable	Control	CT	HIIT
VEGF	1	3.88 ± 1.72	9.12 ± 1.78
HIF-1	1	0.38 ± 0.14	0.11 ± 0.04

VEGF: Vascular endothelial growth factor, ***HIF-1***: Hypoxia-inducible factor 1, **CT:** Continuous Training, **HIIT:** High-Intensity Interval Training.

Table 4. LSD Post Hoc Tests for *VEGF* and *HIF-1* Gene Expression following HIIT and CT Protocols

Variable	Group	Group	Mean±SD	P-value	CV
VEGF	Control	HIIT	8.12±1.56	0.001*	812
		CT	2.88±1.56	0.086	288

	CT	HIIT	5.24±1.56	0.004*	
HIF-1	Control	HIIT	0.880±0.12	0.001*	-89
		CT	0.618±0.12	0.001*	-62
	HIIT	HIIT	0.261±0.12	0.049*	

* signifies that the mean difference is significant at the 0.05 level, ***VEGF*:** Vascular endothelial growth factor, ***HIF-1*:** Hypoxia-inducible factor 1, **CT:** Continuous Training, **HIIT:** High-Intensity Interval Training, **CV:** Coefficient of variation

Discussion

To address the existing discrepancies and ambiguities in the outcomes and the insufficiency of information regarding the impact of High-Intensity Interval Training (HIIT) and Continuous Training (CT) on the gene expression of Hypoxia-Inducible Factor 1 (*HIF-1*) and Vascular Endothelial Growth Factor (*VEGF*) in the left ventricular tissue of male rats' hearts, this study was conducted to assess the response of HIIT and CT on the gene expression of *HIF-1* and *VEGF* in the left ventricular tissue of male rats' hearts. The rationale behind this investigation lies in the essential role of *VEGF* in inducing angiogenesis, particularly crucial under heightened cardiovascular stress, leading to enhanced aerobic capacity. Elevated *VEGF* levels are imperative for cardiac angiogenesis under increased pressure, with endurance exercise further augmenting *VEGF* expression in the heart muscle. Nonetheless, the variations in *VEGF* response to diverse exercise modalities remain largely unexplored. This study, therefore, aims to shed light on the impact of an 8-week regimen of HIIT and CT exercise on alterations in *VEGF* and *HIF-1*α factors in the left ventricular tissue of male rat hearts. The results indicated that a period of HIIT training significantly increased *VEGF* levels compared to group C, which is in line with the findings of Soori & et al. (2019) (21), Ramezani & et al. (2018) (22), Nourshahi & et al. (2018) (23), Żebrowska & et al. (2019) (24), and Ghahramani & et al. (2019) (25). Although Ghahramani and colleagues examined low-intensity interval exercises, they also reported a significant increase in *VEGF* values (25). Hoier & et al. (2013) stated that endothelial cell proliferation and migration are less responsive to vigorous activity than moderate exercise and that vigorous activity is a weaker stimulus for angiogenesis and one of the reasons for *VEGF* reduction in response to vigorous training is probably due to lower *VEGF* leakage from type II muscle fibers than type I fibers (26). However, the findings of the present study are inconsistent with the findings of Yazdanyan (1395) (27) and Shekarchizadeh & et al. (2011) (28). The difference in the results of the present study with the findings of Yazdanyan may be because of the fact that the heart muscle has oxidized fibers, so the ERRα receptor was not affected by the activity Shekarchizadeh, thus preventing the initiation of this cascade and ultimately lack of significant effect on *VEGF* levels was shown (27). Shekarchizadeh and his colleagues did not observe any significant changes in plasma levels of *VEGF* and *VEGF*R1 between the training and control groups after examining 20 Wistar rats in a 4-week resistance training protocol (28). Inadequate training intensity and duration are probably the reasons why training was not effective. According to most of the findings, we can conclude that the longer the duration of the training, the more effectiveness; most

studies that have reported the effectiveness of exercise, follow it over eight weeks. Have used. On the other hand, the type of exercise training these researchers performed was a resistance that might do not induce the necessary intensity.

Another result of the present study is that a period of Continuous Training (CT) exercise significantly increases *VEGF* levels compared to group C, which is in agreement with the findings of Torabimehr & et al. (2019) (29), Vali Zadeh & et al. (2018) (30), Hadi et al. (2016) (31), and Leosco & et al. (2007) (32). Wagatsuma & et al. (2005) also showed that the 9-day short-term swimming protocol increased *VEGF* gene expression in rat heart tissue, which is consistent with the findings of the present study (33). But the results of the present study are inconsistent with the findings of Soltani & et al. (2019) (34) and Shirali & et al. (2017) (35). The researchers reported, respectively, no significant and significant decrease in *VEGF* levels after endurance exercise. Since both study's samples mentioned above were rats with cancer, and tumor cells are dependent on angiogenesis for the supply of oxygen and nutrients as well as the creation of new blood vessels (36), decreased *VEGF* levels indicate a positive effect of endurance exercise. Soltani & et al. also attributed a non-significant decrease in *VEGF* levels to a significant decrease in miR-21 gene expression in this group (34) while Shirali & et al. found that inhibition of cyclooxygenase 2 (COX 2), which plays a key role in the pathway of cancer cells, was the reason for a significant decrease in *VEGF* levels (35). Also, the findings of Mehro & et al. (2014) are inconsistent with the findings of the present study (37). These researchers reported no significant differences in serum *VEGF* levels. Different type of exercise (resistance training) in diabetic rats is probably the reason for this difference in results. Diabetes causes inflammation and resistance exercise has been effective in counteracting this inflammation. On the other hand, considering that the training period was 8 weeks, the severity of the inflammation in the rats was likely to be high, and the resistance exercise training was only able to cope with the decrease in angiogenic factors, and as a result, there was no significant increase in these values in the training group. According to the fact that most studies have highlighted the role of endurance training in angiogenesis improvement, endurance training seems to be more effective than resistance training in angiogenesis processes due to: 1) more changes observed in the peripheral blood circulatory system and, 2) activation of stretching pathways and vascular mechanical stresses, but on the other hand, to compensate muscle atrophy experienced by diabetic patients, taking part in resistance exercises is one of the training requirements for them. So in such a condition, resistance training improves the angiogenesis process by reversing *VEGF* reduction caused by inactivity and inflammation of diabetes. Researchers also observed an insignificant increase in NO levels, which is a stimulus of *VEGF* (37). Besides, the other reason for the above-mentioned insignificant increase may refer to inflammation in rats (38).

Another finding of the current study was that *VEGF* levels increased after the High-Intensity Interval Training (HIIT) more significantly than Continuous Training (CT), which is in line with the findings of Schulze-Tanzil et al. (2011). It seems that another possible cause of a further increase in

VEGF-A in the HIIT group may be the secretion of interleukins such as IL-1, IL-6, IL-10, and TNF-α. There is a positive correlation between *VEGF* secretion and increased IL-1, IL-6, IL-10, and TNF-α after muscle and tendon injuries induced by exercise activity, and it was recorded that the above-mentioned factors are probably increased more in HIIT than CT, and consequently, HIIT increased angiogenesis more than CT (39).

The results of the present study showed a significant decrease in HIF levels after a period of Continuous Training (CT) compared to group C, which is consistent with the findings of Lindholm et al. (2014) (16), Marschner et al. (2019) (17), and Sylviana et al. (2018) (2) but is in disagreement with Mirdar et al. (2014) (40) who observed a significant increase in the lung levels of neonatal *HIF-1*α after the three-week protocol of swimming endurance training in pregnant mice. Because there is an increase in oxygen consumption during exercise, the production of Reactive Oxygen Species (ROS) is increased, which eventually leads to hypoxia and subsequently regulates *HIF-1*α. *HIF-1*α may even increase after a single session of endurance exercise (40).

In the present study, it was shown that HIF expression level is significantly decreased after High-Intensity Interval Training (HIIT) compared to group C. Our finding is supported by those of Thomas Songstad et al. (2015) (41), however, contradict the results found by Abe et al. (2015) (42) which showed a significant increase in *HIF-1*α protein levels in twin rat muscle after acute training (3 hours after a single session HIIT) and after a long-term training period (6-week HIIT). Since the researchers studied the protein level of *HIF-1*α and the synthesis of *HIF-1*α is regulated by the mTOR pathway, intense training affects both mTOR and *HIF-1*α pathway expression. Therefore, it seems that the mTOR pathway is involved in the increase of *HIF-1*α protein induced by exercise activity (42).

In addition, we observed that *HIF-1*α expression was slightly higher in the Continuous Training (CT) group than in the HIIT group. The down-regulation of *HIF-1* as a result of prolonged endurance activity can be affected by negative regulators such as Factor Inhibiting *HIF-1* (FIH-1) and Sirtuin (2). The expression and activity of the above-mentioned *HIF-1* negative regulators may be increased as a result of HIIT exercises, although their levels were not being investigated in the present study.

Conclusion

Our results suggest that exercise training can increase Vascular Endothelial Growth Factor (*VEGF*) and decrease Hypoxia-Inducible Factor 1 (*HIF-1*) levels in male Wistar rats. However, these changes are partly dependent on the type of exercise training, and in this regard, as shown, the role of High-Intensity Interval Training (HIIT) is more prominent. Thus, it seems that increased *VEGF* as a result of exercise training can be considered as an effective factor in improving angiogenesis in male Wistar rats. A more comprehensive study should be performed on larger study populations to confirm our results.

Acknowledgements

We would like to express our gratitude to all colleagues who assisted us in carrying out this research.

Financial support

No funding has been received for this research.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

AS Kh, MF, and MA contributed to scientific management, research concept, participation in module development, and final conclusions. S Kh and MF were responsible for writing the draft and methodology development. N Kh and RM participated in survey development. MF conducted follow-on revisions of the text.

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