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ORIGINAL RESEARCH

The effect of omega-3 fatty acid supplementation on Nitric oxide (NO) serum levels and anthropometric indices in men with cardiovascular disease referred to Tehran Heart Center: A randomized, double-blind, placebo-controlled trial

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Abstract

Objective: Cardiovascular diseases (CVDs) are among the most common non-communicable diseases. They are the leading cause of death worldwide. Omega-3 unsaturated fatty acids are not produced in the human body, so they are essential for humans and are vital for the regulation of human performance. The aim of this study was to evaluate the effect of omega-3 fatty acid supplementation on Nitric oxide (NO) serum levels and anthropometric indices in men with cardiovascular disease.
Materials and Methods: In this double-blind study, 40 male Wistar rats were divided into 4 groups (N: 10). The groups were: Control group, supplement group, exercise group, and exercise + supplement group. At the end, blood was drawn from all the animals, then the serum lipid profile, were measured by ELISA method. They were analyzed with SPSS and a significance level of <0.05.
Results: No statistically significant difference was observed in any of the anthropometric indicators and blood pressure levels (systolic and diastolic) before and after the intervention, as well as within the groups. Also, no significant difference was observed in the serum level of NO before and after the start of the study in both groups, and within each group before and after the intervention, there was no statistically significant difference (P>0.05).
Conclusion: The findings of the present study showed that 10 weeks of supplementing with omega-3 supplementation at a dose of 4 grams per day, didn't cause any significant changes in serum levels of NO.
Keywords: Omega-3 fatty acid, Nitric oxide (NO), Anthropometric indices, Cardiovascular disease

Introduction

Cardiovascular diseases are the major causes of death and disability globally. The formation of atherosclerotic plaques in the coronary artery wall is observed in these patients and leads to clinical symptoms of acute coronary syndromes such as angina and myocardial infarction (1). CVD risk factors include age, male gender, family history of CVD, hypertension, high cholesterol, smoking, diabetes mellitus, socioeconomic status and obesity (2).

Omega-3 fatty acids are a member of essential fatty acids which the human body is not able to synthesize them (3). α -Linolenic acid (ALA) which is a with an 18-carbon chain, is found in vegetable oils such as flaxseed, canola and soybean (4,5). EPA and DHA omega-3 fatty acids, play an important role in primary and secondary prevention of cardiovascular diseases and sudden deaths (6). Epidemiological studies have shown a lower risk of mortality due to cardiovascular diseases among those who consume more fish. EPA and DHA rich diet reduces triglycerides (TG) and oxidative stress and also increases *high-density lipoproteins* (*HDL*) and antioxidant concentration (7).

Several mechanisms have been introduced in this regard, such as anti-inflammatory effects, direct role in gene expression (genes related to immune response, inflammation, lipid and energy metabolism), anti-arrhythmic and anti-thrombosis effects, lowering blood pressure, improve endothelial function, reduces triglycerides in people with a history of *hypertriglyceridemia* and delays in the development of atherosclerotic plaques (8,9).

Oxidative stress is the major factor in the pathogenesis of endothelial dysfunction and many CVD risk factors (10). Several epidemiological studies have shown that diets rich in antioxidants like fruits, vegetables, nuts and whole grains are associated with lower risk of cardiovascular disease and it's mortality (11).

LDL oxidation could be the early stages of atherosclerosis which leads to the formation of foam cells and endothelial injury (12,13). Nitric Oxide (NO) is an internal mediator with vascular expansion features and is known as a mediator molecule in many biological and physiological functions (14). Today NO is known as a key signaling molecule in the cardiovascular and nervous system which plays an important role in the antioxidant defence system (15).

One of the most important substances produced by the endothelium is NO, which plays an important role in vasodilatation, inflammation, and oxidative stress. Suppressing the activity of NO can cause vasoconstriction, which ultimately shows itself as Ischemia. Moreover, suppressing the activity of NO can facilitate the process of vascular inflammation, which contributes to plaque formation in the blood vessels. Reduced production of NO in pathological conditions such as cardiovascular disease, high blood pressure and etc, can lead to serious problems in the integrity of endothelial. That is why the need for drug intervention is increased in order to release NO from the endothelium (16). Previous studies have shown that omega-3 fatty acids can increase the release of NO in diabetic patients (17,18) and athletes (19).

Since the interventions to increase levels of NO is one of the necessary actions to prevent the development of cardiovascular diseases, and because no study has done in these patients, the aim of this study was to investigate the effects of omega-3 fatty acid supplementation on NO and serum levels and anthropometric indices in male patients with cardiovascular disorders.

Materials and Methods

The current study was performed as a double-blind clinical trial. 42 patients of Tehran heart hospital were selected based on inclusion and exclusion criteria. The subjects were divided into two groups of omega-3 and placebo receiver by randomized permitted block. Written consent was obtained from all study participants. A general questionnaire was completed. The blood pressure was measured by a digital sphygmomanometer in the sitting position and after about 5-10 minutes of sitting. Height was measured by a wall stadiometer and without shoes. Weight was measured by a digital Seca scale without shoes and with light clothing. Waist circumference was measured as the midpoint between the last rib and iliac crest with a tape and without making pressure on body surface. Hip circumference was measured based on the largest circumference around the hip using a tape.

Bioelectrical Impedance Analysis (BIA) was used in order to measure the fat mass and fat free mass (FFM). 10 cc venous blood samples

were taken from all subjects and then the serum was separated by centrifuged at 3000 rpm for 10 minutes. At the end, omega-3 supplements at a dose of 4 grams per day, or placebo (oral paraffin) with the same dose, were given to participants for two months. Physical activity questionnaire and 24-hour food recall were completed again after 2 month and weight, waist circumference, hip circumference and blood pressure were measured too. Also another blood sampling was done at the end of study. Serum nitric oxide (NO) was measured by ELISA method at the beginning and end of the study.

In this study, data analysis was performed using SPSS version 25.0 and significance was set as $p<0.05$.

Results

The patients were divided into 2 groups of omega-3 supplement and placebo receiver by random. And each group contained 21 individuals. Of the 42 participants who attended the first visit, 36 subject (16 in placebo group and 20 in supplement group) returned for the second visit.

The participant's mean age was 54.44±1.56 years in supplement group and 56.24±1.56 years in placebo group and the difference was not significant ($p>0.05$).

Also, there was no meaningful difference between two visits in the case of BMI, weight, waist circumference, hip circumference, waist to hip ratio, blood pressure and physical activity level in both groups and within groups. Also, no significant difference was observed in the average of changes (diff) of indexes during the intervention in both groups.

The NO serum level in both groups at the beginning and at the end of study is mentioned in µmol /lit in Table 4. This factor increased slightly only in placebo group, but the difference was not significant statistically.

According to the statistical analysis, which was carried out before and after the study, no significant difference was seen in the amount of NO between two groups and within groups.

Also comparing the average difference (diff) of serum levels of NO did not show significant difference between 2 groups during two visits.

Table1. Anthropometric indices of patients receiving omega-3 fatty acids supplement and placebo; Before and after the intervention [&]

		Omega-3 group N=18	Placebo group N=17	P-value[#]
Height (cm)	before	169.1±1.54	172.1±3.45	0.15
Weight (kg)	before	81.2±8.06	79.1±9.58	0.73
	after	81.2±1.77	81.1±6.07	0.97
BMI (kg/m^2)	before	28.0±3.28	26.0±9.76	0.21
	after	28.0±3.48	27.0±3.56	0.35
WC (cm)	before	99.2±8.01	96.1±9.49	0.33
	after	99.2±3.83	97.1±2.38	0.44
HC (cm)	before	101.1±7.56	100.0±5.29	0.52
	after	101.1±3.34	100.0±1.19	0.49
WHR	before	0.0±9.80	0.0±9.60	0.37
	after	0.0±9.70	0.0±9.70	0.58
FM (kg)	before	20.1±4.06	16.1±8.25	0.16
	after	20.1±7.74	18.1±8.92	0.34
Total FM (%)	before	24.1±5.55	21.1±3.27	0.17
	after	25.1±1.43	23.1±5.82	0.40
FFM (kg)	before	60.1±3.57	61.1±3.85	0.66
	after	60.2±6.81	60.1±8.47	0.95
FFM (%)	before	75.1±4.65	78.1±6.97	0.17
	after	74.1±8.43	76.1±4.12	0.39

[#] Independent t-test
[&] mean ± standard error (SE)
BMI: Body Mass Index; WC: Waist Circumference; HC: Hip Circumference; WHR: Waist (circumference) / Hip (circumference) Ratio; FM: Fat Mass; FFM: Fat Free Mass.

Table 2. Blood Pressure of patients receiving omega-3 fatty acids supplement and placebo; Before and after the intervention [&]

		Omega-3 group N=18	Placebo group N=17	P-value[#]
Systolic blood pressure (mmHg)	before	126.3±2.29	124.4±3.55	0.75
	after	122.3±2.82	128.4±7.15	0.26
diastolic blood pressure (mmHg)	before	80.2±8.35	80.2±1.27	0.84
	after	80.2±6.73	82.2±2.99	0.67

[#] Independent t-test
[&] mean ± standard error (SE)

Table 3. Serum NO level of patients receiving omega-3 fatty acids supplement and placebo; before and after the intervention[&]

		Omega-3 group N=20	Placebo group N=16	P-value[#]
NO (µmol/l)	before	13.1±5.70	11.1±7.14	0.245
	after	13.1±3.92	12.0±3.99	0.520
	P-value[*]	0.889	0.469	----
	average of changes (diff)	0.0±2.16	0.9±6.76	0.586

[*] Paired t test
[#] Independent t-test
[&] mean ± standard error (SE)
NO: Nitric Oxide; diff: difference.

Discussion

This study is the first clinical trial which has assessed the effect of omega-3 supplementation on serum levels of NO in male patients with cardiovascular diseases. The results showed no significant relationship in both case and control groups within and between groups during the intervention in terms of anthropometric indices such as weight, BMI, waist circumference and waist to hip ratio. Also, by comparing the mean changes at the baseline and at the end of intervention in both groups, there was no significant relationship with anthropometric indices.

There are few studies in the field of evaluating the effect of omega-3 fatty acids on anthropometric indices. In addition, there are inconsistent results in different studies.

Hajianfar and his colleagues observed a significant reduction in weight, BMI and waist

circumference after 8 weeks of supplementation with omega-3 fatty acids in people with type 2 diabetes (20).
In Ansari et al. study waist circumference was significantly decreased after 8 weeks of supplementation with omega-3 fatty acids on obese subjects (3).
Another study which was done on obese and overweight subjects found that by increasing the content of omega-3 fatty acids in the diet to 3.6% of total energy expenditure, no change in the weight will be seen (21).
Also in this study, systolic and diastolic blood pressure didn't change significantly in both groups before and after the intervention. Comparison the mean differences at the beginning and at the end of intervention in both groups was not significant in relation to blood pressure.
There are many studies which have addressed the effects of omega-3 fatty acids on reducing blood pressure.
Cabo et al. study showed that consumption of polyunsaturated fatty acids (PUFA) may reduce blood pressure in people with mild hypertension who have not yet started drug therapy (22).
In epidemiological studies, it was observed that consumption of fish oil containing omega-3 fatty acids are beneficial in the prevention of arteriosclerosis and thrombosis (23,24).
Also, Emami Naini et al. study showed that fish oil intake may reduce blood pressure by increasing urinary sodium and decreasing plasma rennin activity (25).
The effect of different doses of omega-3 and omega-6 fatty acids in patients with high blood pressure were examined in one study. The results of this study showed that taking high doses of fish oil reduces blood pressure in men with high blood pressure. Also in this study the vasodilatory effect of prostaglandins was increased (26).
In a review article it was suggested that consumption of omega-3 fatty acids can be effective in the prevention of atherosclerosis and blood pressure control (22).
In line with our results, Donnelly et al. study indicated that supplementation with fish oil capsules in dialysis patients does not cause any change in their blood pressure (27).
In general, the use of different doses of omega-3 fatty acids, in different age groups and with a wide range of diseases, there are conflicting results on the effect of omega-3 fatty acids on systolic and diastolic blood pressure.
The current study found that after 10 weeks of supplementation with omega-3 doses of 4 grams per day no significant difference was observed in and NO. Also no significant difference was seen between the mean change in serum levels of this factors during the intervention.
Ebrahimi et al. divided 120 patients with metabolic syndrome into two groups by random. Sixty patients consumed 1 gram of fish oil capsules containing 180 mg EPA and 120 mg DHA for 6 months. Control group received no supplementation during the same period. The study was completed by remaining 47 patients in the intervention group and 42 patients in the control group. Omega-3 fatty acid supplementation significantly reduced body weight, systolic blood pressure, LDL, total cholesterol, TG, hs-CRP and the heat shock protein-27 antibody titers. No significant change was observed in the control group. In general, it seems that omega-3 fatty acids can improve the risk of cardiovascular diseases in people with metabolic syndrome (28).
Aliasghari et al. divided 90 patients with atherosclerosis into 3 groups of receiving CLA, omega-3 fatty acid and placebo. The intervention was lasted for months. C-reactive protein level significantly decreased in both Groups of CLA and omega-3 fatty acids. IL-6 also had a significant decrease in the group receiving omega-3 fatty acids compared to placebo. Glutathione peroxidase activity in both groups increased significantly and the level of malondialdehyde in both groups showed a significant decrease. As a result, conjugated linoleic acid and omega-3 fatty acids, could reduce inflammation and oxidative stress in patients with atherosclerosis (29).
Kabir and his colleagues showed that taking low doses of omega-3 fatty acids for a period of 2 months, reduces atherogenic markers. Type 2 diabetic patients were divided into two groups of receiving 3 grams of fish oil and control group. It was shown that omega-3 fatty acids reduce the expression of some genes related to inflammation, which were released from fat tissue. This reduction can be accompanied with inflammatory and morphologic changes in adipose tissue (23).
Dawczynski and his colleagues divided 51 individuals to 2 groups of dairy products

fortified with 3 grams of omega-3 fatty acids and usual dairy products. The study lasted for 15 weeks. It was shown that the omega-3 index and the ratio of arachidonic acid / EPA, cholesterol and triglycerides, and the LDL / HDL improved. Also it was shown that dairy products fortified with omega-3 fatty acid, reduce the risk factors for cardiovascular disease (24).

Johansen et al. studied the effect of omega-3 fatty acids on 51 patients with cardiovascular diseases. Finally, they found that omega-3 fatty acid supplementation reduces markers of hemostatic including atherosclerosis, whereas inflammatory markers (VCAM1 _ Eselectin) can be increased (26).

Diego Lopez et al. performed a survey in which rats used corn oil and fish oil for 8 weeks. As a result the production of aortic NO and alpha-tocopherol content were increased and also omega-3 poly unsaturated fatty acids entered LDL and VLDL particles and played an important role in anti-atherogenic effects of fish oil (30).

Umit Mutlu-Tu¨rkog˘lua and his colleagues examined the oxidative changes of lipids, proteins and DNA in 30 patients with CAD and 30 healthy subjects. Plasma levels of MDA and PCO were measured in this study. In the case group compared with the control group, the MDA and PCO level were increased. Also the results showed an increase in oxidative changes of lipids, proteins and DNA in patients with CAD (31).

Veigh et al. examined the effects of fish oil on the release and production of NO in patients with type 2 diabetes. The results showed that 6 weeks of supplementation with fish oil significantly increases the release of NO and improves the endothelial status (18).

In a study the effect of omega-3 supplementation on endothelial function in athletes was assessed. In this study 2.6 grams of omega-3 fatty acids was used daily for 3 weeks. Finally, serum NO level was significantly increased in omega-3 group. Omega-3 supplementation at a dose of 4 grams per day, didn't cause any significant changes in serum levels of NO.

This is the only study which has assessed the effect of omega-3 fatty acids on serum levels of NO in male patients with cardiovascular diseases and it was a double-blind trial also.

Because of the short duration of the study and considering the impact of gender as well as a lower incidence of cardiovascular disease in women, the study was conducted only in men.

Conflict of interest

Author declares no conflict of interest.

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