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The Effect of Acute and Chronic Moderate and High Intensity Aerobic Exercise on Serum Nitric Oxide in Untrained Overweight Males

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Abstract

Background: The present study aimed at comparing the serum concentration of nitric oxide (NO) after 4 weeks of aerobic activity at 2 different intensities in overweight males.
Methods: For this purpose, 20 inactive obese males between 25 and 35 years old were randomly selected as subjects. Their body mass index (BMI) was 29.9 $\pm$ 3.04. They were randomly divided to 2 moderate (n = 10) and high (n = 9) intensity groups. They exercised in accordance of specific instructions and were observed by the researcher. Blood samples were collected 3 times: first, in the morning after an overnight fast, second, after the first session, and third, immediately after 4 weeks of exercise. Concentration of nitric oxide serum was measured using the enzyme linked immunosorbent assay (ELISA). Impedance analysis (Body state) was used to determine the body composition. The collected data were analyzed by statistical analysis using dependent t-test and independent t-test analysis. The level of statistical significance was set at $P < 0.05$. Values were expressed as mean $\pm$ standard deviation (SD).
Results: There was no difference in concentration of nitric oxide serum in the moderate intensity group, after exercise for 4 weeks ($P = 1.0$). Concentration of nitric oxide serum in the high intensity group was significantly higher after 4 weeks ($P = 0.004$) and in comparison with the moderate intensity group ($P = 0.004$).
Conclusions: It seems that short-term aerobic activity with moderate intensity does not induce a concentration of nitric oxide serum in overweight males while nitric oxide increases during a similar period at high intensity.

Keywords: Aerobic Exercise, Nitric Oxide, Overweight

1. Background

Vascular endothelium, a layer on the inner surfaces of blood vessels, is able to create large active molecules. Therefore, it performs as an organ autocrine, paracrine, and endocrine (1, 2). Endothelial cells release powerful active ingredients penetrating to the deep layers of smooth muscle, which leads to a relaxation of arteries. Therefore, endothelium release relaxing factors called EDRF (2, 3). One of these activator factors is nitric oxide (NO), which works as a regulator of vascular tone.

Nitric oxide gas has very high potency (half-life of a few seconds), and is produced by the amino acid L - arginine and a group of enzymes, called nitric oxide synthethase (NOS) (4). Nitric oxide synthase exists in many tissues and cell types, including nerve cells, macrophages, hepatocytes, smooth muscles myocytes, endothelial cells of blood vessels, and kidney endothelial cells (5).

The release of NO by endothelial cells plays an important role against coronary heart disease stems. Nitric oxide prevents structural abnormalities (vasospasm) and plaque accumulation in coronary arteries and the expression of adhesion molecules in endothelial cells. Therefore, NO prevents white blood cells from sticking and penetrating (macrophages), and also prevents releasing and constrictor impact of vessels, such as endothelin. The release of NO is intensified with the presence of blood clots forming, especially with the formation of thrombin and plaque accumulation. If this protective effect of NO is lost, a place will be created for inflammatory responses and this may lead to the formation of atherosclerotic plaques (4, 6).

Obesity is a condition that involves the accumulation of fat in the body. The prevalence of obesity is rapidly increasing around the world (7). It has been suggested that fat tissue may be a source for the production of NO. Previous reports have shown that there is NOS and NOS protein (iNOS) activity in abdominal adipose tissue in humans (8).

It seems that a part of the increase in impaired endothelial function is related to insulin resistance caused by obesity. The dysfunction of the endothelium is iden-

tified by a reduction in relaxation factor NO, supporting the arteries against the development of coronary artery atherosclerotic (9). The most important mechanism that has been proposed, is the deactivation of NO by superoxide anion that leads to the formation of Perry oxy-nitrite. The source of increase in superoxide anion production is both enzymes, such as NADPH oxidase, and also synthase of nitric oxide, which are not paired (10).

Several studies on animals and humans have indicated that obesity reduces bioavailability of NO (11). The risk of atherosclerotic and cardiovascular disease is increased with the increase of fat and is associated with high mortality in all cases. Recent estimates show that a quarter of ischemic heart disease events are associated with weight gain (12).

Losing weight reduces the risk of myocardial infarction-off and increases longevity. Recent studies suggest that NO has a role in regulating food intake in animal experiments (13). Vascular endothelium plays a pivotal role in setting the tone of the arteries, inflammation, and thrombosis functions. Endothelial function is damaged in obesity and this represents the first step in clinical atherosclerotic disease. Abnormalities in endothelial function becomes worse with weight gain because several mechanisms, including impaired glucose tolerance associated with increased fat mass, insulin resistance, metabolic dysregulation, the release of adiponectin (that plays a key role in the assessment of systemic inflammation and cardiovascular disease), occur. Even in patients with diabetes under proper care, a reduction in nitric oxide could damage endothelial dilation (14).

Although endothelial dysfunction is a strong predictor for cardiovascular disease, it reduces the repair of arterial hemostasis (12, 15, 16). By doing aerobic exercise regularly, NOS makes NO continuous release, which definitely improved the control of arterial blood pressure by relaxing the smooth muscle cells (11). Exercise improves NO synthase and it is certainly associated with reduction in antioxidant and blood lactate (17).

Exercise and dietary restriction increases the bioavailability of NO and improves blood glucose and lipid, inflammation and blood pressure, and metabolic syndrome (18). In addition, if the dilation of NO is improved by exercise, it has a protective effect for the heart (19).

According to the results of the above studies, the aim of this study was to investigate the effect of short-term (4 weeks) moderate and high intensity aerobic exercise on the release of NO in obese people.

2. Methods

The present study was classified as an applied research. In addition, a quasi-experimental design was used to meet the research goal.

2.1. Research Sample

Twenty untrained overweight males living in Ahvaz were selected by convenience sampling and were randomly assigned to 1 of the 2 trading groups that performed high intensity aerobic training (n = 10) or moderate intensity aerobic training (n = 10). All samples were healthy and there were no history of heart disease or any chronic discomfort.

Over the past 2 years, both groups had no regular physical activity. Subjects were healthy and without any history of heart disease or chronic discomfort. Demographic characteristics of subjects were recorded and collaborated on research completed consent. Weight and height were measured and body fat percentage was estimated using bioelectrical impedance (3/3 OLYMPIA; manufacturing company Javvn South Korea) and Vo_2Max was measured by the Bruce test treadmill Saturn model (manufacturing Co. hp/cosmuse making Germany).

In this study, intensity was 50% and 75% of maximum heart rate reserve that was arranged in groups of moderate and high intensity exercise. Both moderate and high intensity continuous exercise was carried out outdoor. Intensity of training was set by Polar heart-rate belt made in Germany. Subjects exercised 3 days a week.

Before performing each exercise session, the subjects warmed up for 5 minutes. The subject's heart rate was then increased to meet the training zone and they continued to work out at this intensity to exhaustion. All participants were told that if they felt tired or had any discomfort, they should stop the activity. After each session, the subjects cooled down for 5 minutes. The subjects were trained for an average of 12 sessions. Blood samples were drawn from each volunteer 3 times. In order to avoid the effects of diet on levels of nitric oxide, participants were asked not to eat foods rich in nitrates, such as regular tea, green tea, beer, frozen meat, and fish and chips, one day prior to the sampling procedures.

- First blood: fasting before exercise
- Second stage of sampling: immediately after the first session
- Third stage of sampling: immediately after the last practice session

2.2. Collection and Storage of Samples

To measure human NO, the Cusabio Company kits Made in Japan was used. Both serum and plasma samples

were taken, yet in this study, serum samples were used. Approximately 5 mL of blood was taken from each person and after 30 minutes, they were centrifuged and then the serum was immediately isolated from blood clots and stored in the freezer at -20°C until further testing. Data was expressed as unit nmol/L (ug/dL).

2.3. Statistical Methods and Analysis of Data

In this study, descriptive statistics methods, including mean and standard deviation, were used to calculate age, height, weight, body mass index (BMI), and body fat (BF%). To ensure that data was normal, the Kolmogorov Smirnov test was performed. Paired t-test was used to compare within group differences. Alpha error was considered as $0.5 \geq \alpha$. The SPSS software package version 17 was used for statistical analysis.

3. Results

Tables 1 and 2 show mean and standard deviation of demographic characteristics of research groups.

Table 1. Descriptive Statistic Results for Moderate Intensity Aerobic Group

Group 1: Moderate Intensity Aerobic Exercise	Pretest	Posttest
Age, y	32.5 ± 4.89	-
Height, cm	173 ± 4.62	-
Weight, kg	83.56 ± 3.60	82.43 ± 2.54
VO_2max, mL.kg.min	5.26 ± 2.02	33.85 ± 2.33
BMI	29.9 ± 4./01	27.6 ± 3.66
%PBF	28.5 ± 1.66	26.76 ± 1.33

Table 2. Descriptive Statistic Results for High Intensity Aerobic Group

Group 2: High Intensity Aerobic Exercise	Pretest	Posttest
Age, y	30.12 ± 1.05	-
Height, cm	172.1 ± 6.92	-
Weight, kg	81.82 ± 3.60	78 ± 45.79
VO_2max, mL.kg.min	27.7 ± 3.22	38.21 ± 3.12
BMI	29.9 ± 2.07	27.3 ± 2.04

Table 3 shows statistical paired t-test results before and after one training session.

According to the significance level, concerning the dependent t-test within alpha of 0.05, there was no meaningful difference between pretest and posttest in both moderate (0.34) and high (0.12) intensity groups. Therefore, one session of moderate or high intensity aerobic exercise did not effect the concentrations of nitric oxide serum among obese individuals.

Table 4 shows statistical paired t-test results before and after 4 weeks of training.

According to the significance level concerning the dependent t-test within alpha 0.05, there was no meaningful difference between pretest and posttest in the moderate intensity group (1.0) and there was a significant difference in the high intensity group (0.004). Therefore, high intensity aerobic exercise for 4 weeks effect the concentrations of nitric oxide serum among obese individuals.

4. Discussion

The results show that one session of moderate or high intensity aerobic training does not significantly impact the amount of nitric oxide serum concentration of overweight people. These results do not match the findings of Olszanecka et al. (2008), Bechara et al. (2008), and Goto et al. (2003). The reason was probably related to the duration of training practice and the protocol practice (20-22).

In the training protocol of Bechara, the mice run for an hour with moderate intensity on the treadmill. However, in the current research, participants were obliged to continue moderate intensity training until they get exhausted. The average duration of running with moderate intensity in the current research was approximately 5.15 minutes (21).

In the research of Bechara, the amount of nitric oxide (density of nitrite and nitrate) in the training group in comparison with the control group increased. The results of this research indicate that one moderate intensity training session was able to make the vasoconstrictor response (whether it is dependent on the vascular adrenergic receptors or not) through an enhancement in nitric oxide of mice aorta. One of the major reasons for increment in bioavailability of nitric oxide, is probably enhancement in activity of eNOS enzyme after training. The effects of the training become clear by increasing both the shear stress and the catecholamine levels. More recently, it has been shown that the activity and the expression of the eNOS enzyme is increased by acute exposure of both endothelial cells and mice aorta exposed to shear stress and high levels of adrenergic agonists. In addition, this training was also able to increase endothelial secretion of calcium and release of nitric oxide, dependent on the calcium (21).

The other factor, which can be effective on the increase in nitric oxide bioavailability is the mechanisms related to the signals of super oxide anion, produced by the blood vessels. Superoxide anion is an important factor in the disabling of nitric oxide. In the current research, the sub-

Table 3. Paired t Test Results Before and After One Session of Aerobic Training

Nitric Oxide	Statistical Indicators			
	Diff Mean	T-Test	Df	P Value
Moderate intensity aerobic group	0.3	1	9	0.34
High intensity aerobic group	0.9	-1.711	9	0.12

Table 4. Paired t Test Results Before and After Four Weeks of Aerobic Training

Nitric Oxide	Statistical Indicators			
	Diff Mean	T-Test	Df	P Value
Moderate intensity aerobic group	0	1	9	1.0
High intensity aerobic group	4.5	-3.914	9	0.004

jects practiced to the brink of exhaustion in order to decrease the negative effects of oxidative stress on nitric oxide. In addition, the research showed that oxidative stress and other factors, such as lactic acid, would increase even in moderate intensity training. These can be considered as a reducer factor for releasing nitric oxide. It should be mentioned that the short period of training in the present research probably did not create adequate shear stress for stimulation of the release of NO.

In the research of Goto et al., 8 young males practiced on ergo meter bikes (30 minutes). Moderate intensity training increased the brachial blood flow from 1.1 ± 8.2 to 5.4 ± 1.6 mL/min/100mL. Goto et al. suggested that acute moderate intensity training activates dilation through increasing nitric oxide in human beings. The possible cause of this disparity is the long period of time in Goto's research in comparison with the duration in the current study (22).

Zahorska-Markiewicz et al. (2008) in their research determined the impact of obesity and overweightness on nitric oxide metabolites concentrations (nitrate and nitrite). They also studied the impact of training on activation of nitric oxide production in obese and lean females. The study group included 102 fat subjects, 24 overweight subjects, and 28 control group individuals. All subjects practice on an ergo meter bike and the workload increased every 3 minutes. The practice period for each subject was not more than 9 minutes. The test was finished when the subjects reached 85% of their maximum heart rate or they asked to stop the training due to fatigue and pain. The results of this research showed that the nitric oxide serum concentration was significantly high in fat and overweight groups in comparison with other group. But there was no significant difference in the nitric oxide serum level between fat and overweight groups. During the training, nitric oxide concentration significantly increased in all groups. There was no significant difference in nitric oxide serum level between overweight and fat groups and the control group after training. The value of ΔNO (nitric oxide after practice - rest nitric oxide) in the fat group was lower than others. However, there was no significant difference between fat, overweight, and control groups (20).

The main reason for this disparity between the present research and Zahorska-Markiewicz et al.'s research is probably differences in the training protocol. The training protocol used in the research of Zahorska-Markiewicz et al. was during a longer period and growing type that increased every 3 minutes. Using such a protocol will increase shear stress on vessels. Since the researchers did not find a relationship between nitric oxide and other factors like the concentration of lipid serums, glucose or insulin, it seems that according to the protocol, shear stress on the walls of the vessels is the main factor for increasing nitric oxide. Also, the possibility of releasing nitric oxide by other factors, like fat tissue and its benefit or harm is a theory that needs greater research.

In addition, the reason for the disparity between the present research and Goto et al.'s research is the period of training and protocol type. During Goto's research, the subjects were trained for 12 weeks at moderate intensity, while in this research, the practitioners followed a 4-week protocol. In Goto et al.'s research, the ergo meter bike was used and the duration of each practice session was 30 minutes. In the present research, the subjects continued to run at moderate intensity and until they reached exhaustion (approximately 15 minutes).

In addition, the results of the research showed that 4 weeks of high intensity aerobic training had a significant impact on the rate of nitric oxide serum density in fat individuals. These results match the findings of Gomes et al.

(2008) and Currie et al. (2009), and do not match the research done by Goto et al. (2003) (22-24).

Gomes et al. (2008) examined the relationship of nitric oxide in patients with metabolic syndrome. The intensity of training was 10% lower than aerobic threshold of the participants. The training was done using an ergo meter bike and the research period was 3 days a week and for 3 months. Each training session was 45 minutes. The increased production of nitric oxide in the practice group was significant. The training increased overall NO concentration and cGMP. Also it decreased the amount of both oxidative stress and ADMA concentration in circulation. The main training activity mechanisms, which lead to an increase in nitric oxide formation, have not been fully detected. The increase in vascular shear stress is certainly a major factor in the enhancement of nitric oxide concentration. Additionally, ADMA is a deterrent androgenize for nitric oxide synthesis. Recent evidence suggests that the increase in ADMA concentration enhances the risk of cardiovascular diseases. In this research, the ADMA levels in circulation were the same in patients with metabolic syndrome and healthy individuals of the control group before the training. However, these levels were significantly reduced after training, with at least some part being related to incensement in nitric oxide production. A similar reduction in the ADMA concentration after training in patients with diabetes mellitus has been reported (23).

In Goto et al.'s research (2003), the nitric oxide concentration in high intensity training did not significantly change in young healthy males. They suggested that the effect of increase of nitric oxide concentration in high-intensity training faded by the increment in oxidative stress. In this research, high intensity training was used until subjects reached the point of exhaustion. Therefore, it might decrease the effect of oxidative stress deterrence on the amount of nitric oxide release (22).

The SI unit for magnetic field strength H is A /m. However, if one wishes to use units of T, they must either refer to magnetic flux density B or magnetic field strength symbolized as μ_0H. The center dot is used to separate compound units, e.g. "A.m^2."

4.1. Conclusion

The results of this study showed that nitric oxide (NO) release in obese individuals was effected by the intensity of the training and the duration of the training. However, in regards to intensities, it was shown that both moderate intensity and high intensity trainings were effective on nitric oxide release, whether in human or animals, obese or non-obese cases. In regards to fat subjects, whether one session of activity is performed or a long term training, the high-intensity trainings impact on nitric oxide release. The mechanisms of nitric oxide release have not been fully identified. Generally speaking, most researches mentioned shear stress as the most important factor for nitric oxide release from endothelial cells.

Also, it seems that the most important factor for deterrent of nitric oxide release during training is incensement in oxidative stress and decrement in antioxidants. The negative effect of cardiovascular risk factors, such as triglycerides, oxidized low density lipoprotein (LDL), the amount of blood sugar, and insulin sensitivity on nitric oxide release, has been found. However, some researchers have shown that the increase in the intensity of nitric oxide occurs whether cardiovascular risk factors change or not. In the current research, it seems that the most important factor in nitric oxide release in high intensity training was shear stress created by the blood flow.

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