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The effect of eight weeks of HIIT on some angiogenesis indices of apelin-13 in elderly male rats

Golbano Blori[1], Hossein Abednatanzi[1*], Hojjatallah Nikbakht[1]

1. Department of Physical Education and Sport Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran

***Corresponding author:** Tel: +98 9126107064 Fax:+98 -
Address: Department of Physical Education and Sport Sciences,Science and Research Branch, Islamic Azad University, Tehran, Iran.
E-mail:abednazari@gmail.com
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Abstract

Introduction: Aging causes changes in metabolic, cardiovascular, and neuromuscular functions. By developing aging process, physical activity decreases, which is mainly due to fundamental changes in body composition and lack of angiogenesis for which some indicators of angiogenesis have been found in older male mice. The purpose of this study was to investigate the effect of high-intensity interval training (HIIT) on angiogenesis indices in elderly male rats.
Materials and Methods: The studied sample included 20 elderly male mice, which were randomly divided into two groups of control (n = 10) and experimental (n = 10). The experimental group repeated the HIIT in the specified intervals. The exercises were repeated after two minutes of rest, with 80% of the maximum speed in the first week, 90% in the second week, 100% in the third week, and 110% from the beginning of the fourth week, until the end of the training. To evaluate the research variables (VEGF, FGF21, NO, apelin-13), 3 ml of peripheral blood was drawn from the tail of elderly male mice 72 hours before and after the last session of the training.
Results: Eight weeks of HIIT significantly increased VEGF and NO (P = 0.001). It also increased the FGF and apelin-3 levels significantly (P = 0.0001). No significant change was observed in the control group.
Conclusion: The results of this study showed that eight weeks of HIIT significantly increased angiogenesis levels in elderly male rats. Therefore, HIIT can be used as an appropriate and especially alternative exercise to increase angiogenesis in the elderly individuals.

Keywords: HIIT, VEGF, FGF21, Apelin-13, Angiogenesis, Elderly

Introduction

Cardiovascular disease risk factors increase 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 diseases.
Due to the improvement of health status, life expectancy incerement, and mortality reduction, more attention is needed to the elderly population. In 2010, more than 600 million people in the world will be over 60 years old, and this number is expected to increase to about 2 billion in 2050 (1). In Iran, according to the 2006 census, more than 6.5 % (7.2 million people) of the population were over 60 years old; if this life trend continues in about 1410, there will be an explosion of aging in the country, and 25-30 % of the population will be over the age of 50 (2).

Aging causes changes in metabolic, cardiovascular, and neuromuscular functions. Physical activity decreases with age; this process is mainly associated with fundamental changes in the body composition (3). By increasing the age, the heart experiences a stiffening of its muscle, due to an increase in the size of the ventricular muscle cells along with decrease in the number of these cells. Also, the intercellular matrix and collagen increase, and the heart muscle develops fibrosis (4).

Cardiovascular diseases are one of the most common leading causes of death in the world. The disease is caused by the gradual deposition of cholesterol (one of the types of blood fats) and other fats and substances in the inner wall of the arteries, and causes plaques; this process will lead to narrowing and hardening of the arteries walls (5). Another important change associated with aging is muscle wasting and decreased endurance capacity and musculare weakness (6). These fundamental changes are also due to dysfunction of the body's arteries; during this period, angiogenesis often occurs in old age (6).

One of the most important angiogenic factors is vascular endothelial growth factor (VEGF), which is the strongest growth mitogen for endothelial cells with a molecular weight of 35-45 kDa. Physical activity can be an important stimulus to increase VEGF. Stimulation of VEGF causes growth, migration, and survival of endothelial cells, and consequently further proliferation of retinal vessels, but the stimulation of antiangiogenic factors leads to decrease in the process of angiogenesis (7).

Clinical studies have shown the role of antiangiogenic in the treatment of diseases of the elderly;

now antiangiogenic therapy is used as an effective treatment, of which endostatin treatment is one of the most effective ones. Antiandrogens are affected and reduced during exercise, which can increase physical strength in the elderly. Another factor in angiogenesis is nitric oxide (NO), which secreted from vascular endothelium, and has a great effect on maintaining vascular health and the function of vasoconstriction and dilation. NO is one of the ten smallest molecules in the world, with the molecular weight of 30 kDa; it is produced by the NO synthase enzyme from L-arginine (8). Some studies have shown, that NO decreases with age. It has been shown, that the highest rate of NO depletion is in the age group of 46-60 years.

Inactivity and lack of exercise are two risk factors, which increase the pathological condition in the elderly. Researchs have shown, that Aplin-13 reduces in the cardiovascular patients (9). Currently, regular and long-term exercises are used as a therapeutic intervention for the treatment of many diseases, including cardiovascular diseases, diabetes, and obesity (10).

Behjati et al., studied the effects of a resistance training course on old men. The results showed that resistance training increases the rate of angiogenesis in old men (6). Also, Nourshahi et al. investigated the effect of a course of aerobic exercise with cinnamon supplementation on male rats. The results of Nourshahi’s study showed that short-term exercise had no effect on angiogenesis indices. Studies have shown, that exercise can be a positive factor in stimulating the process of angiogenesis in both physiological and pathological conditions (8).

Sedentary lifestyle and lack of exercise increase the risk of these pathological conditions in the elderly. The results of studies have shown, that exercise causes vasodilation, which causes blood flow and delivery of nutrients to the tissues, resulting in the physical health of the elderly (8). Exercise, by increasing blood flow, causes mechanical stimulation in the arteries, and if

endothelial cells act well, it leads to increased production and release of NO (9).
Fibroblast growth factor (FGF) is also an important indicator of angiogenesis in the elderly, which plays an important role in physiological conditions. It is a protein, that is involved in many cellular processes, such as cell division, embryonic development, angiogenesis and many others (10). So far, no Iranian study has been conducted on the effects of sports activities and FGF. Therefore, medical and sports experts believe that the elderly should do sports before getting sick. Since a previous study examined the direct effects of exercise on vascular growth factors in elderly, and the negative results has yielded, the researcher intended to investigate the effect of eight weeks of high-intensity interval training (HITT) on angiogenesis apelin-13 indices in aged male rats.

Materials and Methods

Animals

In this study, 20 old male Wistar ratsas with the age of 21-monthe were purchased from Razi Institute. They were kept in special polycarbonate cages with an average temperature of 22 ° C, humidity 55 and a 12:12 light-dark cycle. All animals had access to water and food. Subjects were randomly divided into two groups of control and experiment. The experiment group expirenced HITT, for 10 minutes and five times a week; the subjects in the experimental group were taught how to run on the treadmill. After 48 hours of rest following the last session, an exhaustive exercise test was taken to measure the maximum speed. Due to the compatibility of the animals with the exercise, the exhaustive exercise test was taken every four weeks; the intensity of exercises was determined based on the results of the tests. This experimental study was approved by the Research and Bioethics Committee of The Islamic Azad University.

Maximum Oxygen Uptake Speed Test

To determine the maximum rate of oxygen uptake, the designed standard incremental test by Hoveida et al., was used for standardized Wistar rats. The test consists of 10 three-minute stages. The speed in the first stage was 0.3 km/h, and in the next stages 0.3 km/h was added to the speed of treadmill. Considering that the five exhaustive exercise test methods, which have been introduced by Reynolds et al. has different slopes, the slope of zero was used in this study to determine the maximum oxygen uptake. The speed obtained in the last stage (when the animal was not able to run) was recorded as the maximum running speed (26).

Exercise Protocol

The protocol of periodic training included three parts of warm-up, interval training, and cooling down. The rats first warmed up to 40-50 % of the maximum speed on the treadmill for 5 minutes, then did interval training, and finally cooled down to 40-50 % of the maximum speed. Periodic training consisted of a combination of high and low-intensity intervals. Repeating the high-intensity interval included a time span of two minutes with 80% maximum speed in the first week, 90% maximum speed in the second week, 100% maximum speed in the third week, and 110% maximum speed since the beginning of the fourth week.
High-intensity intervals included two intervals in the first week, four intervals in the second week, six intervals in the third week, and eight intervals since the beginning of the fourth week. Therefore, the total training time was 16 minutes in the first week, 24 minutes in the second week, and 40 minutes in the third week and the beginning of the fourth week on wards (24).

Data were analyzed both descriptively and inferentially. At the descriptive level, indicators such as mean and standard deviation were used. The normality of data distribution was investigated using Kolmogorov-Smirnov test. In the inferential level, independent and dependent t-test were used for intergroup and intragroup evaluation. SPSS software (v.16) was used for analysis and conducting statistical tests at a significance level of 0.05.

Results

The results obtained from the control group indicated no significant difference between the two stages (P = 0.739). A comparison of VEGF changes in the pre and post-test, between the control and experimental groups showed a significant difference (P = 0.0001). As depicted in Table 1, comparing the means of the pre- and post-test of the experimental group showed, that the subjects' FGF level increased significantly after the interval training, which was significant according to the obtained t-value of 2.93 and significance level of P = 0.0001.

In the control group, the obtained results indicated no significant difference between the two stages (P = 0.731). Comparing the means of the pre- and post-test showed that the subjects' NO level was significant after the interval training.

The comparison of NO changes between the control and experimental groups showed a significant difference (P = 0.0001). Also, according to the information in Table 1, by comparing the means of the pre-test and post-test of the experimental group, it was found that the level of apelin-13 had a significant increase after interval training, which was a significant change (P = 0.0001). In the case of the control group, the obtained results indicated no significant difference between the two stages (P = 0.581).

Table 1. Comparison of the effects of eight weeks high-intensity interval training (HIIT) in the experimental and control groups under study.

Variable	Group	Pre-test	Post-test	Pwithin group	P between group
Weight (g)	Control	435.21 ± 5.22	400.44 ± 4.20	0.000	P = 0.0001
	Experimental	438.14 ± 4.53	416.11 ± 4.53	0.556	
VEGF(pg/mL)	Control	5.93 ± 0.03	6.00 ± 0.09	0.739	P = 0.0001
	Experimental	7.96 ± 1.19	14.19 ± 1.55	0.0001	
NO (µmol/l)	Control	3.25 ± 0.16	3.27 ± 0.12	0.735	P = 0.0001
	Experimental	4.89 ± 0.83	8.92 ±1.36	0.0001	
FGF (pg/mL)	Control	2.518 ± 0.33	2.62 ± 0.36	0.713	P = 0.0001
	Experimental	3.55 ± 0.044	7.26 ± 0.55	0.0001	
Apelin-13	Control	9.95 ± 0.05	9.99 ± 0.06	0.581	P = 0.0001
(pg/Ml)	Experimental	11.73 ± 1.89	19.98 ± 2.12	0.0001	

VEGF: Vascular endothelial growth factor. NO: Nitric oxide. FGF: Fibroblast growth factor.

Discussion

This study aimed to evaluate the effect of eight weeks of HIIT on angiogenesis indices in the elderly rats. The results of this study showed that eight weeks of HITT significantly increased apelin-13 levels in the aged male rats. The results of this study are consistent with the results of Farzanegi et al., (10). They showed that two methods of aerobic exercise increase the levels of apelin and its receptors in the heart tissue of aged rats with chronic kidney disease (10). It is also consistent with the results of Kazemi's study which examined the effect of eight weeks of moderate-intensity aerobic exercise on apelin in diabetic male rats (11). However, the results of this study were not consistent with the results of Azali et al. (12). The results of Azali et al. revealed, that eight weeks of aerobic exercise significantly reduces the level of apelin in middle-aged women (12). It is also inconsistent with the results of a study by Kazemi et al., that examined the effect of six weeks of HIIT training on the level of apelin in overweight boys (13). The most important reason for increasing apelin-13 in HITT is related to increased insulin sensitivity; apelin 13 increases insulin sensitivity, which leads to increased apelin, too (14).

Arteries and capillaries play a vital role in health by providing oxygen and nutrients to the metabolic tissues, and removing metabolic wastes. Normally, there is a balance between angiogenic and angiostatic factors. However, in physiological and pathological conditions, the balance is disturbed (15). In the present study, the effect of interval training on the angiogenesis impressive factors has been investigated. These factors can play an important role in the quality of life of the elderly. As mentioned above, eight weeks of aerobic exercise significantly increased the rate of angiogenic factors in aged men.

Studies in the field of sports have shown, that activities which have sufficient intensity and duration increase serum VEGF (16). In this regard, Gevin et al. reported that with increasing intensity of exercise, VEGF-mRNA levels increase further (17). Also, Lloyd et al. showed that angiogenesis increases in a relatively short period after exercise; so, the number of capillaries around each fiber increases to 0.025 in the Gastrocnemius muscleafter during 12 days of training (18). VEGF secreted in response to stimuli such as: ischemia, hypoxia, shear stress, and metabolites, including adenosine and lactate, and also vasodilators, such as NO, adipokine, platelets, and thymus (19).

Exercise-induced angiogenesis improves the transfer of oxygen and nutrients to the muscles by increasing capillary density in the muscle fibers. Increasing capillary density through increasing the level of diffusion, increases the time of exchange between blood and tissue, and reduces the diffusion distance, and provides more fatty acids (FFA); so that, muscle fibers can have access to FFA. Vascularization of the brain and heart tissues also reduces stroke and heart attack. Taheri et al. (2011) stated that a period of exhaustive exercise increases the serum VEGF protein content of the active men (20). Conversely, in another study, Ranjbar et al. reported, that there was no difference between the serum VEGF protein content of the active men and women at rest, and in response to the submaximal exercise (21).

Another variable of this study, that increases VEGF is NO. It secretes locally by the endothelium of muscle vessels and muscle fibers during contraction in response to the increased blood flow, or in other words, shear stress. The main source of NO production in endothelial cells is eNOS, which is activated during the exercise. Resistance training affects present mechanical sensors (G-protein) in endothelial cells membrane, and then activates eNOS and ultimately produces NO. During the early stages of angiogenesis, upregulation of VEGF depends on the shear stress and NO release; but in the later stages, NO involvement in the angiogenesis process is independent of VEGF (22). According to the studies by Carlos Alberto et al. (2016) performing HIIT increases NO and vasodilation in diabetic individuals (25).

Adenosine is another factor, that increases VEGF due to exercise. Adenosine is a product of ATP metabolism. In conditions of hypoxia or muscle contraction, a significant amount of adenosine is produced. Researches have shown, that increased adenosine increases muscle vasodilation, promotes energy balance, increases the expression of growth factors, and proliferation and migration of endothelial cells, and forms new blood vessels in various tissues (7).

Since exercise is associated with cyclic stretching and static stretching, it can be concluded, that in this study one of the reasons for angiogenic factors incerement is cyclic stretching and static stretching. Unlike shear stress, that only affects vascular endothelial cells, stretching activates a wide range of cells, including skeletal muscle myocytes, satellite cells, interstitial fibroblasts, vascular smooth muscle cells, pericytes, and endothelial cells. The secretion of angiogenic factors has been shown to vary depending on the type of applied stimulus, and the secretion of MMP causes formation of new blood vessels, only when the muscle is stretched (22).

The results of this study also showed, that eight weeks of HIIT increases the level of apelin-13 in aged male rats. Continuous moderate-intensity training may stimulate apelin-13 and its receptors by stimulating the cardiovascular system. Apelin connects to its receptors, phosphorylates eNOS through Akt activation, then releases NO through L-arginine, followed by an increase in cyclic guanosine monophosphate, and finally resulting in vasodilation (23).

Conclusion

In general, the results of the study showed, that HIIT increased the number of angiogenic factors. According to the above-mentioned reasons, due to the growth of the elderly population, it is important to pay attention to these people. Some researchers working in this field believe, that aging begins in people when muscle weakness or sarcopenia happens. Sarcopenia happens when less blood reaches the tissue due to the lack of angiogenesis. Therefore, according to the results of this study, HITT increases angiogenic factors.

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