The effect of 12 weeks of intermittent aerobic exercise on endothelin-1 concentration of plasma in adult rats

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Abstract:
Introduction: Endothelin is a vasoconstrictor which is released from the endothelium. The aim of the present study was to investigate the effect of intermittent aerobic exercise on the endothelin-1 concentration of plasma as an important indicator of cardiovascular diseases, especially hypertension, in Sprague-Dawley rats.

Materials and Methods: The subjects of this study were 20 adult male Sprague-Dawley rats with the average weight of 250±4 g and age of 20±2 months. The rats were randomly assigned to two groups of experimental (10 rats) and control (10 rats). The subjects of the experimental group participated in selected intermittent aerobic exercises for 12 weeks. Blood samples were taken 24 hours before and after the training sessions.

Results: The results of this study revealed that 12 weeks of intermittent aerobic exercise has a significant effect on decreasing endothelin-1 concentration of plasma (p=0.001), while no significant change was observed in the control group (p=0.24); endotheline-1 fluctuations were significantly different in the exercise and control groups (p=0.022).

Conclusion: Intermittent aerobic exercise can be effective in prevention of hypertension or other kinds of cardiovascular diseases through influencing endothelin-1.

Keywords: Endothelin-1, Aerobic Exercises, Sprague-Dawley Rats

Introduction
Endothelin was discovered in 1985 by Yanagisawa M. Endothelins are a family of amino acid peptides with three distinct isoforms obtained from cultured aortic endothelial cells from pigs. Endothelins are vasoconstrictor factors that are released from endothelium and the main location for their synthesis is endothelial cells. All endothelins have 21 amino acids and the type of amino acid is the only difference between them. Among them, endothelin-1 has higher concentration than others, it is the most abundant isoform, and more information is available on it (1). So far, two types of human endothelin receptors, i.e. endothelin receptor type A and endothelin receptor type B are known.
Endothelin receptors type A are mostly in heart and vascular smooth muscles, but endothelin receptors type B are more extensively distributed and found mainly in the kidney, uterus, the central nervous system and vascular endothelial cells. Endothelin type A is the main endothelin-mediated vasoconstriction, while endothelin receptors type B stimulation causes production of nitric oxide (2). Endothelin-1 is in healthy lungs besides plasma and is secreted mainly in pulmonary vascular endothelium, vascular smooth muscle cells and airways epithelium (3). Systemic and topical hemostasis such as regulation of blood flow, volume, pressure and viscosity, renal volume homeostasis such as regulation of renal blood flow, urinary excretion of water and electrolytes, cardiovascular homeostasis such as regulation of cardiac output, coronary blood flow, positive inotrope and chronotrope properties are all known physiological and biological effects of endothelin-1 (4). Also vascular smooth muscles relaxation and stimulation of cell proliferation are other physiological effects of this substance (3-4). It has become clear that relaxing factor induced by vascular endothelium which is similar to nitric oxide is somewhat effective in redistribution of tissue blood flow during exercise. (5). Endothelin's loss of function is not only characteristic of diseases such as blood pressure, increased cholesterol and arteriosclerosis, but also is associated with increasing age (6). Aging causes endothelial dysfunction in the aorta and decreased vascular resistance. Changes in endothelial function due to aging can signal clinically significant points and symptoms of cardiovascular diseases (7). Endothelin-1 is involved in the development and progression of atherosclerosis. In atherosclerotic vessels, certain cellular changes are associated with abnormality in calcium ions' transport process (1, 8).

Regular aerobic exercise reduces the incidence of cardiovascular diseases. One of the potential outcomes of any regular aerobic exercise is reducing the risk of cardiovascular diseases and positive and beneficial effect on vasomotor function. Furthermore, improvement of coronary vasomotor function is associated with reduction of cardiovascular events. In addition, several studies have shown that aerobic exercise increases endothelin-dependent vasodilation in healthy older men and in patients with hypertension (9). For example, 12 weeks of moderate-intensity aerobic exercise had reverse effects compared to aging in reduction of endothelial vasoconstrictive function in sedentary men. Recent studies have demonstrated that moderate intensity aerobic exercise can often lead to a decrease in endothelin-1. Exercise also reduces endothelin-1 system activation which may be significantly correlated with the beneficial effects of exercise in preventing and treating hypertension as well as reducing the risk of atherosclerosis (10-11). Some studies have shown that exercise reduces the endothelin-1 concentration of plasma and increases endothelin in internal tissues and, thus, in addition to reducing blood pressure, it reduces the risk of this disease. However, conflicting results are also seen regarding the effects of exercise on endothelin-1. For example, Seiji et al. and Shen et al. have found conflicting results in their studies: some studies have shown the ineffectiveness of exercise and some have indicated the effects of exercise on increasing endothelin-1 (12-13). Likewise, Emory et al. showed the effect of exercise on endothelin (12, 14). Exercises are usually performed in resistance, anaerobic or aerobic methods. In addition, aerobic exercise is done in two continuous and intermittent ways, where people mostly welcome the intermittent type due to the availability of rest time.

Given that most studies have been done on resistance and anaerobic exercise, and long-term aerobic exercise is effective on intracellular adaptations and various
hormones secreted from endothelial cells, the present study investigated the effect of 12 weeks of intermittent aerobic on endothelin-1 as one of the common exercise methods (15).

Materials and methods
In this experimental study, 20 male Sprague-Dawley rats, with the average weight of 250±4 g and age of 20±2 months were randomly selected and were kept in animal laboratory under strictly controlled conditions at temperature of 25±1°C, humidity 20%, light:dark cycle of 12:12 hours. The rats were randomly divided into two experimental (10 rats) and control groups (10 rats). All environmental and dietary factors were similar for both groups. 24 hours before the onset of exercises, blood samples were taken from tail veins of rats in each group at 10 am. After blood centrifugation, plasma was extracted and then frozen and stored at –70°C. After 24 hours, exercise was started in the experimental group. The exercise protocol in this study was 12 weeks, 5 sessions in every week and exercise intensity and duration were determined in accordance with principle of overload. In the first two weeks, speed of exercise was 12 meters per minute and then for the third to the tenth week, an increase of one meter per minute was determined in each week. The speed of exercise in the eleventh and twelfth weeks increased from 21 to 23 meters per minute. Exercise duration on the treadmill in the first two weeks was 1, 1.5 minutes, respectively, and in the ninth to tenth weeks, it increased two minutes each day. Exercise duration was kept constant for the last two weeks. At this stage, intensity of activity was determined only based on speed. In other words, duration of activity from 10 minutes in a day reached to 80 minutes at the beginning of the eleventh week and then remained constant. The exercises were done twice a day in the first four weeks, three times a day in the fifth to the eighth weeks and four times a day in the ninth to the twelfth weeks. Intervals of rest between exercise shifts were considered by a ratio of four to one (4:1). To warm up, the animals ran at the beginning of each exercise session for three minutes at the speed of seven meters per minute and then to achieve the desired rate-per-minute, the speed of treadmill was increased two meters per minute. Due to warm body, the speed of warm up was increased in the next periods between exercise instances with more speed and 3 to 4 meters per minute was added to the treadmill speed. To cool down the body at the end of each exercise instance, the treadmill speed was reduced reversely to reach the initial speed. All stages of exercise were performed on a treadmill without the incline. This exercise program was designed according to the oxygen cost and total distance of the exercise was calculated 74010 meters considering the warming up and cooling down of the body in the experimental group (16). For equalizing conditions, all rats in the experimental group started and finished the exercise on a large treadmill together. During this time, the rats in the control group with the similar diet and environmental conditions to the experimental group were kept in their cages without any physical exercise. To avoid the possible influence of unwanted factors in results of the study, including sexual intercourse, only male rats were selected for each group. 24 hours after the last exercise session, blood samples were taken from tail vein of rats again. Then the blood samples were centrifuged in the laboratory and their plasma was extracted and was frozen at –70°C. All blood samples before and after exercise were sent to a specialized laboratory to measure the plasma concentration of endothelin-1. Endothelin concentration was measured by ELISA diagnostic kit (Glory, the United States) with the accuracy of one picogram on milliliter (pg/ml).
In all stages of the research, standard conditions were adhered to for nutrition and health status of rats, duration of
dark:light cycle, air condition and locations for keeping rats. Due to the potential effect of stress on the results of the study, creating any extra environmental stress on the subjects was prevented during the research. Dependent t-test was used to compare the difference of plasma concentration of endothelin-1 in the groups, and independent t-test was used to compare concentration difference in the experimental and control groups before and after 12 weeks of exercise. Statistical significance level was considered less than 0.05. Data analysis was performed in SPSS software.

**Results**

After conducting Kolmogorov–Smirnov test to confirm normal distribution of data, dependent t-test was used to compare the intragroup mean changes in endothelin-1. The results showed that the mean of endothelin before (2.17±0.16) and after (2.15±0.13) the study period was not significant in the control group (P=0.24), but the mean in the experimental group before the test changed from 2.16±0.11 to 2.05±0.10. This finding represents the significant change in reduced endothelin-1 concentration in the experimental group before and after 12 weeks of aerobic exercise (P=0.001) (Table 1). Concentration difference before and after exercise in the experimental and control groups was calculated 0.107±0.069 and 0.028±0.071, respectively, and differences were significant (P=0.022) (Table 1).

**Table 1: Descriptive indicators and comparing changes in endothelin-1 in experimental and control groups**

<table>
<thead>
<tr>
<th>Endothelin-1 (PgL)</th>
<th>Experimental group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean and standard deviation of pre-test</td>
<td>2.16±0.11</td>
<td>2.17±0.16</td>
</tr>
<tr>
<td>Mean and standard deviation of post-test</td>
<td>2.05±0.10</td>
<td>2.15±0.13</td>
</tr>
<tr>
<td>p-value in intragroup changes (dependent t)</td>
<td>0.001*</td>
<td>0.24</td>
</tr>
<tr>
<td>Mean difference in concentrations</td>
<td>0.107</td>
<td>0.028</td>
</tr>
<tr>
<td>p-value in intragroup difference (independent t)</td>
<td></td>
<td>0.022*</td>
</tr>
</tbody>
</table>

* Statistically significant

**Discussion**

The results of this study indicate that 12 weeks of intermittent aerobic exercise with a specific pattern has a positive effect on reduced plasma levels of endothelin-1 in rats. Seiji et al., Anthony et al., Yoshifumi Kawanabe et al. have referred to ineffectiveness of exercise and Yusha'u et al., Maeda et al., Gray et al., Samanta et al. have referred to the effects of exercise on the reduced endothelin-1 concentration of plasma (17-23). It seems that the reason for the contradiction in the results is the type of exercise used. Most of the above studies are based on a limited number of movements in resistance exercise or inadequate levels of percentage of maximal oxygen consumption (VO2MAX). The results of the present study is similar to studies on moderate and increasing intensity exercise and therefore the protocol used by the researcher to reduce the plasma concentration of endothelin-1 and as a result reduce the symptoms caused by the presence of large amounts of this substance in the blood is appropriate. The exact mechanism that leads to reduced plasma levels of endothelin-1 after exercise is unknown. It seems that...
regulation of body tropic hormones due to physical activity or changes in body weight and total fat mass and also increase in strength and power of skeletal muscles surrounding blood vessels all reduce the body’s need to function of vascular endothelial cells. As a result, the substances secreted by these cells decrease in plasma. Low concentration of endothelin-1 in the blood may reduce blood pressure, the risk of heart diseases, the risk of atherosclerosis and inhibit renal ischemia and other cases due to the presence of high levels of endothelin in blood. Furthermore, different blood rheological factors and neural -blood factors such as angiotensin II, arginine and vasopressin are involved in the production of endothelin-1. Therefore, the mentioned factors affected by aerobic exercise will probably be reduced, which is a reason for the claim that significant drop in plasma concentration of endothelin-1 may reduce high blood pressure, the symptoms and risk of high blood pressure and also control the concentration of angiotensin etc., and thus indirectly reduce the symptoms induced by the high concentration of these substances (3).

Conclusion
Due to the increase in plasma levels of endothelin-1 by aging and also its relationship with cardiovascular diseases including high blood pressure, it can be said that intermittent exercise will cause reduced endothelin-1 and as a result low blood pressure (9). However, recommendation for intermittent exercise program with specific intensity, duration and program in humans requires further investigation in the future.

Acknowledgement
Hereby, all people who helped the researchers in this study are appreciated.

Conflict of interest
The authors declare no conflict of interest in this study.

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15. Öunis O, Elloumi M, Zouhla H, et al. Effect of an individualized physical exercise program on resting cortisol and growth hormone levels and fat oxidation

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