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Comparison of Continuous and Periodic Exercise on Serum Nitric Oxide Level and Vascular Endothelial Growth Factor in Old Rats

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ABSTRACT

Background and objectives: Considering the importance of aging and the associated physiological changes, as well as the effects of exercise on angiogenesis and cardiac index, this study aimed to compare continuous and periodic exercise in form of high-intensity interval training (HIIT) on serum levels of nitric oxide (NO) and vascular endothelial growth factor (VEGF) in old rats.

Methods: In this study, 30 old male rats were randomly divided into three groups: continuous training (n=10), HIIT (n=10), and control group (n=10). Interventions were performed for 8 weeks. To evaluate the research variables, 72 hours before the first training session and after the last training session, 3 ml of blood were taken from the tails of the rats. One-way analysis of variance was used to analyze the findings and Levene's test was used for assessing the homogeneity of variance. All statistical tests were performed using SPSS 17 software at a significance level of 0.05.

Results: Both training exercises significantly increased NO and VEGF levels compared to the control group ($p<0.05$).

Conclusion: The results of this study showed that 8 weeks of continuous and interval training cause a significant increase in the level of angiogenic factors in old rats. Therefore, these exercises and especially alternative exercises can be used as a suitable way to increase angiogenesis in the elderly.

Keywords: Exercise, Vascular Endothelial Growth Factors, Nitric Oxide.

INTRODUCTION

The risk for cardiovascular disease increases with age and obesity. Aging causes endothelial dysfunction in the aorta and decreases vascular resistance. Changes in endothelial function following aging may contain clinically important implications and lead to cardiovascular disease (1). Aging is a known risk factor for cardiovascular disease. There is also evidence of an increased incidence of thrombotic disease associated with aging (2). It has been shown that older people who have more daily activities have a better quality of life (3). Regular exercise can moderate many risk factors. Resistance training is a beneficial but often overlooked approach compared with endurance training (4). Angiogenesis is a process that directs the function of the endothelium to create new blood vessels by horning the blood vessels of the heart (5). In the process of angiogenesis, the endothelium shows different functions in response to various stress stimuli mediated by angiogenesis activators and inhibitors (6). One of the most important releasing factors of endothelium is nitric oxide (NO), a free radical generated by the conversion of L-arginine catalyzed by NO syntheses. This molecule is involved in various processes such as neurotransmission, vascular function, defense, and inflammation. By doing 8 weeks of continuous training, NO dilates blood vessels and also prevents platelet aggregation and leukocyte adhesion (7). Considering the importance of aging and the associated physiological changes on the cardiovascular system and the beneficial effects of exercise on angiogenesis and cardiovascular health, this study aimed to compare the effects of continuous and periodic exercise on NO and vascular endothelial growth factor (VEGF) levels in old rats.

MATERIALS AND METHODS

Thirty 21-month-old male rats were purchased from the Razi Institute in Tehran, Iran. The rats were kept in groups of 3, in special polycarbonate cages, under a controlled environment: average temperature of 22 °C, 55% humidity, and a 12:12 light–dark cycle. All animals had free access to water and food. After one week of familiarity with the laboratory environment, the subjects were randomly divided into three experimental groups : moderate-intensity continuous exercise, high-intensity intermittent exercise (HIIT), and a control group. Then, the animals of the endurance groups were subjected to treadmill running every day for 10 minutes, five times a week. The maximum velocity measurement was taken. Then, both continuous and intermittent endurance groups performed continuous aerobic and high-intensity intermittent training, five sessions a week for 8 weeks. Due to the adaptation of the animals to exercise, at the end of every four weeks, the animals were tested for fatigue, and the intensity of animal training was determined based on the new fatigue test.

The periodic exercise protocol consisted of three parts: warm-up, exercise including interval repetitions, and cooling. Next, the subjects performed the maximum cooling rate with an intensity of 40-50%. Periodic training consisted of a combination of high-intensity and low-intensity interval repetitions.

High-intensity interval repetition including 2 minutes at 80% of maximum speed in the first week; 90% maximal in the second week, 100% maximal in the third week, and 110% maximal velocity from the beginning of the fourth week to the end of the workout. The number of high-intensity interval repetitions was two in the first week, four in the second week, six in the third week, and eight from the beginning of the fourth week onwards (8).

In the continuous aerobic training protocol, the rats first ran on a treadmill for 40 minutes with an intensity of 40-50% of the maximum speed to warm up. The training intensity increased to 60% of the maximum speed in the first week, 65% in the second week, and 70% from the third week onwards. The training protocol was adjusted so that the total distance travelled was equal for both periodic and continuous training groups. Finally, the rats cooled down for 5 minutes at 40-50 % of the maximum speed (8).

Data were expressed using descriptive statistics (mean and standard deviation). Data were analyzed using analysis of variance and post hoc tests in the SPSS software (version 17). The statistical significance level was set at 0.05.

Measurement of biochemical parameters

Subjects in each group were anesthetized by intraperitoneal injection of ketamine (70 mg/kg) and xylazine (3-5 mg/kg).

Tail blood (2 ml) was taken and then immediately sent to the laboratory for

measurement of VEGF and NO using commercial kits.
First, the *Shapiro–Wilk* was used to determine the normality of the data, then the one-way ANOVA test was used to compare inter-group differences. *Tukey's* test was used to determine the significance of the variables.

RESULTS
Table 1 shows the weight, body mass index (BMI), and maximum speed test of rats in each group. Table 2 shows the mean level of VEGP and NO in the study groups. The percentage of changes in angiogenesis factors in the periodic group was more than that in the continuous group (Figures 1 and 2).

Table 1- Characteristics of the subjects before and after the intervention

Variables	Groups	Posttest	Pretest	*p*-value
Weight (g)	Control	4.2±4.400	5.2±5.435	0.000
	Continuous exercise	5.4±1.438	5.4±1.424	0.633
	Intermittent exercise	5.4±1.430	1.2±5.419	0.000*
Average speed (m/min)	Continuous exercise	20.2±4.34	50.2±4.38	0.001
	Intermittent exercise	58.2±8.33	780.2±4.44	0.000*

*Statistically significant difference

Table 2- Comparison of VEGF and NO between the study groups

Factors	Ss	Degrees of freedom	Ms	Statistical value F	*p*-value
VEGF (pg/mL)	224.52	2	112.26	9642.16	<0.001
NO (mg.mol)	40.50	2	20.25	468.97	<0.001

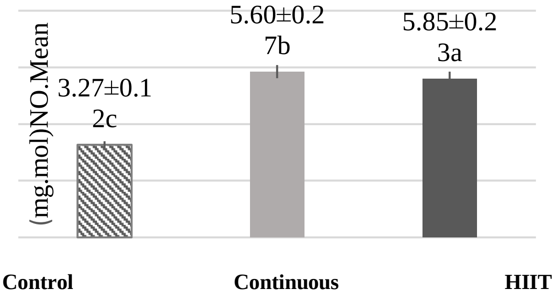

Figure 1-Mean NO level in the study groups

Figure 2- Mean VEGF level in the study groups

According to the findings, the mean level of NO increased significantly in both training groups ($p<0.001$). The result of the Tukey post hoc test shows that continuous exercise was more effective than HIIT ($p<0.05$).

DISCUSSION
In the present study, the effects of continuous and intermittent exercise on NO and VEGF were compared. These factors can play an important role in the survival of the elderly and in improving the quality of life of these individuals. In this study, 8 weeks of continuous and intermittent exercise both caused a significant increase in the level of these angiogenic factors in older rats. These results are consistent with the results of a study by Taher Gorabi and Khazaei (2012) which showed that 6 weeks of endurance training affected the level of VEGF and endostatin in tumor tissue of rats with breast cancer (9). Similarly, Nourshahi et al. (2012) showed that 8 weeks of endurance training on a treadmill reduced endostatin levels and increased VEGF

in the serum of rats (10). However, a study by Amani-Shalamzari et al. (2013) demonstrated that endurance training reduced VEGF in rats with breast cancer (11).

Studies have shown that activity of sufficient intensity and duration can increase the VEGF level in the capillaries (12). In this regard, a study reported that mRNA-VEGF levels increase further with increasing intensity of exercise (13). Lloyd et al. also showed that angiogenesis begins to increase in a relatively short period after exercise so that the amount of capillaries around each strand increases in the twin muscles after 12 days of training (14). Weston et al. showed that swimming exercises for 8 weeks can significantly increase the amount of VEGF in the heart muscle of rats (15). Hong et al. have also shown the positive effects of exercise on VEGF (16).

In another study, capillary capacity and interstitial VEGF increased in response to acute exercise, while tissue inhibitor of metalloproteinases 1 (TIMP-1) was not affected. Moreover, endothelial NO synthase increased by 0.5-fold after acute exercise in young men (17).

Inconsistent with this finding, Nourshahi and Ranjbar found no difference in the serum VEGF levels between both sedentary and active men and women at rest and after sub-maximal exercise (18).

Since sub-maximal exercise activity temporarily reduces the main factor involved in angiogenesis in healthy and inactive men, low-intensity exercise might not address the major factors involved in angiogenesis, such as providing tissue hypoxia and shear stress. In addition, this reduction may be due to the binding of serum VEGF to its receptors (19). Brixius et al. (2008) showed that VEGF levels in older obese men did not change after several months of regular aerobic physical activity. The reason for this discrepancy can be due to the characteristics of subjects and the duration of exercise (20). Husain showed that swimming exercises for 8 weeks significantly increased the amount of VEGF protein in the heart muscle of rats (21).

One of the mechanisms concerning the improvement of endothelial function is the increase in VEGF. It has been proven that regular exercise increases several angiogenic factors, such as VEGF and stimulates the phosphorylation of endothelial NO synthase (22). In this regard, in a study by Maleki et al., 2 to 4 days of exercise by electrical stimulation inhibited NO synthase and decreased VEGF expression, capillary proliferation, and capillary-to-fiber ratio in rat opener muscle. In general, secreted dilators participate in the regulation of gene expression, and NO causes upregulation of VEGF, although angiogenesis in turn activates endothelial NO synthase and NO production (23). Therefore, it can be said that there is a reciprocal relationship between NO and angiogenesis (22).

Another possible physiological stimulus for the production of NO is increased blood flow in the venous duct, the acute effect of which is to increase NO synthase and modulate vasodilation to balance pressure. These findings reinforce the possibility that because regular exercise frequently increases pulse pressure and pulsation, it may thereby increase the bioavailability of NO (24). In this regard, Naghavi et al. (2002) stated that 8 weeks of aerobic exercise (4 sessions per week for 60 to 90 minutes) increases the concentration of NO at rest after a period of exercise (25), which is in agreement with our findings.

As mentioned earlier, VEGF is secreted in response to stimuli such as ischemia, hypoxia, shear stress, metabolites such as adenosine and lactate, as well as vasodilators such as NO, adipokines, smooth muscle, platelets, and thymus (26). Exercise-induced angiogenesis improves the transfer of oxygen and nutrients to muscles by increasing capillary density in muscle fibers. The increased vascular bed has special benefits for obese people. Increasing the level of diffusion through capillaries by increasing the exchange time between blood and tissue and reducing the distance of diffusion causes more fatty acids to be called from adipose tissue and more muscle fiber to have access to free fatty acids (27). Vascularity of brain and heart tissue also reduces stroke and heart attack, respectively (28).

One of the reasons for the increase in angiogenic factors in this study can be angiogenic stimuli, a set of factors that stimulate the formation of new blood vessels. The most important angiogenic stimuli are hypoxia, hemodynamic forces, metabolites, vasodilators, muscle contraction, some cytokines, and some types of stretching (29). One of the reasons for the aerobic exercise-induced increase in VEGF is the increase in adenosine, a product of adenosine triphosphate metabolism. Significant amounts of adenosine are produced in the absence of oxygen or muscle contraction. Research has shown that increased adenosine increases muscle vasodilation, promotes energy balance, increases the expression of growth factors, increases the proliferation and migration of endothelial cells, and ultimately results in the formation of new blood vessels in various tissues (28).

CONCLUSION

Continuous and periodic exercises increase the level of angiogenic factors such as VEGF and NO, which at first glance, is desirable and useful for the elderly and those with cardiovascular disease. It can be also concluded that HIIT has an advantage over continuous endurance training in terms of time and workload.

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DECLARATIONS

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Ethics approvals and consent to participate

This research has been approved by the Research and Bioethics Committee of the Tehran University of Science and Research under the number IR.IAU.SRB.REC.1397.018.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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