

Research Paper:

Effect of Endurance Training Under Microgravity Condition on Vascular Endothelial Growth Factor



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ABSTRACT

Background and Aim: Exposure to microgravity conditions is associated with changes in the cardiovascular system. Physical activities are employed to reduce deleterious physiologic effects of long-duration microgravity exposure. The purpose of the current study was to evaluate the effect of endurance training on serum levels of Vascular Endothelial Growth Factor (VEGF) under simulated microgravity condition.

Materials and Methods: A total of 42 male Wistar rats were randomly selected and divided into five groups of SST (suspension and suspension training, n=10), SET (suspension and endurance training, n=6), S (suspension without training, n=10), ET (endurance training, n=6), and C (control, n=10). Serum VEGF levels were measured by ELISA kit before and after training. One-way ANOVA with a Bonferroni post hoc analysis was employed to test the research hypothesis.

Results: Our results showed that six weeks of endurance training in simulated microgravity increased serum VEGF levels in the SST group compared to the S and control groups ($P \leq 0.001$), while six weeks resting in simulated microgravity condition did not significantly affect serum levels of VEGF ($P > 0.999$).

Conclusion: Endurance training in simulated microgravity could affect VEGF and angiogenesis. In addition, endurance training in simulated weightlessness condition could be effective in rehabilitating patients with cardiovascular diseases.

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1. Introduction

The study of exercise in microgravity condition is difficult due to various reasons. First of all, there are non-standardized tests. Secondly, the collected data are obtained from a small group because the number of crew members in a spacecraft is limited. These limitations highlight the importance of ground tests. Spaceflight causes several stressful environmental factors, including microgravity, radiation, loss of circadian rhythm, and confinement. Microgravity affects various physiological systems such as musculoskeletal [1], cardiovascular [2], immune [3], vestibular, nerves [4], nutrition, and metabolism [5]. In a microgravity environment, blood and body fluids shift towards the head and move into the thoracocephalic region. The overall fluid redistribution and the effects of the reduced gravity gradient dramatically influence the cardiovascular system [6]. These changes decrease total blood volume [7] and adaptive responses of endothelial cells. Also, they alter the regulation of proangiogenic factors and angiogenesis [8].

Several investigations have shown the beneficial effects of physical activity on cardiovascular health. Regular exercise has also been shown to prevent the development of some non-cardiovascular chronic diseases, including type 2 diabetes mellitus [9], osteoporotic problems [10], depression [11], obesity [12], hypertension [13], and some types of cancers such as breast [14], lung [15] and colon cancer [16]. Exercise modifies both forms of vascular remodeling, including angiogenesis and arteriogenesis [17]. These findings suggest the protective effects of physical activity against coronary heart diseases and peripheral arterial diseases mediated by vascular remodeling [18].

Vascular Endothelial Growth Factor (VEGF) is one of the most important angiogenic factors for stimulating the formation and growth of vessels. Based on the previous studies, endurance training significantly increases VEGF serum levels and expression of VEGF mRNA [19-21]. It has been reported that one session of dynamic physical activity has boosted the expression of the VEGF gene in skeletal muscle [22]. Also, VEGF is an essential survival and growth factor for vascular endothelial cells that stimulate the propagation and migration of these cells [23]. Evidence suggests that exercise restores vascular endothelial dysfunction, too [17].

As mentioned above, microgravity affects the cardiovascular system and induces cardiovascular deconditioning.

Based on recent studies, endothelial cells are very sensitive to microgravity, and changes in vascular endothelial cells contribute to microgravity-induced cardiovascular dysfunction [24, 25]. Furthermore, Wang et al. observed that the expression of VEGF increases in the hippocampus after exposure to a simulated microgravity environment for 28 days [26]. The activation of VEGF and other angiogenic factors could increase vascular density [27].

Few studies have been conducted to determine the protective effects of physical activity on microgravity-induced physiological changes, both in a real and simulated microgravity environment [17].

Limited studies prove that endurance training could reduce deleterious physiologic effects of long-duration microgravity exposure. Hence, endurance training as an angiogenesis stimulus could regulate VEGF and angiogenesis in simulated microgravity. In addition, endurance training in simulated weightlessness condition could effectively rehabilitate patients with cardiovascular diseases [28]. Also, studying the cardiovascular system under microgravity condition leads to a better understanding of problems concerning the cardiovascular system and possible ways to deal with them during space explorations.

Considering all these aspects, the purpose of this study is to evaluate the effect of six weeks of endurance training under simulated microgravity condition on the serum levels of VEGF in male Wistar rats.

2. Materials and Methods

This study was authorized by the Ethics Review Committee on lab animals of the University of Tehran and was performed following the rules for animal care and ethical principles [29]. Forty-two 6-week-old male Wistar rats (average weight: 175 ± 15 g) were obtained from a licensed laboratory animal vendor in Razi Vaccine and Serum Research center (Ministry of Jihad Keshavarzi, Karaj, Iran). On arrival at our laboratory, all animals were housed in an environmentally-controlled room ($23 \pm 1^\circ\text{C}$ temperature, $55 \pm 5\%$ relative humidity; 12:12 h light-dark photoperiods, lights on 09:00–21:00 hours). They were fed with standard rat chow and given water ad libitum. Before the training period, all animals were familiarized with the laboratory, simulated microgravity condition, and treadmill for one week. The animals were randomly selected and divided into 5 groups as follows: SST (24 h suspension per day and suspension training, $n=10$), SET (12 h per day suspension and endurance training, $n=6$), S (24 h suspension per day without training, $n=10$), ET (endurance training, $n=6$), and

C (control, n=10). Training groups performed treadmill running three days per week for 6 weeks. The duration and speed of exercise started with 15 min and 10 m/s in the first week. Then, it increased and peaked at 30 min and 25 m/s in the last week (Table 1). All animals were sacrificed after the last training session.

Simulated microgravity (Hindlimb Suspension [HLS])

Ground-based models of simulated microgravity play a crucial role in galactic exploration because they prepare data crucial for the plan of human space travel missions. We have used the hindlimb suspension model with a suspension cage. The scientific communities have accepted this rat model as the rodent model of choice to simulate some aspects of spaceflight [30]. Briefly, the rat was placed under restraint which allowed its easy access to the tail. The tail was cleaned, made sticky by spraying a tincture of benzoin, and allowed to air dry. Rats were suspended by their tails using wide strips. The strips were placed laterally along the proximal two-thirds of the tail and secured with three rings of Elastoplast wrapped around the tail at equidistant points. A wire tether was attached at one end to the strips on the tail and, at the other end, to a swivel and a ring that slid easily at the top of the cage. It allowed the rat a free 360° range of movement around the cage. The height of the hook was adjusted such that only the forelimbs were in contact with the ground, and the body made a 30° angle with the floor of the cage. This situation affects the cardiovascular system [31].

Endurance training under microgravity condition

Besides the suspension cage, we prepared another cage that rats were trained under simulated microgravity condition. The cage can be placed on the treadmill, and rats (SST group) were trained (Figure 1).

Table 1. Endurance training protocol

Familiarization		Week					
		1	2	3	4	5	6
Age (wk)	6	7	8	9	10	11	12
Speed (m/min)	10	10	15	15	20	25	25
Duration (min)	10	15	15	20	25	25	30
Slope (degree)	0	0	0	0	0	0	0
Session (d/wk)	3	3	3	3	3	3	3

Blood sample preparation

After the last training session, all Wistar rats were anesthetized by ketamine (90 mg/kg)–xaillysin (10 mg/kg) mixture administration [32]. Then, their blood samples were obtained by cardiac puncture, and serum samples were immediately separated by centrifugation at 3000 rpm for 10 min and were stored at -80°C. Serum VEGF levels were measured by ELISA kit (Rat VEGF, ELISA, Hangzhou Eastbiopharm, USA, Assay rang 10 - 3000 ng/L, sensitivity: 5.01 ng/L).

Statistical analysis

All obtained data were reported as the Mean±SEM. To analyze the data, firstly, the Shapiro-Wilk test and Levene's test were performed to test the normality of data distribution and homogeneity of variances. Then, 1-way-ANOVA test and Bonferroni post hoc test were employed to compare intergroup changes. Statistical analyses were performed in IBM SPSS version 24. The level of statistical significance was set as P≤0.05.

3. Results

Table 2 presents descriptive statistics concerning the amount of serum VEGF measured by the ELISA method (ng/L) and its changes before and after training. One-way ANOVA results revealed a significant difference between group means (P≤0.001).

Pairwise comparisons result of VEGF means demonstrate a significant difference between the SST group and the control group (P≤0.001). However, there was no significant difference between the S group and the control group. Although the amount of VEGF in the S group was higher than that of the control group, this difference was not significant (P>0.999). Also, the results showed a



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Figure 1. Endurance training under microgravity condition

significant difference between VEGF mean values of the SET group and the control group ($P \leq 0.001$) (Table 3).

4. Discussion

Endothelial cells of the cardiovascular system are susceptible to various physiological stimuli, including biochemical factors, metabolic factors, neural mediators, and circulating hormones. Endothelial cells are also sensitive to various mechanical forces, such as stretch and compression of vessels wall, fluid shear stresses, and hydrostatic pressures. One of the mechanical forces is microgravity that affects cell growth and physiology. Based on recent studies, endothelial cells can respond to microgravity, and changes in vascular endothelial cells

contribute to microgravity-induced cardiovascular dysfunction. Besides, physical activity is recommended as a component of healthy living, and endurance training improves endothelial dysfunction and promotes cardiovascular health. Also, endothelial cells respond differently in microgravity condition. VEGF as a proangiogenic factor significantly affects endothelial cell survival, proliferation, and migration [33].

In this research, we inquired about the effects of endurance training under microgravity condition on serum levels of VEGF in young male Wistar rats. We employed the hindlimb suspension model to simulate microgravity condition in adult rats. Our data showed that endurance training increased serum VEGF levels in the SST, SET, and ET groups compared to the S and control groups after 6 weeks of training (Table 3). We could not detect any differences in serum VEGF levels between the S and control groups after 6 weeks of suspension. Former research which confirmed the effects of various exercise training protocols on VEGF and other proangiogenic factors did not consider the effect of microgravity condition. Studies showed that VEGF levels increased after endurance training [34]. The up-regulation of angiogenic regulators through physical exercise raises questions on the underlying mechanisms. Two stimuli seem to contribute to the liberation of the angiogenic modulator: decreased oxygen supply in biological tissues [35] and mechanical forces [36]. For this purpose, former research indicates that oxygen pressure decreases inside skeletal muscle during a workout, and this alteration triggers the angiogenesis process [37]. Also, endurance training can extremely enhance blood flow and shear stress at the vessel wall, as well as the mechanical stretch of skeletal muscle and its capillaries [38]. On the other hand, the

Table 2. Descriptive statistics

Group	VEGF (Mean±SD)		
	Pre-test	Post-test	Cohen's Effect Size
SST	13.62±3.68	14.43±3.28	0.10±0.04
SET	13.72±5.40	14.14±5.15	0.07±0.01
ET	13.72±5.40	14.72±6.04	0.04±0.03
S	13.12±4.04	12.72±4.14	-0.07±0.01
C	13.22±3.90	12.38±2.52	-0.10±0.02


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SST: 24 h suspension per day and suspension training; SET: 12 h per day suspension and endurance training; S: 24 h suspension per day without training; ET: Endurance Training; C: Control.

Table 3. Bonferroni post hoc test results

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Standard Error	Sig.
Percentage change	SST	S	0.17764	0.03999	0.001*
		ET	0.05523	0.04898	1.000
		SET	0.03097	0.04898	1.000
		C	0.20840	0.03999	0.001*
	S	ET	-0.12241	0.04898	0.173
		SET	-0.14667	0.04898	0.050*
		C	0.03076	0.03999	1.000
	ET	SET	-0.02426	0.05656	1.000
		C	0.15317	0.04898	0.035*
	SET	C	0.17743	0.04989	0.009*

SST: 24 h suspension per day and suspension training; SET: 12 h per day suspension and endurance training; S: 24 h suspension per day without training; ET: Endurance Training; C: Control. *Significant difference.

endurance training in this study was under microgravity condition that changes the regulation of VEGF levels [8].

Cornachione et al. investigated the effect of eccentric and concentric training on capillarization and myosin heavy chain contents after hindlimb suspension. Based on their experiment, concentric training increased the capillary/fiber ratio [39].

The result of our study demonstrates no significant difference between the S group and the control group serum VEGF levels after six weeks of suspension. Although the S group mean change was higher than that of the control group, this difference was not significant. The results of previous studies were different about the VEGF levels under simulated microgravity condition [8, 40]. Infanger et al. in 2006 evaluated the effect of exposure to VEGF in simulated spaceflight condition. They showed that vascular endothelial growth factor performs a cell-protective effect on EC exposed to simulated weightlessness. VEGF is promoted after 4 h of clinorotation and more increased after 12 h. Also, they have shown that modeled gravitational unloading by three-dimensional clinostat induced downregulation of VEGF in human endothelial cells [41]. These differences in VEGF responses to microgravity condition could be related to the duration of exposure to low gravity condition. We examined VEGF levels after six weeks of exposure to microgravity condition that might be a reason for the adaptation of endothelial cells to this condition.

Differences in the type of device that used to simulate microgravity condition could affect the results. The hindlimb-suspension rat applies 50% of its body weight to its forelimbs when the angle between the torso and the floor of the cage is 30°, but the clinostat is an effective, ground-based tool that can be usually used to generate hypogravity. The weightlessness created by a clinostat is often regarded as simulated microgravity. Under these conditions, cells cannot feel gravity; the gravity vector escapes its detection machinery. In addition, we investigated VEGF levels in serum that can be an overall outcome of whole body tissues, not in a specific tissue such as human ECs from the umbilical vein.

5. Conclusion

Like formerly published articles on mice and humans, we showed that endurance training under microgravity condition increases the serum levels of VEGF in adult male Wistar rats. It seems that endurance training in simulated microgravity could affect VEGF and angiogenesis. Besides, endurance training in simulated weightlessness condition could be effective in rehabilitating patients with cardiovascular diseases. Studying other proangiogenic factors and using other training such as resistance training in microgravity condition can help understand better the effects of training under microgravity condition.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Tehran University.

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The paper was extracted from the MSc. thesis of the first author at the Department of Exercise Physiology, Faculty of Physical Education, Kharazmi University of Tehran.

Authors' contributions

Conceptualization and supervision, funding acquisition and resources; data collection: Vahid Nikbakht and Mohammad Asadi Golzar; Methodology: Ali Kazemi; Investigation, writing – original draft, and writing – review & editing: All authors; Data analysis: Vahid Nikbakht and Zahra Hajebrahimi.

Conflict of interest

All authors declared no conflict of interest.

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