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Research Paper

# The Effect of Eight Weeks of Resistance Training on Some of the Angiogenesis Indices in Elderly Men

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## ABSTRACT

**Background and Aim:** Aging is a process in which the physiological capacity of the body decreases continuously after the age of 30. One of these major changes in body mass (sarcopenia) is caused by a reduction in blood flow due to angiogenesis disorder. Therefore, the purpose of this study was to examine the effect of eight weeks of resistance training on some angiogenesis indices in elderly men.

**Materials and Methods:** The sample of this study was 30 elderly men (average age 61.65 and average weight 68 kg) who were randomly divided into control (15) and experimental (15) groups. The experimental group received 8 weeks of aerobic exercise, which included 55-50% MHR in the 1st week, with a high 80%-75% MHR in the eighth week. Studies were performed 48 hours before vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and nitric oxide (NO) and after the last session of the 5-cc protocol of the blood from the elderly's arms. To analyze the findings, independent and dependent T test was used for inter-group and intra-group evaluations. Statistical tests were performed using SPSS 16 software at a significance level of $\alpha=0.05$.

**Results:** Eight weeks of aerobic training significantly increased the levels of VEGF ($P=0.000$), NO ($P=0.000$), and FGF ($P=0.000$) in elderly men. There were no significant changes in the control group.

**Conclusion:** The results of this study showed that eight weeks of aerobic training increased significantly in the angiogenesis levels of the elderly. Therefore, aerobic exercise can be used as an appropriate method for increasing angiogenesis in the elderly.

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## 1. Introduction

Aging is one of the most prominent phenomena in recent years in the field of global health. Many nations consider the 21-century phenomenon to be the rapid growth of the elderly population. The world population of the elderly over 60 is estimated at more than 605 million and it is estimated that by 2050, this number will reach 2 billion, which is more than the growth of the child population. In developing countries such as Iran, the growth rate of the elderly population is higher than in developed countries [1]. More than 59% of the elderly population lives in developing countries and is estimated to reach 71% by 2030 [1]. With age, people's ability to function in daily life decreases [2]. Decreased physical activity is dangerous for the health of the elderly [3]. Aging is a physiological process that is influenced by environmental and genetic factors and the mechanisms involved are unknown [4].

Aging is a process in which the physiological capacity of the body continuously decreases after the age of 30. Demographic changes in the population showed that the number of people over 60 years old in Iran has increased and the structure of the age pyramid is inverted [5]. The inability to perform daily activities of life is the most important problem of this group. The percentage of disability increases with age. Among the factors that occur with aging in the aging process is the incidence of cardiovascular disease, so the rate of heart disease in men is five times higher than in women. Decreased arterial capacity and impaired vascular endothelium are two factors affecting vascular endothelium. As the body gets older, angiogenesis insufficiency increases with age, and vascular disorders increase [6]. Aging is associated with reduced genetic processes, mitochondrial degeneration, cell death, and loss of enzymatic activity. In particular, in some angiogenesis-related tissues, vascular endothelial growth factor (VEGF), which is the most basic and dominant regulator, decreases with age. Vascular disorders appear to be an important factor in the development of atherosclerosis, hypertension, and cardiovascular disorders in the elderly. Angiogenesis refers to the formation of new blood vessels from existing ones. With age, the body also develops atrophy, which causes imbalance and reduced quality of life. These symptoms are usually clearly related to the reduced blood supply and reduced angiogenesis [6].

VEGF with a molecular weight of 35 to 45 kDa is one of the most important angiogenic factors, which is the strongest growth mitogen for endothelial cells. Physical activity can be an important stimulus for VEGF. This stimulation of VEGF causes the growth, migration, and survival of endothelial cells and as a result, further expansion of the vascular network, but more stimulation of anti-angiogenic factors than angiogenic factors reduces the process of angiogenesis in old age, which has various pathological complications [7].

Clinical studies have shown the role of antiangiogens in the treatment of diseases of the elderly and now anti-angiogenic therapy is used as an effective treatment, of which endostatin treatment is one of the most effective. Antiangiogens are affected and reduced during exercise, which can increase physical strength in the elderly. Another factor in angiogenesis is nitric oxide (NO). NO is one of the substances secreted from vascular endothelium, which 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 a weight of 30 kDa, which is produced by the enzyme nitric oxide synthase from L-arginine [8]. Studies have shown that the greatest reduction in nitric oxide is at the age of 46 to 60 years, and inactivity and lack of exercise as risk factors increase this pathological condition in the elderly. Currently, regular and long-term exercise as a therapeutic intervention is used to treat many diseases, including cardiovascular disease, diabetes, and obesity in the elderly. Behjati et al. (2015) studied the effect of a resistance training course on older men. The results showed that resistance training increases the rate of angiogenesis in older men [6].

The effect of a course of aerobic exercise with cinnamon supplementation in male rats was also investigated. The results of this study showed that short-term exercise does not affect angiogenesis indices. Studies have shown that exercise can be a positive factor in stimulating the process of angiogenesis in physiological conditions, even in pathological conditions [9]. A sedentary lifestyle and lack of exercise as risk factors increase this pathological condition in elderly people [8]. Exercise increases mechanical blood flow, causes mechanical stimulation in the arteries, and healthy endothelial leads to increased production and release of nitric oxide [9]. Also one of the important indicators of angiogenesis in the elderly, which plays an important role in physiological conditions, is fibroblast growth factor (FGF), a protein that is involved in many cellular processes such as cell division, embryonic development, angiogenesis, and many other processes [10]. Blori et al. (2020) showed that continuous aerobic exercise increases FGF in elderly male mice [11]. Therefore, medical and sports experts believe that the elderly should do sports before getting sick. Therefore, the researcher intends to investigate the effect of eight weeks of aerobic exercise on some angiogenesis factors in elderly men.

## 2. Materials and Methods

The present study is a quasi-experimental study conducted in Varamin city. A total of 40 people with an average age of 65 to 60 years retired from education were selected according to the research criteria. The inclusion criteria included full consciousness, ability to answer questions, physical activity, ability to perform normal and daily activities of life without dependence on others, and lack of a history of regular exercise. The exclusion criteria included neurological disorders (stroke, Parkinson's disease), cardiovascular disorders, high and uncontrolled hypertension, respiratory disorders, and muscle dizziness during exercise. After coordination and initial studies, 62 people were eligible to participate in the study their phone numbers and address was registered and they were invited to participate in the study. Of these, 40 people agreed to cooperate and were met at the training site. On the appointed day, after providing the necessary explanations and explaining the goals and stages of the research, the consent form for participation in this research was completed by all participants and a 1st-class family member. Then, these people were randomly divided into two groups of control (15 people) and an aerobic exercise group (15 people). To homogenize the two groups before the interventions, they were compared based on age, height, weight, duration of disease, body mass index (BMI), and aerobic fitness and it was found that there is no significant difference between them (Table 1). All participants received written information about the research and after studying, they were asked to sign a written consent. The ethical licenses required to conduct this research were obtained from Qom University. Also, the present study was performed under the supervision of a physician and sports physiologists.

### Physiological tests and body composition

BMI was calculated as an overweight index by measuring weight and height at the beginning and end of 8 weeks. Body weight was measured by a digital scale with an accuracy of 0.1 kg at the beginning and end of 8 weeks. To determine the cardiovascular fitness of the subjects, 48 hours before and after the implementation of the protocol, the ACSM (2000) Rockworth walking test was calculated using a polar heart rate monitor and the related equation [11]:

(heart rate×1194/0)-(time×1/4537)-(gender×892/8) + (weight (pounds)×0957/0) -768/88=Vo2max oxygen consumption was considered as Vo2max of subjects in mL/kg/min.

### Exercise protocol

The experimental group performed aerobic exercise for 8 weeks and 3 sessions per week according to the protocol. The subjects' maximum heart rate was calculated using a formula (220-age) and then the subjects in the experimental group performed an exercise protocol that included running at 55% to 50% of their maximum heart rate for 30 minutes in the 1st week, up to 75. The 80% maximum heart rate (MHR) was reached for 60 minutes in the eighth week. At the end of the 1st 4 minutes, the MHR was recalculated and the endurance training program for the 2nd 4 weeks was designed based on the new MHR. Using the caronene formula, the heart rate equivalent to 70% of the reserve heart rate of each individual was calculated and provided to the subjects.

### Blood sampling and measurement of biochemical variables

To examine the research variables, a blood sample of 5 ccs was collected 48 hours before and after the last training session. Blood samples were centrifuged at 2000 RMP for 10 minutes to separate the serum. The serum was then distributed in special Eppendorf containers for further analysis of the indicators and immediately stored in a -80°C freezer. To measure VEGF, the American Sigma Aldrich kit with a sensitivity of 3.6 pg/mL and with an in-group sensitivity of 3.1%-3.5%, to measure NO, the measurement kit of German Booker company prepared by Padgin Teb Company with a sensitivity of 1.1 µmol/L and to measure FGF, Hangzhou China Company with a sensitivity of 0.39 pg/mL and with CV 3.8%-6.5% were used. All research variables were measured using ELISA STAT FAX 2100 made in Japan and with special kits.

### Data analysis

Data were analyzed at both descriptive and inferential levels. At the descriptive level, indicators such as Mean±SD were used. By the Kolmogorov-Smirnov test, the normality of data distribution at each factor level was investigated, and in the inferential statistics section, independent and dependent t-tests were used to analyze the findings for intergroup and intragroup evaluation. SPSS software version 16 was used for analysis and statistical tests at a significance level of $\alpha$=0.05.

**Table 1.** Comparison of changes in measured variables before and after eight weeks of aerobic exercise intervention.

| Variables | Groups | Pre-test | Post-test | P | |
|---|---|---|---|---|---|
| | | | | Within the Group | Between Groups |
| Weight | Control group | 80.15±3.25 | 81.15±3.75 | 0.0756 | 0.0621 |
| | Aerobic exercise group | 81.87±2.57 | 76.25±2.11 | 0.0557 | |
| BMI | Control group | 24.11±1.66 | 25.75±1.45 | 0.0714 | 0.0001* |
| | Aerobic exercise group | 25.35±1.74 | 23.24±1.1 | 0.0001 | |
| VEGF (pg/m) | control group | 129.15±9.11 | 128.08±8.55 | 0.0731 | 0.0001* |
| | Aerobic exercise group | 132.29±15.45 | 192.66±21.46 | 0.0001 | |
| NO (mL/L) | Control group | 13.1±2.1 | 13.9±2.17 | 0.0636 | 0.0001* |
| | Aerobic exercise group | 13.3±1.11 | 19.5±3.19 | 0.0001 | |
| FGF (pg/m) | Control group | 5.1±1.22 | 5.06±1.83 | 0.0713 | 0.0001* |
| | Aerobic exercise group | 5.3±2.33 | 9.2±2.16 | 0.0001 | |
| Vo2max | Control group | 22.19±1.9 | 22.18±1.3 | 0.0789 | 0.0001* |
| | Aerobic exercise group | 32.63±3.33 | 22.97±1.23 | 0.0001 | |



## 3. Results

This study aimed to evaluate the effect of eight weeks of aerobic exercise on some angiogenesis indices in elderly men. Biologically, increasing age is associated with a decrease in capacity to develop strength, and power, decreased cardiovascular, and respiratory fitness, and ultimately decreased functional capacity [12]. Decreased muscle mass, decreased endurance capacity, and muscle weakness in the aging process all lead to decreased physical activity and eventually lead to complications such as cardiovascular disease [13]. Studies have shown that one of the main reasons for these processes is the reduction of angiogenesis in the elderly [6]. Blood vessels and capillaries play a vital role in function and health by providing oxygen and nutrients to metabolic tissues and early metabolic wastes from these tissues. Normally, there is a balance between angiogenic and angiostatic factors. However, in physiological and pathological situations, the balance between these hormones is always disturbed [14].

In the present study, the effect of resistance training on the effective factors of angiogenesis has been investigated. These factors can have an effective role in the survival of the elderly and the quality of life of this segment of society. As mentioned above, 8 weeks of aerobic exercise significantly increased the rate of angiogenic factors in older men. The results of this study are consistent with the results of Nourshahi et al. which examined the effect of 6 weeks of endurance training on the level of VEGF and endostatin in tumor tissue in rats with breast cancer [15]. The results of this study are also consistent with the research of Nourshahi et al. who showed that eight weeks of endurance training on a treadmill reduced endostatin levels and increased VEGF in the serum of rats [16] and also with the results of Amani Shalamzari et al. The effect of endurance training on VEGF in mice with breast cancer is inconsistent [16].

The results of this study are inconsistent with the results of Jones et al. and Aghaalinejad et al. (2014). Jones et al. used human adenocarcinoma cell transplantation in mice. While in the present study, human samples did not have any disease. Also, the type of research protocol is different. The intensity of training in the above research is also different [17].

## 4. Discussion

One of the reasons for the increase in angiogenic factors in this study can be considered angiogenic stimuli. Angiogenic stimuli are a set of factors that stimulate the formation of new blood vessels. The most important of these hypoxia factors are hemodynamic forces, metabolites, vasodilators, muscle contraction, some cytokines, and types of stretching [8].

Concerning sports activity, studies have shown that activity that is of sufficient intensity and duration increases serum VEGF [18]. In this regard, Gwyn et al. reported that with increasing intensity of exercise, mRNA-VEGF levels increase further [19]. VEGF is secreted in response to stimuli such as ischemia, hypoxia, stress milk, metabolites such as adenosine, lactate, and vasodilators such as nitric oxide, adipokines, smooth muscle, platelets, and thymus [20]. 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 to 0.025 in the twin muscles after 12 days of training [21]. Exercise-induced angiogenesis improves the transfer of oxygen and nutrients to the muscle by increasing capillary density in muscle fibers. The increased vascular bed has special benefits for obese people. Increasing capillary knowledge by increasing the level of diffusion, increasing the exchange time between blood and tissue, and reducing the distance of diffusion cause more fatty acids (FFA) to be called from adipose tissue and more muscle fiber to have access to FFA [22]. Vascularity of brain and heart tissue also reduces stroke and heart attack, respectively [23]. Taheri et al. also stated that one round of strenuous exercise increases the serum VEGF protein content of active men [24].

Hussain et al. also showed that swimming exercises for eight weeks significantly increased the amount of VEGF protein in the heart muscle of rats [25]. Studies in both humans and animals have shown positive changes in the VEGF factor following exercise. Haier et al. conducted a study entitled "Response of protein and anti-angiogenic factors of human skeletal muscle to acute exercise and training". This study investigated the effect of acute training and 4 weeks of aerobic training on skeletal muscle gene and protein expression of protein and angiogenic factors in 14 young men. The exercise included 60 minutes of cycling (60% of maximum oxygen consumption) 3 times a week. Intramuscular fluid was collected at the beginning of the 1$^{st}$ week and the end of the 4$^{th}$ week. Exercise increased capillary capacity and interstitial VEGF concentrations increased similarly before and after exercise in response to acute exercise. TSP-1 and matrix tissue inhibitor metalloproteinase-1 (TIMP1) were not affected by muscle exercise, while endothelial nitric oxide synthase protein levels were increased by approximately 50%. As a result, acute exercise causes a similar increase in protein gene expression and angiogenic factors in trained and untrained muscles [25].

In another study, Ranjbar et al. reported that there was no difference between the serum VEGF protein content of active men and women at rest and in response to sub-maximal exercise. There was also no difference between the serum VEGF protein content of inactive men and women at rest and in response to sub-maximal exercise [26]. Since sub-maximal exercise activity temporarily reduces the main factor involved in angiogenesis in healthy and inactive men, it is possible that low-intensity exercise may not be the main factor involved in angiogenesis such as tissue hypoxia and stress milk. In addition, this decrease may be due to the binding of serum VEGF to its receptors [26] and the findings of the present study are inconsistent with the results of Brexion et al. (2008), Prior et al. [10]. VEGF levels in obese older men do not change after several months of regular aerobic physical activity. The reason for this discrepancy can be due to the type of subjects and the duration. Brixon used elderly people in his study. Also, the duration of their training protocol was longer [26]. In this study, however, rats were used and the duration of the training protocol was shorter than in Brixon's study.

Contrary to the results of the present study, Prior et al. reported that one training session (one hour of training on a treadmill at a speed of 18 m/min and a slope of 10 degrees) could not increase angiogenesis activity without a change in capillary density, lack of time and intensity. Exercise can be predicted [27]. Another variable in this study that has increased VEGF is NO. NO is secreted locally by the endothelium of muscle vessels and muscle fibers during contraction in response to high blood flow or, in other words, increased stress milk. The main source of NO production in endothelial cells is eNOS, which is activated during exercise and stress milk. Resistance training by activating mechanical sensors (G-protein) present in the endothelial cell membrane activates eNOS and ultimately produces NO. During the early stages of angiogenesis, the upregulation of VEGF is dependent on stress release and NO release, but in the later stages of angiogenesis, NO involvement in the angiogenesis process is independent of VEGF [8].

One of the reasons for the increase in VEGF due to aerobic exercise is the increase in adenosine. Adenosine is a product of ATP metabolism. In conditions of hypoxia or muscle contraction, a significant amount of adenosine is produced. Research has shown that increased adenosine increases muscle vasodilation, promotes energy balance, increases the expression of growth factors, increases the pro-

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liferation and migration of endothelial cells, and ultimately the formation of new blood vessels in various tissues [7]. Eder et al. showed that 50% to 70% of the angiogenic response in hypoxia is mediated by adenosine. Concerning other metabolites, since resistance training produces some lactate, lactate production promotes collagen synthesis by stimulating the production of VEGF and fibroblasts by endothelial cells and macrophages. In this regard, Beckett et al. showed that lactate indirectly mediates endothelial cell migration by increasing VEGF production. Since aerobic exercise is associated with cyclic stretching and static stretching, it can be concluded that the causes of increased angiogenic factors in this study are cyclic stretching and static stretching. In contrast, stress milk, which affects only vascular endothelial cells, exerts a load or stretch that activates a range of cells, including skeletal muscle myocytes, satellite cells, interstitial fibroblasts, vascular smooth muscle cells, and pericytes to become endothelial cells.

The secretion of angiogenic factors has been shown to vary depending on the type of stimulus applied, and the secretion of MMP levels only results in the formation of new arteries through germination when the muscle is stretched [28]. In general, skeletal muscle contraction is one of the mechanical forces outside the arteries that stimulates the angiogenesis process in skeletal muscle independently of increased muscle blood flow. Therefore, skeletal muscle contractions in power movements can be the cause of angiogenesis in these people [17].

## 5. Conclusion

As stated in the result, aerobic exercise has increased the amount of angiogenic factors. Due to the increase in the number of elderly people in society, there is a need for more importance and attention to these people. Less blood begins to reach the tissue due to a lack of angiogenesis. Therefore, according to the results of this study that aerobic exercise increases angiogenesis factors, it is recommended that older people do aerobic exercise in the range of intensity to increase their health.

## Ethical Considerations

### Compliance with ethical guidelines

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### Authors' contributions

All authors contributed equally to preparing this article.

### Conflict of interest

The authors declare no conflict of interest.

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