The Effect of 8 Weeks Aerobic Training on Angiogenesis (VEGF) and Angiostatic (ES) Factors In Sedentary Women

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Received 10 Nov, 2016, accepted for publication 17 Jan, 2017

Abstract

Background & Aims: It is more important to know which of physical activities could be the most effective way to cause angiogenesis. In this regards, we have investigated an 8-week aerobic training on vascular endothelial growth factor (VEGF) and Endostatin (ES) in sedentary women.

Materials & Methods: 20 volunteer inactive women were chosen and were randomly divided in 2 groups of aerobic and control. Aerobic training programme consisted of 3 days per week for 8 weeks, each consists of 30 minutes with intensity of 65-70% of maximum heart rate was performed.. Before and after training, we have tested aerobic training and control group after an overnight fast. Blood Samples were used to conduct Enzyme-linked immunosorbent assay (ELISA). Independent and Dependent T-Test t were performed to see the significance level of (P<0.05).

Results: Statistical analysis revealed that aerobic training increased VEGF and decreased ES to a significant level. Consequently, the results of Independent T Test revealed that there is an obvious difference (P<0.05), between the amount of VEGF and ES in control and intervention group.

Conclusion: The results show that the equilibrium between angiogenesis and angiostatic agents. As a result, aerobic training is shifted toward angiogenesis factors. This result helps us to understand the process of angiogenesis consistence with aerobic training activities.

KeyWords: Aerobic training, Angiogenesis, VEGF, ES, Sedentary women

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Introduction

Sport activities have an important influence on factors which cause cardiovascular diseases. These activities improve the general density of lipoprotein, general fat content (1), insulin sensitivity to, glucose release and glucose tolerance(2). Also, sport activities control blood pressure in individuals on the verge of high blood pressure(3). Aerobic exercises decrease blood pressure and heart beat. Aerobic exercises increase V02 max and aerobic enzymes activity and extend capillary network in heart and skeletal muscles(4).

The extension of this network is done through arteriogenesis and angiogenesis processes on the
vascular bed of skeletal muscle which decrease or
omit the stress caused by sport act in ties(5).
Arteriogenesis refers to an increase in the diameter
of existing arterial vessels (6). However,
Angiogenesis means the formation of new
capillaries from the previous capillary(7). In
angiogenesis process, endothelium acts differently
in reaction to different stress incentives because of
angiogenic factors (vessel-make factors) and
angiostatic factors (controlling factors)(8).
Angiogenic factors are those factors involved in the
formation of new capillaries either directly or
indirectly and help making and evolving of vessels.
The lack of any of these factors disturbs the stages
in making and evolving capillaries(9). The most
important growth factor in angiogenesis process is
vascular endothelial growth factor (VEGF)(10).
The 45–kDa glycoprotein factor provides necessary
ground for the survival, multiplication,
immigration, and penetrability of cardiovascular
endothelial vessel through increasing adjustment of
anti-apoptotic components, DNA synthesis,
destruction of basic membrane and
phosphorylation of intercellular adhesion
molecules and finally causes the formation of new
vessels (11).

On the other hands, angiostatic factors are factors which
enter the angiogenic process in a certain stage and
hamper it. Endostatin (ES) is a 20-KDa part which
is released from the terminal C of XVIII collagen
and inhibits angiogenesis. In its prevention
mechanism, endostatin connects to a VEGF
angiogenic factor and prevents it for function and
in this way Inhibits endothelial cell proliferation.
Also, endostatin prevents the destruction of the
vessel’s basic membrane which eventually prevents
endothelial cells from immigration. In fact,
endostatin prevents the multiplication and
immigration of endothelial cells and by doing so
prevents the growth of capillary network(12).

In normal situations, there is a balance between
angiogenic and angiostatic factors. However, this
balance is always disturbed in physiological and
pathological situations and one of these situations
is exercise(13).

The effect of exercise on serum amounts of angiogenic
factors leads to contradictory results. As Ravasi et
al (2014) showed in their study of the effect of two
kinds of physical activities (one session of
progressive aerobic activity and one intensive
interval) on the serum response of VEGE on non –
athlete men. These two factors can change the
levels of VEGF angiogenic factor with the same
degree(14). Nourshahi et al (2013) studied the
effect of six-week resistance training on VEGF
and endostatin on tumor texture in rats inflicted
with breast cancer and showed that these training have
no effect on the angiogenesis pattern of tumor
studied the effect of high intensity resistance
exercise (knee extension) on serum amount of
VEGF and insulin-like growth factor and
concluded that serum IGF-1 was increased after
exercise while serum levels of VEGF remain the
same(16). Gavin et al (2007), in a study entitled
“acute resistance exercise increases skeletal muscle
angiogenic growth factor expression” asked
participants to do resistance training in the form of
knee extension for three times with 10 repetitions,
each time with 60 to 80 percent 1RM. Blood and
muscle samples were taken from them before and
after the activity. The results showed that exercise
increased the mRNA and VEGF protein in skeletal
muscle(10). Nourshahi et al (2012) investigated the
effect of six weeks of endurance training on the
amount of VEGF and endostatin of tumor texture
in rats inflicted with breast cancer and showed that
endurance training increases VEGF significantly but the increase of endostatin was not significant\(^{(17)}\). Shekarchizadeh et al\(^{(2012)}\) in their study of the effect of 4 weeks (3 days a week) of resistance training on the plasma level of nitric oxide, endothelial growth factor and its type-one receptor in male rats, showed that resistance training have no effect on the plasma level of effective factors on angiogenesis including NO and VEGF in healthy animals\(^{(18)}\). Also, Ranjbar et al\(^{(2011)}\) in their study of the effect of gender and physical activity on serum vascular endothelial growth factor at rest and response to submaximal exercise, showed that gender and the amount of physical activity aren’t among the effective factors on the amount of VEGF while resting and in response to endurance activities\(^{(19)}\). Our investigations show that studies about the effect of long term exercise on angiogenesis factors are rare and most studies have focused on short and one-session exercise. It should also be mentioned that in the investigated researches, both angiogenic (VEGF) and angiostatic (ES) factors have not been studied simultaneously and in the form of an aerobic training plan. Thus, this study sets to answer the effect of 8 weeks aerobic training on VEGF and ES in sedentary women.

**Materials and Methods**

The present study is a pre-test - post-test quasi-experimental test with a control group. The population of this study was sedentary women from Urmia with ages 30-40. The inclusion criteria were as follows: not having medical record, not taking the medicine while taking part in the research, not having a regular sport record (at least six months before the research). The exclusion criteria were: not being interested in the research, taking medicine and nutritional supplements, not participating in the session regularly, and injury. In this study, from the initial 60 sedentary women, 20 with ages 30-40 were randomly selected after filling out the satisfaction form and health questionnaire. These women were categorized into two equal groups of aerobic exercise and control.

**Measuring the demographic variables:**

These variables include age (years old), height (cm / with meter), weight (kg / with digital weight), and BMI (dividing weight by the square of height). Omron (Omron BF306 Body Composition Monitor) made in Korea was used to measure the percentage of body fat. Participants, while standing, grabbed the sensors with both hands. Then, start button was pressed and body fat was measured.

**Training plan:**

In the briefing session, the way of doing training and the research time schedule were explained to the participants. Then, they were ready to take part in the training plan.

- **Aerobic training**

Participants did 8 weeks of training on the treadmill for 3 times per week, each time 30 minutes, with intensity of 65-70% of Maximum heart rate (age-220). To control the intensity of exercise, heart rate monitor was used. Each training session included: 10 minutes stretching and active warm-up, 30 minutes aerobic training on the treadmill, and final 5 minutes cool-down and recovery (Table 1).

- **The intensity of the aerobic training**

The participants in the aerobic training group did activities before the main training with the intensity of 50-60 % maximum heart rate to gradually adapt to the real pressure of the activity. Then, the real training began in 65 to 70 % maximum heart rate. While doing the aerobic training and if the increase or decrease of intensity was required, the necessary feedback was given to the participants (Table 1).
Table 1. Intensity and duration of aerobic training (3 sessions per week)

<table>
<thead>
<tr>
<th></th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
<th>5th week</th>
<th>6th week</th>
<th>7th week</th>
<th>8th week</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intensity</strong> (MHR)</td>
<td>50 %</td>
<td>55 %</td>
<td>60 %</td>
<td>65 %</td>
<td>70 %</td>
<td>70 %</td>
<td>70 %</td>
<td>70 %</td>
</tr>
<tr>
<td><strong>Time</strong> (Min)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

- **Control group**
  The participants in this group had no regular sport activity and their physical activity was controlled based on their own reports.

**Blood sampling:**
Blood sampling was done after 12 hours of fasting in two stages. In the first stage, based on the guidelines for blood sampling conditions, participants were asked to avoid doing any intense physical activity, stressful situations, and taking medicine for 3 days before sampling. The resulted serums were frozen in -70 centigrade. The second time of blood sampling was done 48 hours after the last training session. VEGF and ES were measured by the enzyme-linked immunosorbent assay (ELISA) with a special kit.

**Statistical method:**
Descriptive statistics were used to analyze the research data for measuring the central indexes and dispersion. The K-S test was used to identify the normal distribution of data. The Leven test was used to determine the homogeneity of variances. The Paired T test was used to investigate the changes from pre-test to post-test. The independent T test was used to investigate the changes between groups. Data were analyzed using SPSS version 21 and the level of significance was p < 0.05.

**Results**
Physical and physiological traits of the participants are shown in Table 2.

Table 2. Physical and physiological traits of the participants, before and after 8 weeks of training

<table>
<thead>
<tr>
<th>Group</th>
<th>Aerobic training</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Pre-test</td>
<td>Post-test</td>
</tr>
<tr>
<td>Age (years)</td>
<td>*34.5 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.5±5.02</td>
<td>165.7±5.14</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.9±4.2</td>
<td>61.45±4.03</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.6±0.82</td>
<td>22.05±0.48</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>32.98±4.04</td>
<td>30.4±4.15</td>
</tr>
</tbody>
</table>

* The amounts are indicated as standard deviation ± average.

The average and standard deviation of dependent variables, before and after 8 weeks of training, and the results of the paired T test and the independent T test are shown in Table 3.

The results of the paired T test showed that 8 weeks of aerobic training change the VEGF (P=0.001) and ES (P=0.001) in sedentary women while no significant difference was observed in the control group (P≥0.05). Also, the result of independent T
test showed that in the amount of VEGF and ES, there is a significant difference between aerobic and control groups (Table 3).

### Table 3. The results of paired and independent T tests in determining the difference between the research variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Level of significance</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerobic training</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>VEGF (ng/l)</td>
<td>Pre-test</td>
<td>134.9±28.67</td>
<td>128.37±28.58</td>
</tr>
<tr>
<td></td>
<td>Post-test</td>
<td>215.6±43.39</td>
<td>128.97±36.95</td>
</tr>
<tr>
<td></td>
<td>Level of significance</td>
<td>¯ 0.001</td>
<td>0.871</td>
</tr>
<tr>
<td></td>
<td>The difference</td>
<td>80.7±14.72</td>
<td>0.6±8.37</td>
</tr>
<tr>
<td></td>
<td>Between pre and post</td>
<td></td>
<td>* 0.001</td>
</tr>
<tr>
<td>ES (ng/ml)</td>
<td>Pre-test</td>
<td>11.11±3.04</td>
<td>10.82±2.45</td>
</tr>
<tr>
<td></td>
<td>Post-test</td>
<td>8.58±1.84</td>
<td>10.72±2.40</td>
</tr>
<tr>
<td></td>
<td>Level of significance</td>
<td>¯ 0.001</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>The difference</td>
<td>2.53±1.2</td>
<td>0.1±0.05</td>
</tr>
<tr>
<td></td>
<td>Between pre and post</td>
<td></td>
<td>* 0.031</td>
</tr>
</tbody>
</table>

# Significance as compared to pre-test amounts

* Significance as compared to aerobic group. The amounts are indicated as standard deviation ± average.

### Discussion and Conclusion

Blood vessels and capillaries provide oxygen and food for metabolic tissues and remove waste and thus have a crucial role in body function and health. In normal conditions, there is a balance between angiogenic and angiostatic factors. However, in physiological and pathological situations, this balance is disturbed and one of these conditions is exercise(13).

It is shown that VEGF plays a crucial role in angiogenesis resulted from the exercise. This glycoprotein factor provides the necessary ground for the survival, multiplication, immigration, and penetrability of endothelial cell through the increasing adjustment of anti-apoptotic components, DNA synthesis, destruction of basic membrane and phosphorylation of sticky endothelial inter-vessel components and finally cause the formation of new vessels(11). In the present study, 8 weeks of aerobic training increased VEGF in sedentary women significantly. Also, there was a significant difference between the aerobic training and control groups. The increasing trend shows that VEGF and its receptors VEGFR1 and VEGFR2 and NPR-1 increased in response to a sport activity (20-21).

Long term sport training with moderate intensity is regarded as the positive regulator of VEGF mRNA expression and the protein content. For instance, Gavin et al (2004) showed that 60 minutes of cycling with the intensity of 50% VO2 max increases VEGF around 4.5 times (22). Hoier et al
(2012) showed that 60 minutes of cycling with the intensity of 60% VO₂ max leads increase in inter-
tissue density 6 folds compared to the resting conditions (23). On the other hand, Brixius et al 
(2008) showed long term aerobic training in both 
groups (60 minutes running and 90 minutes cycling 
with the average intensity for six months, three 
times a week) doesn’t lead to the change of VEGF 
level in the obese(24).

Different factors affect the production rate of VEGF as 
a result of exercise. Among these factors, hypoxia, 
shear stress (hemodynamic force as a result of the 
friction between blood flow and vessel wall), 
contraction and stretching of the muscle and 
disturbance of metabolism are the most important 
one(25-26). Exercise leads to the higher speed of 
the blood circulation in the active muscle. Aerobic 
training increases blood circulation 5-6 folds(27). It 
seems that aerobic training increases Interstitial 
Calciumions (CaN and CaMK)(28), NO (eNOS 
and nNOS) (29) and hypoxia (HIF-1) and thus sets 
the VEGF(30).

The reason for the increase in VEGF in the present study 
is most probably was due to the decrease of ES. ES 
which prevents the increase in VEGF (31) by the 
induction of apoptosis in endothelial cells(32) , 
prevents the immigration of endothelial cells(31), 
and suppressing the messaging of Nitric oxide(33).

Local discharge of cathepsins and extracellular matrix 
metalloproteinases (MMPs) through motivated 
endothelial cells leads to the release from XVIII 
collagen, prevention of angiogenesis, and decrease 
in the release of cathepsins and MMPs(12). In the 
present study, 8 weeks of aerobic training 
decreased ES in sedentary women significantly. 
Also, there was a significant difference between 
aerobic and control groups. Little information is 
available about the response of ES to exercise. In 
this regard, Rullman et al (2007) showed a single 
bout of exercise, 65 minutes of cycling with 65% 
VO₂ max increases artery plasma endostatin 1.7 
times in 17 minutes of exercise which is higher than 
the vein plasma ES (1.6 times). However, in 57 
minutes of exercise it gets to the resting amounts 
and stays in the basic amounts until 120 minutes. It 
is interesting that vein plasma ES had a two-fold 
increase in 57 minutes of exercise which is 
significantly high in comparison with artery plasma 
ES. This suggests that absorption has occurred from 
blood circulation to muscle (34).

Also, it is shown that long term exercise like the one in 
this study can increase angiogenesis through 
that long term aerobic exercise in both groups (60 
minutes running and 90 minutes cycling with the 
medium intensity for 6 months, 3 times a week) 
decreases basic amounts of endostatin 14 %. Also, 
endostatin was decreased in both training groups as 
compared to the control group (24). Nourshahiet al 
(2012) investigated the effect of six weeks of 
endurance training on the amount of VEGF and 
endostatin of tumor tissue in rats inflicted with 
breast cancer and showed that endurance training 
increases VEGF significantly but the change in 
endostatin was not significant(17).

It seems that aerobic training in the present study 
increases angiogenesis through decreasing ES, 
because endostatin decreases angiogenesis by 
preventing the multiplication and immigration of 
endothelial cells (31), preventing the activity of 
angiogenesis inducers (such as VEGF and FGF- 
2)(35), disturbing the activity of eNOS and 
decreasing the release of NO resulted from 
VEGF(33), preventing cyclin D1 (stopping G1 of 
cell cycle and apoptosis)(32), and preventing the 
activity of extracellular matrix metalloproteinases 
(MMP-2, MMP-9 and MMP-13)(36). However, the 
decrease in ES in response to the exercise is not
clear yet. It is probable, though, that exercise decreases the change in extracellular matrix and this can lead to the prevention of the release of endostatin from collagen\(^\text{37}\).

In short, the results showed that VEGF increases and ES decrease as a result of adapting to the aerobic training. These findings show that aerobic training changes the balance between angiogenic and angiostatic factors toward angiogenic factors.

Lack of control over the excitements and other mental factors in participants, as well as their sleep and resting conditions, were among the limitations of the present study. Also, since ES was decreased significantly in this study, and this was not observed in previous studies, interpreting and applying the findings to other studies create some limitations.

Since the increase in angiogenesis has a positive effect on people’s health, this study suggests that individuals make use of aerobic training for motivating angiogenesis and improving their health.

Acknowledgement

Authors feel obliged to thank all the women taking part in the study. We are also grateful to the Laboratory of Pathobiology center of Urmia.

References


