Effects of 8-week Aerobic Exercise Training on Vascular Endothelial Growth Factor among Postmenopausal Women

Elham Shakoor 1, Ahmad Qassemian*1, Maryam Koushkie Jahromi 1, Ahmad Mehrez 1

1 Department of Sport Physiology, School of Physical Education and Sport Sciences, Shiraz University, Shiraz, Iran

*Corresponding Author:
Address: Ahmad Qassemian, Department of Sport Physiology, School of Physical Education and Sport Sciences, Shiraz University, Shiraz, Iran.
Email: ahmadqassemian@gmail.com

ABSTRACT:
Introduction: Vascular endothelial growth factor is essential for health of vascular wall integrity and vasomotor tone and sedentary postmenopausal women suffer from vascular endothelial dysfunction and have a higher risk of cardiovascular disease. Objective: The aim of this study was to evaluate the effect of a 8-week exercise training on plasma concentration of vascular endothelial growth factor and its relationship with blood pressure in postmenopausal women.
Method: twenty four postmenopausal women [mean age of 67.85±5.67 years, height of 153.50±7.70 cm, weight of 66.16±11.96 kg, BMI of 28.15±4.98] were purposefully selected and randomly divided into two control [n=12] and experimental [n=12] groups. The experimental group performed eight weeks of aerobic training with 60-70% of maximum heart rate three days a week. The level of vascular endothelial growth factor at rest as well as systolic and diastolic blood pressure was measured and recorded before and after eight weeks of training. Paired t-test was used to evaluate intra group variance, independent t-test to assess inter group variance.
Results: The results showed that 8-week exercise training significantly [p=0.026] reduce plasma concentration of vascular endothelial growth factor in post-menopausal women. In addition, aerobic training significantly reduced the systolic [p=0.04] and diastolic [p=0.001] blood pressure in postmenopausal women. However, there was no significant correlation between concentration of vascular endothelial growth factor with systolic and diastolic blood pressure.
Conclusion: It seems that a period of 8-week exercise training is effective in reduction of blood pressure as well as concentration of plasma vascular endothelial growth factor [cardiovascular disease risk factors], in postmenopausal women.

KEYWORDS: Aerobic Training, Vascular Endothelial Growth Factor, Postmenopausal Women, Elderly
INTRODUCTION

The elderly population is rapidly increasing in the world, especially in developing countries [1]. The growth rate of elderly population in developing countries, including Iran, is higher than the developed countries [2]. The population of aged people is rapidly increasing in our country and there are currently five million and one hundred thousand elders in Iran [3]. The high cost of health care is a rapidly growing overall concern of aging population [4]. Menopause and subsequent loss of steroid sex hormones as well as increased body fat, especially abdominal fat, has increased the possibility of cardiovascular and metabolic diseases in women [5]. In general, the risk factors of cardiovascular diseases are increased with increasing age and obesity [6]. Aging impairs the endothelial function in aorta and lowers the resistance of vessels [7]. Changing endothelial function with aging may have important clinical implications in development of cardiovascular diseases [8].

Due to the advancement of technology and its influence on human life, humans have been faced with the problem of poor physical activity, which is associated with several troubles in old age. Daily training programs can contribute to performance of adults in old age [9] and can reinforce muscle strength, skeletal muscle and bone mass in old age, improve physical performance [10] and prevent acute and infectious diseases associated with aging [2]. Exercise training is associated with increased strength [11], maintenance of muscle mass [12], functional capacity [13] as well as reduced cardiovascular risk factors [14] and plays an important role in maintaining health [15]. During the last decade, aerobic and resistance exercise have been used as common training methods for improvement of physical fitness [16], performance [17], prevention of injury and increase in muscle size [18].

Despite considerable progress in medical science, atherosclerosis is still a main cause of the majority of cardiovascular diseases [19]. Carotid arteries are among the vessels subject to atherosclerosis along with coronary arteries. Progressive atherosclerosis leads to impaired blood supply to the brain, increasing the level of different vasoconstrictors such as endothelin in these arteries, which eventually contributes to the development of atherosclerosis [20]. Study of Wen et al, demonstrated effect of aerobic exercise on bone metabolism and functional fitness of postmenopausal women [21]. Vascular endothelial growth factor (VEGF) is a 45 kDa homodimer heparin-binding protein with proangiogenic activity both in vivo and in vitro [16]. VEGF has seven isoforms of A, B, C, D, E, F and PIGF that are generated due to different splicing of VEGF gene. These isoforms are different in terms of molecular weight and biological properties [17]. It seems that VEGFA is the most important isoform performing the functions of VEGF. Overexpression of this isoform results in potent angiogenic effects in different target tissues, increasing vascular permeability and vasodilation [18]. VEGF is involved in migration, proliferation, matrix degradation, formation of vascular network as well as generation and release of nitric oxide in endothelial cells. In addition, it exerts an anti-apoptotic effect on endothelial cells, resulting in the expression of BCL2 anti-apoptotic protein in these cells [19]. VEGF is one of the most important specific regulators of angiogenesis. The biological function of vascular endothelial growth factor on target cells is mediated via interaction with tyrosine kinase receptors on plasma membrane of the cell. These receptors are dimerized and autophosphorylated following binding with their ligands, which ultimately generates an intracellular cascade. VEGF-A is the main angiogenic factor exerting its effect via activation of VEGFR-1 and VEGFR-2 receptors [20]. VEGF activates the synthesis of nitric oxide via phosphorylation of AKT as well as phosphorylation of protein kinase activator of AMP [21].
Effects of Aerobic Exercise Training on VEGF among Postmenopausal Women

Various factors affect the level of VEGF production, including hypoxia, shear stress, muscle contraction and tension, decreased blood glucose level, several cytokines as well as HIF-1 [Hypoxia Inducible Factor]. Research has indicated that cytokines such as IL-10 and IL-13 could inhibit VEGF release [22, 23]. VEGF promotes the growth, proliferation, survival and migration of endothelial cells as well as increased vascular permeability via receptor tyrosine kinases [VEGFR-1, VEGFR-2, VEGFR-3] [24, 25].

The physical exercises stimulate the angiogenesis process [26]. Aerobic exercises is able to stimulate the angiogenesis process by increasing the expression of angiogenic factors such as VEGF as well as reducing the expression of angiostatic factors like endostatin [27]. On the other hand, there are conflicting results concerning the effect of exercise on serum VEGF level. Otrock et al [2007] showed that serum VEGF level is increased following exercise [28] while Kojda et al [2005] reported no change in serum VEGF concentration after exercise [29]. The study of Vincenti et al [1996] indicated decreased VEGF serum concentration after exercise [30]. Due to limited and inconsistent results on the effects of exercise training on concentration of vascular endothelial growth factor, this study aimed to assess the impact of aerobic exercise training on vascular endothelial growth factor concentration in elderly women.

METHODS

Research designs and participation

Twenty four 20 postmenopausal women [mean age of 67.85±5.67 years, height of 153.50±7.70 cm, weight of 66.16±11.96 kg, BMI of 28.15±4.98] participated in this study. The inclusion criteria were: postmenopausal women age 60 years and above, the participants without history of regular exercise and able to do low level of aerobic exercises. The exclusion criteria were: cardiovascular problem and musculoskeletal disorders. After filling out the consent form to participate in the study, the subjects were randomly divided into two groups: aerobic exercise training group [n=10] and control group [n=10]. General health, physical activity and medical history questionnaire was completed by subjects to assess their preliminary situation. The weight and height of subjects was measured and body mass index was determined by the following formula:

\[
BMI = \frac{weight\ (KG)}{square\ height\ (M)}
\]

Exercise protocol

The intervention group performed eight weeks of aerobic training with 60-70% of maximum heart rate three days a week. All sessions of exercises were started with 10 minutes warming up on treadmill and upper and lower body stretching program. with 60-70% of maximum heart rate to responds the nerve signals for quick and efficient action[31]; and end of exercise participants were performed cool-down program such as stretching, running with a slow Pace, shaking and arm, and walking [32]. The principle of overload was observed as follows: first week training session for 20 minutes and exercise intensity of 60% maximum heart rate, second week with the same intensity and duration of 22 minutes, third, fourth and fifth weeks with intensity of 65% maximum heart rate within 24, 26 and 28 minutes, respectively, sixth and seventh weeks with 70% intensity within 30 and 34 minutes, respectively and finally the eighth and final week at 75% intensity within 40 minutes. In each session, there were two separate warm-up and cool-down rounds each lasting 10 minutes. It should be noted that the exercise protocol of this study was designed according to Bagheri et al protocol [2009] [9] that was used in the elderly. Specific recommendations of American College of Sports Medicine [ACSM] for older people were also considered in this study [33].
All the stages of training were performed under direct supervision of female fitness and bodybuilding educators. To obtain maximum heart rate, the value of 220 was subtracted from the age of subjects and Karonen method was used to determine the exercise intensity at each training session. Rockport 1 mile walk test was used to obtain maximum oxygen consumption. This test has been reported as an appropriate valid approach to measure the aerobic capacity of the elderly given the specific conditions of the majority of older people. The subjects walked a mile with maximum power and then their heart beat was measured. Their heart rate was determined and maximum oxygen consumption was calculated as follows [3]:

\[
\text{VO}_2\text{max} [\text{L/M}] = (\text{final heart rate} \times 0.0115) - [0.2240 \times \text{time in minute}] - [0.5955 \times \text{gender}] + [0.0275 \times \text{age}] - [\text{weight per LB} \times 0.0091] + 6.9652
\]

**Test procedure**

To measure of vascular endothelial growth factor, fourthly eighth hours before and eight weeks after exercise training, 5 ml venous blood was taken from antecubital vein of subjects. Blood samples were collected at 8 AM after 12 hours of fasting in physician’s room of Elderly Home. Blood samples taken before the exercise program were immediately sent to laboratory in order to separate plasma by centrifuge in accordance with kit instructions to be stored at -70°C. After drawing blood samples following eight weeks of aerobic training and separation of plasma, the samples were collectively sent to clinical laboratory for analysis and measurement of plasma VEGF concentration using ELISA kit of US BOSTER Company with an accuracy of 0.1 picogram per milliliter [pg/ml].

Blood pressure was measured before and after exercise. Exercise HRs was verified periodically during each training session. Blood pressure measurements were taken every other minute using a standard sphygmanometer.

**Data analysis**

Mean and standard deviation of variables were calculated and recorded using descriptive statistics. Paired t-test was used to examine the effect of 8-week exercise training on variables within each group. The Shapiro-Wilk test was used to assess the normality of data. Data analysis was done using SPSS software version 22. The results were evaluated at significance level of p<0.05.

**STATISTICAL RESULTS**

The characteristics of participants are illustrated in Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>66.1±11.9</td>
</tr>
<tr>
<td>Age</td>
<td>67.8±5.6</td>
</tr>
<tr>
<td>Height</td>
<td>153.5±7.7</td>
</tr>
<tr>
<td>BMI</td>
<td>28.1±4.9</td>
</tr>
</tbody>
</table>
Table 2: Pre and post-test for VEGF concentrations, systolic and diastolic Blood pressure for both groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Experimental group</th>
<th>Control Group</th>
<th>P Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre</td>
<td>post</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>131.3±15.8</td>
<td>126.2±11.1</td>
<td>0.04</td>
<td>138.5±9.8</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>85.80±3.5</td>
<td>80.6±1.9</td>
<td>0.001</td>
<td>87.6±5.4</td>
</tr>
<tr>
<td>VEGF concentration</td>
<td>31.56±3.9</td>
<td>27.2±2.7</td>
<td>0.026</td>
<td>29.4±2.9</td>
</tr>
</tbody>
</table>

According to the Table 2, P value in the experimental group equals 0.026. As can be seen in the Table, since P<0.05, mean difference of VEGF concentration in pre- and post-test is significant in the experimental group. While in the control group, p=0.784, which is higher than the level of significance [α=0.05], indicating that there is no significant difference between pre- and post-test. The results of the independent t-test to compare the "pre-test and post-test VEGF concentration difference" between experimental and control groups is shown in Table 2. In other words, the independent variable [aerobic exercise training] has had a significant impact in reducing the concentration of VEGF in plasma of the experimental group. Therefore, VEGF plasma concentration has been significantly decreased following eight weeks of aerobic training.

DISCUSSION

The results of this study showed that eight weeks of aerobic exercise training had a significant impact on reducing the plasma concentration of vascular endothelial growth factor in elderly women. There have been contradictory results on the effect of exercise on serum VEGF Concentration. Van Craenenbroeck in 2008 demonstrated that serum VEGF concentration was increased following intensive exercise. This exercise was performed on bicycle ergometer and the results closely approached the significance level while none of the VEGF levels remained unchanged and the change was visible at all levels [34]. However, Suhr et al [2007] reported no change in serum VEGF concentration following intensive physical activity [23]. The subjects were 12 male cyclists and each subject performed 4 cycling sessions under normal conditions [NC] without vibration [fluctuation], normal conditions with fluctuation, normobaric hypoxic conditions without vibration, and normobaric hypoxic conditions with fluctuation. Each session lasted 90 minutes and the sessions were randomly distributed during the week days. The results showed significant increase in VEGF level immediately after exercise only in fluctuations induced from outside [34]. On the other hand, Gu et al [2004] reported decreased concentrations of serum VEGF. They also found that exercise increases the level of circulating endostatin in bloodstream of healthy volunteers. The exercise was performed on treadmill and VEGF level was decreased within half an hour, 2 hours and 6 hours after training [41].
Malamitsi et al [2000] demonstrated that serum VEGF level was higher in women than men during their lifetime. The results showed that women had a higher serum VEGF level than men. Women in all age groups showed a higher VEGF level compared to men, although VEGF level did not differ in their secretory phases [42]. However, Scott et al. [2005] as well as Kraus et al [2004] showed that plasma VEGF level was not different between active and inactive men at rest and in response to exercise. The results showed that high-intensity training in male athletes undergoing resistance training significantly increased VEGF levels in male athletes immediately after training and one hour after exercise [12,38]. However, no increase was observed in VEGF levels at any point in time in sedentary men [12]. In a study measuring VEGF level, Kraus et al showed increased VEGF level after 2 and 4 hours of physical activity in active and inactive people [38]. The difference in results can be attributed to the intensity, duration and type of physical exercise, nutrition of subjects, and preparation level of subjects, individual differences and age of subjects.

Reduced level of serum vascular endothelial growth factor following exercise training does not mean that physical exercise can reduce the production of vascular endothelial growth factor, but it is possible that exercise-induced temporary reduction in vascular endothelial growth factor is due to vascular endothelial growth factor binding to receptors on endothelial cells, which is a stimulus for angiogenesis in skeletal and cardiac muscle [39]. It has been shown that VEGF transcription level in skeletal muscle is the most important factor in regulation of serum VEGF after two hours of activity [40]. Furthermore, increased serum VEGF level two hours after exercise may be due to mobilization of skeletal muscle VEGF into the bloodstream. This study for the first time showed that VEGF is present in human skeletal muscle and that the exercise increases the concentration of VEGF as well as other factors such as unidentified angiogenic factors.

In addition, reduced VEGF level may be due to binding with other proteins, including heparin sulfate [42] and EPC [Endothelial progenitor cell] [43]. Suher et al [2007] showed that VEGF plays an important role in recruitment of EPC from the bone marrow [41]. It should be noted that EPC level will be increased following exercise. Therefore, decrease in VEGF level is likely to be due to increased endostatin level [43].

CONCLUSION

The results of this study showed that a period of 8-week exercise training is effective in reduction of blood pressure as well as concentration of plasma vascular endothelial growth factor [cardiovascular disease risk factors], in postmenopausal women, and can be used a practical way to improve the health level of this population.
REFERENCES


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