Angiogenesis and Nitric Oxide in Elderly Men after Continuous and Interval Aerobic Training

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Introduction

Aerobic trainings, which are especially prescribed by healthcare workers for keeping healthy and treating metabolic diseases, cause an increase in the capillary density (3). The vascular structure of the skeletal muscles changes to provide the

Abstract

Objective: The reasons that some of these studies have offered for aerobic training’s lack of tangible effect on the level of angiogenesis, are subjects’ readiness and the conditions of their diseases, while others have detected the intensity and the frequency of doing the aerobic trainings as the factors preventing the effectiveness of them on the level of angiogenesis. Thus, the present study studied the effects of taking interval and continuous aerobic trainings on the degrees of angiogenesis and nitric oxide.

Materials and Methods: Sixty eligible old men in Kahrizak nursing home among volunteers were selected and studied in three groups (Continuous, Interval and Control). First, the initial blood samples were gathered to record of serum WEGF by ELISA kit. In the second stage NO in saliva were measured by conversion of nitrate to nitrite and by Griess reaction. After the 48 hours, experimental group in both continuous and interval carried out training for 6 weeks and the control group continued their daily activities. Post-test data measured and recorded in the same conditions with the pre-test. Obtained data were analyzed by using one-way ANOVA, Scheffe post hoc and dependent T-test.

Results: The results showed that WEGF serum and salivary NO levels after performing the aerobic training to both continuous and interval methods was not any significantly different (P>0.05). However, the percentage change recorded data for the experimental group than in the control group despite the lack of significant difference of nearly a significant increase in the WEGF serum and saliva levels of NO were observed (No: Continuous and Interval≈ 0/05 increase, Control: 0/01 increase, VEGF: Continuous= 0/03 Increase, Interval= 0/05 Increase, Control= 0/02 Decrease). According to the paradox observed in obese and diabetes subjects can be said that apply the aerobic training in elderly subjects with such conditions requires specific design and likely to be that the implementation of traditional aerobic trainings will not have a significant effect on the WEGF serum and NO saliva levels.

Conclusion: In the present study, we found that doing aerobics training in both continuous and interval courses had no significant effects on the level of angiogenesis and nitric oxide in the old men under study. However, we can maintain that taking aerobic trainings by subjects who suffer from diabetes, hypertension and obesity, based on results from previous studies has no positive effects. Patients suffering from diabetes, hypertension and obesity would see no significant change in their angiogenesis rate after doing continuous or interval aerobic trainings.

Keywords: WEGF, NO, Continuous aerobic training, Interval aerobic training
muscles needs. Two main arteriogenesis and angiogenesis changes take place in the vascular structure of the skeletal muscles. The changes cause decrease or removal of the conditions caused by the intensity and time of doing sports (4). Arteriogenesis is the increase of the distal veins size and numbers. This process involves the growth of present arthritis and also demands the duplication of smooth and endothelial muscles of blood vessels (5). As some sources make plain, tensile force is the main stimulant for arteriogenesis (6).

Angiogenesis, on the other hand, is the growth and appearance of new capillaries in the muscles. This process is done along with the duplication and move of endothelial cells, and it takes place by the duplication or sprouting of the capillaries present in the tissues (7). Increase of capillaries is a complex process which involves the inter-involvement of cells, signaling routs, growth factors, and receptors (8).

Certain angiogenic agents were so far known among which the most important ones are acidic fibroblast growth factor (AFGF), basic fibroblast growth factor (BFGF), transforming growth factor-a (TGF-α), transforming growth factor-β (TGF-β), hepatocyte growth factor, tumor necrosis factor-a (TNF-α), angiogenin, and interleukin-8 (IL-8). Past studies have all pointed out the main role of vascular endothelial growth factor (VEGF) in producing the normal or abnormal angiogenesis. Thus, among the angiogenic regulating factors, VEGF is known to be the strongest mitogen specific to the endothelial cells (9). In less recent studies, it was documented that taking regular aerobic trainings would slow down the decline in the functioning of physiologic organs of the body, and thus, would increase human health and life expectancy (12,13). About the effects of taking aerobic trainings on angiogenesis, previous studies showed contradictory results. While some recent studies reported the positive effects of taking aerobics trainings on angiogenesis (14), others have denounced the possible effects of such trainings on the level of angiogenesis (5). The reasons that some of these studies have offered for aerobic training’s lack of tangible effect on the level of angiogenesis, are subjects’ readiness and the conditions of their diseases, while others have detected the intensity and the frequency of doing the aerobic trainings as the factors preventing the effectiveness of them on the level of angiogenesis. Thus, the present study studied the effects of taking interval and continuous aerobic trainings on the degrees of angiogenesis and nitric oxide.

Materials and Methods
The present study is a semi-experimental based on a pre-test and post-test comparison. It has been conduct on two experimental groups and control. Sixty old men were randomly selected from Kahrizak nursing home residents to participate in three 20-members groups. Group 1: continuous aerobic training, group 2: interval aerobic training and group 3: control. The subjects were first asked to participate in a short briefing class. They were then asked to give a written consent as to their voluntary participation in the experiment, to fill out a form about their qualifications and health status regarding any previous case of disease (physical or mental). In the brief cession, information about the aims, and methods of conducting this research, kinds of food to be used by the subjects, and blood sampling were given to them.

In the first stage of the study, subjects were directed to sit on a chair for 30 minutes before the first blood sampling was done from the antecubital vein so as to measure the level of VEGF through the use of ELISA kit. In the second stage, the amount of NO in the saliva from the subjects’ mouths was measured through changing nitrate to nitrite and based on the Griess reaction. Anthropometric measurements (height, weight, subcutaneous fat around the chest, stomach and thighs through Jackson and Pollack method) were done. Their physiological cardiovascular studies (maximum heartbeat rate, maximum oxygen intake) were also measured and
recorded. After 48 hours form the first blood sampling, the two training groups were led to the chosen site for taking the aerobic trainings, while the controls went back to their routine life.

The final protocol of continuous aerobic training was designed as three following steps (table 1):

1- The trainings were safe and practicable for the elderly subjects of this study.

2- For adding up the load of the trainings, first the size then the intensity of them were increased.

3- The basic formation of the protocol was based on the previous studies (15-17).

Table 2 showed the Interval aerobic trainings course.

At the beginning of the trainings, both groups of continuous and interval aerobic subjects were led to take a 10-minute jogging and extension as a warm-up, and for increasing the heartbeat rate at the end of training sessions. Also, 5-minute extension training was done for cooling down and decreasing the heartbeat rate.

The blood samples were taken to the laboratory for the study of endocrine glands and metabolism for isolating serum and measuring the primary factors of the endothelial growth of arteries. The samples were kept at –80 degrees centigrade and were then centrifuged by Eppendorf machine for ten minutes and with 3000 RPM velocity, in 4 degrees centigrade. For analyzing the data of VEGF, the China-made (by USCN Life Science Institute) ELISA Kit was used. For measuring the general thickness of NO in the saliva, the Enzo No parameter assay kit was applied based on Griess reaction, through which NO was analyzed by detecting the enzyme changes of nitrate to nitrite and at the presence of reeducates enzyme (18).

First, for finding about normality of the data, the Kolmogorov-Smirnov test was applied. After that, the data achieved in pre-test for each of the dependent variables among the three groups were compared by the simple ANOVA test. Whenever the pre-test data were found to be insignificant, and for the simplicity of comparing the effects of the two training methods, the post-test data were first subtracted from the pre-test data and their differential was recorded as D (difference). Using the one-way ANOVA test and Scheffe post hoc test, the differences between the data from pre and post-tests of the three groups of the subjects for each of the dependent factors, were compared. And finally, for finding about the meaningfulness of the pre- and post-test data, the t test was employed. All statistical analyses were done in SPSS version 22, with \( \alpha \leq 0.05 \) of meaningfulness level.

**Results**

Table 3 presents the personal features of the subjects such as age, weight, height etc. Using the one-way ANOVA test, it was found that there were no significant differences between the personal features of the subjects from the three groups in pre-test. Table 4 presents the recorded data for NO variables and VEGF in both pre and post-test sessions.

For the statistical analysis of the data, first Kolmogorov-Simonov test was used for making out the normality of the data distribution and then the variance homogeneity test of Levine was used for attesting their homogeneity.

<table>
<thead>
<tr>
<th>Time</th>
<th>Onset week</th>
<th>Two week</th>
<th>Three week</th>
<th>Four week</th>
<th>Five week</th>
<th>Six week</th>
</tr>
</thead>
</table>

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<tr>
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<th>Onset week</th>
<th>Two week</th>
<th>Three week</th>
<th>Four week</th>
<th>Five week</th>
<th>Six week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training</td>
<td>4 sets Running 5 min with 55% Intensity</td>
<td>5 sets Running 5 min with 55% Intensity</td>
<td>6 sets Running 5 min with 55% Intensity</td>
<td>6 sets Running 5 min with 60% Intensity</td>
<td>6 sets Running 5 min with 65% Intensity</td>
<td>6 sets Running 5 min with 70% Intensity</td>
</tr>
</tbody>
</table>
For deciding about the differences between the data from pre and posttest stages of the study, the dependent T-test was used the results of which are given below:

Upon using the paired T-test for determining the differences between the means of VEGF in the subjects of continuous aerobic training group in the pre and posttest stages it was found that there was no significant differences between the pre and posttest data ($t=1/361$, $P=0.195$).

Upon using the paired t test for determining the differences between the mean degrees of nitric oxide in the subjects of continuous aerobic training group in the pre and posttest stages it was found that there was no significant differences between the pre and posttest data ($t=1/578$, $P=0.137$). Thus, by refuting the assumption of the study, the zero assumption was approved of based on which doing continuous aerobic training has no effect on the nitric oxide in the subjects.

Upon applying the paired t test for determining the differences between the pre and posttest mean degrees of VEGF in the subjects of interval aerobic training group, it was found that there was no significant differences between the pre and posttest data ($t= 1/141$, $P=0.272$). Thus, doing interval aerobic training has no significant effects on WEGF.

After applying the paired t test for determining the differences between the pre and posttest mean degrees of NO variable in the subjects of interval aerobic training group, it was found that there was no significant differences between the pre and posttest data ($t=1/731$, $P=0.104$). Thus, upon denouncing the possible effects of interval aerobic training on NO, the zero assumption was approved.

For testing the difference in the effects of interval and continuous aerobic trainings on the degree of VEGF, the values achieved in posttest were subtracted from those of pretest and the received differential was recorded as D. therefore, by having 3 different groups in the columns of the table and only one row for D, the one-way ANOVA test was used for statistical analysis.

Using the one-way F test for the VEGF variable of the subjects showed that there is no significant difference among the three continuous, interval and control groups in VEGF ($F=2/879$, $P=0.067$). Recorded data of VEGF for none of the subjects in the three groups had significant differences in pre and posttest stages of study.

For testing the difference in the effects of interval and continuous aerobic trainings on nitric oxide, the values achieved in posttest were subtracted from those of pretest and the received differential was recorded as D. therefore, by having 3 different groups in the columns of the table and only one row for D, the one-way ANOVA test was used for

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Table 3. The personal features of the subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Characteristics</th>
<th>Interval Training ($n=16$)</th>
<th>Continuous Training ($n=15$)</th>
<th>Control ($n=18$)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Year)</td>
<td>59.40± 7.17</td>
<td>61.13± 5.59</td>
<td>61.80± 6.73</td>
<td>0.065</td>
<td></td>
</tr>
<tr>
<td>Height (Cm)</td>
<td>173.20± 5.18</td>
<td>175.53± 5.69</td>
<td>176.13± 3.85</td>
<td>0.081</td>
<td></td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>81.66± 5.86</td>
<td>84.33± 5.53</td>
<td>85.33± 4.71</td>
<td>0.132</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>27.34± 3.65</td>
<td>28.52± 4.12</td>
<td>25.31± 2.86</td>
<td>0.417</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Mean and standard deviation of the recorded data for NO and VEGF variables in pre and post-test stages

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Pre Test</th>
<th>Post Test</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>NO</td>
<td>740±107</td>
<td>775±102</td>
<td>0/137</td>
</tr>
<tr>
<td></td>
<td>VEGF</td>
<td>82.86±7.56</td>
<td>85.60±6.93</td>
<td>0/195</td>
</tr>
<tr>
<td>Interval</td>
<td>NO</td>
<td>739±104</td>
<td>773±107</td>
<td>0/104</td>
</tr>
<tr>
<td></td>
<td>VEGF</td>
<td>84.43±7.95</td>
<td>88.63±6.23</td>
<td>0/272</td>
</tr>
<tr>
<td>Control</td>
<td>NO</td>
<td>76±85</td>
<td>709±92</td>
<td>0/666</td>
</tr>
<tr>
<td></td>
<td>VEGF</td>
<td>86.29±8.72</td>
<td>84.17±7.91</td>
<td>0/204</td>
</tr>
</tbody>
</table>
statistical analysis. Using the one-way F test for the NO variable of the subjects showed that there was no significant difference among the three continuous, interval and witness groups (F=16/869, P=0.102). Recorded data of NO for none of the subjects in the three groups had any significant differences in pre and posttest stages of study.

In figure 1, the changes of NO in the three groups of continuous, interval and witness in pre and posttest times are presented. In figure 2 the changes of WEGF in the three groups of continuous, interval and witness in pre and posttest times are presented.

Discussion
The present study investigated the degrees of angiogenesis and nitric oxide in old men after taking a term of continuous and interval aerobic trainings. To this end, the subjects were selected from Kahrizak nursing house and were divided into the three groups of continuous, interval and control. Results from our study showed that taking aerobic training courses in either continuous or interval formats had no significant effects on VEGF and NO among the subjects.

In past studies, it was claimed that the effects of aerobic trainings on angiogenesis is affected by the health conditions of the subjects such as obesity and diabetes. Brixius et al. (2007), for instance, have studied the responses of VEGF in a group of inactive obese men (50 to 60 years old). The project was done during a 6-month endurance training course. The first group undertook a running training of 3 times a week, each session for 60 minutes. The second study group did the bicycling training for 3 times a week each time for 90 minutes.

Results from this study showed that endurance trainings had no effect on the VEGF plasma
level in three groups of running (1.3 versus 1.5 ng/ml), biking (1.6 versus 1.5 ng/ml) and control (2.5 to 2.1 ng/ml). Thus, it was found that endurance training did not change the VEGF serum level in obese people (19). The angiogenesis process begins itself in the adipose tissue while the no angiogenesis action happens in the skeletal and cardiac muscles. At this stage, an increase and concentration of VEGF in the blood can be due to angiogenesis process in the adipose tissue which is a good sign after the training. Similarly, Cristina et al (2013) in their study concluded that type 1 WEGF receptors increase significantly after training in obese and thin women, while after the training course and returning to the pre-course state, only the obese women show a stable increase in the type 1 WEGF receptor. This study also found that WEGF is an important indicator of obesity, while obesity along with an increase in WEGF receptors can cause the onset of cancer (20). Yet, Jinki et al. (2010) showed that despite a significant decrease of systolic and diastolic blood pressure and the fat mass, the WEGF serum had a significant increase (21). The subjects of this study were also in pre-diabetes state, blood sugar of 97-112. As we know, diabetes and angiogenesis process have negative relationship.

Also, results from the studies of Arman et al (2016) have proved that obesity and hypertension, left coronary heart disease, increase in triglyceride, decrease of HDL, more resistance to insulin and type 2 diabetes have led to cardiac diseases and increase death rates among human beings in recent years. Their studies have also proved that by stimulating angiogenesis and preventing obesity and hypertension can prevent the onset of nearly all such diseases. And the best way for stimulating the angiogenesis is through doing training and sport activities (22).

In their study the results of which were somewhat opposed to those of this study and many of the previous ones, Michael et al (2014) studied the increase of serum endostatin after taking training in both healthy and diabetic men and women. They concluded that endostatin had a significant increase in both healthy and diabetic groups, while the increase of WEGF is restrained by endostatin in both groups. Thus, catching diabetes has no significant effects on changes in serum WEGF after physical activities (23).

In still another study, Van Craenenbroeck et al (2016) studied the relationship between endothelial progenitor cells (EPC) and VEGF and profile cardiovascular factors like LDL following a course of physical activity. To this end, 25 healthy persons (20-30 years old men for the first group and 20-50 years old men for the second group) were selected to take a sport test of cardiovascular characteristic in which subjects were ordered to act up to a frustration on an ergometer that started was started with a 40- watt round to which 20 watts were added every one minute. Blood sampling was done before and 10 minutes sharp after the maximum active turn. There appeared increase in EPC in both groups; in the first one up to 76% (27.2± 13.7 cells/ml, \( P=0.01 \) to 15.4± 10/7 cells/ml) and in the second group up to 69% (30.9± 14.6 cells/ml, \( P=0.03 \) to 52.5± 42.6 cells/ml). The VEGF level also showed a strong tendency to significantly (\( P=0.055 \)) increase with physical activity. This study showed that increase in EPC and VEGF is positively correlated to LDL (24).

A review article by Martian et al (2014) as a summary of its results showed that among the 10 researches about the effects of sport trainings on the viscosity of WEGF, only four had reported significant changes in WEGF after taking training, while the other six ones had no significant changes in WEGF after training (25).

Conclusions

In the present study, we found that doing aerobics training in both continuous and interval courses had no significant effects on the level of angiogenesis and nitric oxide in the old men under study. However, we can maintain that taking aerobic trainings by subjects who suffer from diabetes,
hypertension and obesity, based on results from previous studies has no positive effects. Patients suffering from diabetes, hypertension and obesity would see no significant change in their angiogenesis rate after doing continuous or interval aerobic trainings.

References


22. Erman H, Gelisgen M, Cengiz O, Tabak F, Erdenen H. The association of vascular endothelial growth...