

# The Effect of a 12-Week Regular Aquatic Exercise on Plasma Levels of Nitric Oxide, Endothelin-1, and some Platelet Indices in Healthy Young Men

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## ABSTRACT

Nitric oxide is a potent vasodilator, and endothelin-1 is a potent vasoconstrictor peptide, which is produced by endothelial cells. The present study was conducted to examine the effect of a 12-week regular aquatic exercise program on plasma levels of nitric oxide, endothelin-1, and some platelet indices in healthy young men. The subjects were 24 healthy young men, who were randomly assigned to two groups of experimental (n=12) and control (n=12). Both groups were similar regarding their age and body mass index. Practice protocol in the experimental group included a 12-week swimming program (three sessions a week) with an intensity of 55-75% of the maximum heart rate. Participants in the control group did not take part in any regular physical activity program during the study. Blood samples were taken 48 hours after the initiation of the practice protocol for measuring levels of nitric oxide, endothelin-1, and some platelet indices. Independent and dependent t-tests were run to compare the means of the examined parameters ( $\alpha=0.05$ ). The results indicated that, in the experimental group, the level of nitric oxide increased ( $p=0.003$ ); but, the level of endothelin-1 ( $p=0.029$ ), platelet count ( $p=0.004$ ), mean platelet volume (MPV) ( $p=0.29$ ) and platelet count percentage ( $p=0.001$ ) significantly decreased. Between-group comparison showed that changes in the level of nitric oxide ( $p=0.035$ ) and platelet count percentage ( $p=0.003$ ) were significant, while those of platelet count ( $p=0.066$ ) and MPV ( $p=0.62$ ) were not significant statistically. Based on the results of this study, aquatic aerobic exercise can reduce the risk of hypertension in healthy young men by increasing the level of nitric oxide and decreasing the level of endothelin-1.

**Keywords:** Aquatic Aerobic Exercise, Nitric Oxide, Endothelin-1, Platelet Indices

## Introduction

The cardiovascular system functions in a way to maintain the levels of homeostasis necessary for the functioning of the body, and be responsive to its threatening factors. Heart disease and

stroke are the most common causes of death among all races and nations. The rate of mortality caused from cardiovascular diseases increases with age.<sup>[1]</sup> The cause of most cardiovascular diseases is inflammation. Reduced arterial elasticity and vascular endothelial dysfunction are two important factors affecting vascular function. Endothelial dysfunction seems to be a major contributing factor in the incidence of atherosclerosis, hypertension and heart diseases.<sup>[2]</sup> Vascular stimulation and its damages, caused by the consumption of certain materials, directly -and sometimes indirectly (e.g., increased shear stress)- increase the sensitivity of vascular endothelial cells such as nitric oxide (NO) and endothelin-1 (ET-1).<sup>[3]</sup> As a vasodilator factor secreted from vascular endothelium cells, NO plays a key role in regulating cerebral blood flow and maintaining the health of vascular walls.<sup>[4]</sup> NO plays an important role in protecting

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vascular walls and regulating vascular constriction and dilation; nitric oxide synthases produce NO from L-arginin.<sup>[5]</sup> Physical activity can protect the cardiovascular system by reducing risk factors for cardiovascular diseases (e.g., normalizing high blood pressure). Reducing risk factors also reduces the rate of mortality, which is partly achieved due to the direct effects of physical activities on vascular health.<sup>[6]</sup> Sequential changes in shear stress and its increase due to regular physical activities lead to increased NO bioactivity and improved vascular endothelial function.<sup>[7]</sup> Physical activities improve vascular endothelial function, which is one of the indicators of endothelial function, i.e., vasodilation response due to increased blood flow.<sup>[8]</sup>

On the other hand, ET-1 is a potent vasoconstrictor peptide, which is produced by endothelial cells; it has a strong contractile effect on endothelial cells of human arteries.<sup>[3]</sup> ET-1 is involved in the development of atherosclerosis. In arteries narrowed due to atherosclerosis, cellular changes are followed by a disrupted calcium ion transfer process.<sup>[9]</sup> Results of various studies have shown that physical activity has different effects on ET-1 concentration. Some researchers have shown the effectiveness of a 12-week regular aquatic exercise in reducing the concentration of ET-1 in mice plasma,<sup>[10]</sup> while others have emphasized that physical activity does not affect the level of ET-1.<sup>[11]</sup> Therefore, researchers have not yet come to a consensus on the effectiveness of physical activity in reducing ET-1 in human plasma. It seems that regulating body's tropic hormones by physical activity, changing body weight and total fat mass, and increasing the strength and capacity of skeletal muscles around body vessels all reduce the body's need for the function of vascular endothelial cells, and thus, they reduce their secretory function.<sup>[12]</sup>

The main physiological function of platelets is to form hemostatic plaques.<sup>[13]</sup> In physiological conditions, platelets, moving in the bloodstream alongside the vascular walls, are inactive due to the protective effect of the single-layer set of endothelial cells and the release of inhibitory mediators such as NO and PGI<sub>2</sub> from healthy endothelium tissue.<sup>[14]</sup> Some researchers have examined the effects of the intensity of various physical activities on platelets.<sup>[15]</sup> They investigated the relationship between platelet response and NO changes in inactive people following the implementation of severe and moderate endurance exercises and found that severe-intensity endurance exercise increases the ADP platelet stimulants, collagen, and platelet aggregation, while moderate-intensity endurance exercise reduces the sensitivity and reactivity of platelets to the stimulants and platelet aggregation.<sup>[16]</sup> Physical exercises usually are preformed as endurance, anaerobic or aerobic activities. In most of the previous studies, land-based physical activities have been examined; however, most people prefer to do aquatic exercises due to the difficulties and damages they experience while exercising on land. People experience less pressure on their lower body parts and joints in water due to the upward force that water exerts on their bodies. Moreover, long-term aerobic exercises have positive effects on intracellular adaptation and various hormones

secreted from endothelial cells. Hence, the present study aimed to investigate the effect of a 12-week regular aquatic exercise on plasma levels of NO, ET-1 and some platelet indices in a group of healthy young men.

## Methodology

The present study was quasi-experimental field research. Among people willing to participate in the present study, 24 healthy young men, who could regularly participate in the designed training program and met the study's inclusion criteria, were selected and randomly divided into the two groups of external (n=12) and control (n=12). The most important inclusion criteria included having no specific disease (e.g., heart or metabolic diseases), blood pressure higher than 140/90 mmHg and having a Body Mass Index (BMI) in the range of 18.5-25 Kg/m<sup>2</sup>. Participants in the experimental group participated in a 12-week (three sessions a week) practice protocol, while those in the control group continued their inactive lifestyle. Every participant, fasting for 12 hours, was present at the laboratory between 8:00 and 10:00 AM two days before the initiation of the workout program. To determine the levels of examined factors, 10 ml of blood was taken from each participant in a resting position (pre-test). Participants in the experimental group started their aquatic aerobics 48 hours after their blood samples were taken. The practice protocol included a 12-week (three sessions a week) aquatic aerobic exercise program (i.e., walking in water) with an intensity of 55-75% of the maximum heart rate (HRmax). A polar watch was given to each participant to measure his heart rate and the intensity of his exercise. Each participant's HRmax was calculated based on the formula 'age-220=HRmax'. In line with the overload principle, both intensity and duration of the workout sessions increased in five stages: in the first stage (the first eight sessions), the intensity and duration of the exercise were respectively 55% of HRmax and 40 minutes; in the second stage (the second eight sessions), the intensity and duration of the exercise were respectively 60% of HRmax and 45 minutes; in the third stage (the third eight sessions), the intensity and duration of the exercise were respectively 65% of HRmax and 50 minutes; in the fourth stage (the fourth eight sessions), the intensity and duration of the exercise were respectively 70% of HRmax and 55 minutes; and finally, the last stage (the last four sessions), the intensity and duration of the exercise were respectively 75% of HRmax and 60 minutes. The aquatic aerobic exercise was performed in the semi-deep part of a pool, and at the temperature of 26-28° C. Participants in the control group did not participate in any regular training program during the study period. To avoid the possible effect of training on the results, 48 hours after the last session of the workout program, blood samples of all participants were taken again in a similar condition to the pre-test condition (post-test). The measurement of variables was done simultaneously in the two groups of experimental and control.

At each stage of blood sampling, blood samples were immediately poured to EDTA-containing tubes. After being

centrifuged at 3000 rpm for 10 minutes, blood plasma was isolated and stored in special microtubes and at the temperature of  $-80^{\circ}\text{C}$ . NO plasma levels were measured with the sensitivity of  $10\ \mu\text{mol/L}$  and based on the ELISA technique and using a Chinese kit made by the Bosterbio Corporation. ET-1 level was determined via the ELISA technique and a Japanese kit made by the Cusabio Corporation. The factors PLT, MPV, PCT and PDW, were measured via the Chinese cellular reader Mindray 5500-5150. Results of the Shapiro-Wilk test showed that the examined parameters were distributed normally.

Dependent and independent t-tests were run at the  $\alpha=0.05$  level to compare the means of examined parameters.

## Results

Anthropometric and aerobic fitness characteristics of both groups are presented separately in table (1). As shown in table (1), weight and BMI significantly decreased in the experimental group.

**Table 1: Mean and SD of a general profile and VO<sub>2</sub> max in subjects**

Variables	Control group			Exercise group		
	Pre-test	Post-test	P	Pre-test	Post-test	P
Age (year)	21.1±1.9	-	-	20.9±1.6	-	-
Height (m)	1.75±0.6	-	-	1.75±0.5	-	-
Weight (kg)	74.5±5	74.4±4.9	0.085	74.1±4.9	73.1±4.3	0.001
BMI (Kg/m <sup>2</sup> )	24.3±0.8	24.2±0.8	0.093	24.2±0.9	23.9±0.8	0.001

Results of within-group comparison (table 2) showed that the resting level of NO significantly increased ( $p=0.003$ ) and the level of ET-1 ( $P=0.029$ ), platelet count ( $p=0.003$ ), MPV ( $p=0.029$ ), and platelet count percentage ( $p=0.001$ ) significantly decreased in the experimental group after a 12-week aquatic aerobic exercise program.

Results of between-group comparison (table 2) indicated that changes in NO ( $p=0.035$ ) and platelet count percentage ( $p=0.003$ ) were significantly different between the two groups; but, the level of ET-1 ( $p=0.81$ ), platelet count ( $p=0.066$ ), and MPV ( $p=0.62$ ) were not statistically different between the two groups.

**Table 2: a comparison of intergroup and within group variations of biochemical indexes in subjects**

Variables	Group	Pre-test	Post-test	P	P
		Mean and SD	Mean and SD	In-group	intergroup
NO ( $\mu\text{mol/L}$ )	Exercise	11.48±1.45	12.52±1.52	*P=0.003	¥P=0.035
	Control	11.01±1.45	11.14±1.48	P=0.383	
ET-1 (pg/mL)	Exercise	1.47±0.4	1.38±0.33	*P=0.029	P=0.81
	Control	1.42±0.3	1.41±0.32	P=0.961	
PLT ( $10^3/\mu\text{L}$ )	Exercise	245±38	216±27	*P=0.003	P=0.066
	Control	244±39	243±31	P=0.064	
MPV (fL)	Exercise	10.3±1.1	10.1±0.96	*P=0.029	P=0.62
	Control	10.3±1.1	10.2±1.1	P=0.754	
PCT	Exercise	0.25±0.02	0.22±0.01	*P=0.001	¥P=0.003
	Control	0.25±0.02	0.24±0.02	*P=0.031	
PDW	Exercise	16.5±0.4	16.5±0.5	P=0.27	P=0.85
	Control	16.5±0.4	16.5±0.4	P=0.674	

\*In-group Statistical significance; ¥ intergroup Statistical significance

## Discussion and Conclusions

Reduced arterial elasticity and vascular endothelial dysfunction are two important factors affecting vascular function.<sup>[13]</sup> Endothelial dysfunction seems to be a major contributing factor in the incidence of atherosclerosis, hypertension and heart diseases.<sup>[17]</sup> Based on the results of this study, the concentration of NO significantly increased in the experimental group after a

12-week aquatic exercise program. The increase in NO concentration was also observed in between-group changes, which was in line with results of <sup>[18]</sup> and <sup>[19]</sup>, but not consistent with results of <sup>[20]</sup>. There are only a few studies on the concentration of NO in healthy men. Most of the studies, conducted on subjects with NO-related cardiac and vasodilation problems, have shown an improvement in conductive or resistance function of the arteries; but, studies on healthy

subjects have not shown similar levels of improvement, indicating that reduced endothelial function in sick subjects is more likely to increase than normal endothelial function in healthy subjects.<sup>[5]</sup> In their study, Kazemi et al. found that the level of NO significantly decreased following a severe exercise program; while a light exercise program led to an increase in plasma NO level. Thus, they concluded that light exercise program with moderate intensity increased NO and improved vascular endothelial function.<sup>[21]</sup> Physiological stimulants have been considered a probable cause of NO production and blood flow increase in the vessels; the acute effect of physiological stimulants increases nitric oxide syntheses and regulates vascular dilation to balance blood pressure. These results reinforce the possibility that regular exercise may increase NO bioactivity as it repeatedly increases the heart rate.<sup>[5]</sup> Another mechanism for improving endothelial function is increasing vascular endothelial growth factor. It has been proven that regular physical activity increases various angiogenic factors, such as vascular endothelial and fibroblast growth factors. Moreover, physical exercise causes angiogenesis, stimulates endothelial NO enzyme phosphorylation and increases NO production by increasing the volume of vascular endothelial growth factor. Researchers believe that regular physical activity improves endothelial function by reducing oxygen free radicals.<sup>[22, 23]</sup>

On the other hand, the 12-week aquatic exercise program used in this study significantly reduced the level of ET-1 in the experimental group. Researchers have not yet come to a consensus on how physical activity affects ET-1 level in human plasma. It seems that regulating body's tropic hormones by physical activity, changing body weight and total fat mass, and increasing the strength and capacity of skeletal muscles around body vessels all reduce the body's need for the function of vascular endothelial cells, and hence, they reduce discharging substances into the plasma. Decreased ET-1 concentration in the blood probably lowers blood pressure, decreases the risk of cardiac diseases and atherosclerosis and prevents renal ischemia and other problems associated with increased ET-1 level in the blood. Moreover, various rheological and neuromuscular factors (e.g., angiotensin ii, arginine, & vasopressin) are involved in the production of ET-1. The mentioned issues may be reduced by aerobic exercises, which is a reason supporting that a significant decrease in plasma ET-1 may reduce the risk of hypertension and its symptoms and control the concentration of angiotensin and similar substances, and thus, indirectly reduce symptoms caused by high concentration of those substances.<sup>[12]</sup> These results were consistent with the results of<sup>[24]</sup> and<sup>[25]</sup> concerning the effectiveness of physical activity in reducing plasma ET-1 concentration. However, these findings were not in line with results of<sup>[26]</sup> and<sup>[27]</sup> who reported that physical activity did not affect the concentration of ET-1.

According to the results of this study, the 12-week regular aquatic exercise program significantly reduced platelet count, MPV and platelet count percentage in the experimental group. Results of previous studies, whose results were in line with those of the present study, indicated that platelet count

decreased at the end of endurance exercises. Regular endurance exercise can improve antioxidant defense mechanism, increase levels of HDL-C and reduce platelet aggregation rate by simultaneously increasing the amount of L-arginine transfer and levels of plasma and intraplatelet NO.<sup>[28]</sup> It is worth noting that there is still a controversy about the effect of exercise on platelet count and platelet activation indices.<sup>[29]</sup> Such differences can be attributed to factors such as exercise stress and physiological, nutritional, and even psychological and neurological factors.<sup>[30]</sup> Moreover, the duration and intensity of exercise along with the time of blood-taking process may affect the results.<sup>[31]</sup>

Changes in the levels of PCT are not related only to the release of new platelets. Indeed, they are also related to the type of physical activity, its intensity and duration, training time and blood sampling method.<sup>[32, 33]</sup> Changes in PCT during the training period can be due to the time and condition of blood sampling (resting position in the case of the present study) and also body temperature of the subjects relative to the time of their activity.

There is much evidence that MPV is a major biologic variable and that larger platelets, which are characterized by an increase in MPV, have more thrombotic potentials. Thus, MPV has the potential to be used as a factor for platelet activation. Generally, long-term exercises decrease MPV by increasing prostacyclin and NO secretion and antioxidant activities and reducing LDL oxidation and the effects of increased ADP and Ca.<sup>[34, 35]</sup> The results of this study showed that aquatic aerobic exercise significantly decreased MPV. Therefore, one of the essential platelet indices, showing the formation of thrombosis in blood vessels, decreased, indicating the positive effects of the aerobic program on platelet indices. Among possible mechanisms, reduced oxidative stress and the effects of ADP and Ca can be mentioned.<sup>[34]</sup> Furthermore, regular and long-term exercises increase NO and improve endothelial function, leading to the increase of vascular dilatation and, consequently, improvement of blood flow and reduction of MPV.<sup>[35]</sup>

## Conclusion

Results of the present study showed that aquatic aerobic exercise increased the NO level, decreased the ET-1 level, and improved platelet indices. These results showed that aquatic aerobic exercise had positive and therapeutic effects on factors causing cardiovascular diseases among young men. Considering the role and importance of the examined indices, more studies are needed to determine the long-term effects of physical activity on cardiovascular risk factors among young men.

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