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The Effect of Omega-3 Fatty Acids on Serum Apelin Levels in Cardiovascular Disease: A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Background: Cardiovascular disease (CVD) is the leading cause of mortality worldwide. Omega-3 fatty acids have been shown to have both anti-atherogenic and anti-inflammatory effects through inducing the expression and production of adipokines. Adipokines such as apelin, have been observed to play a protective role in the incidence and progression of CVD. The aim of this study was to assess the influence of omega-3 fatty acids supplementation on the serum apelin levels in patients with cardiovascular disease.

Methods: Forty-six male patients with CVD participated in the study. Patients were randomly allocated into two groups receiving either omega-3 fatty acids or a placebo. Participants received 4 g of omega-3 fatty acids (EPA: 720 mg, DHA: 480 mg) or a placebo (edible paraffin) for 8 weeks. Serum apelin levels, high sensitive C-reactive protein (hs-CRP), and lipid profiles were measured. Dietary intake, anthropometric parameters, body composition, systolic and diastolic blood pressure were evaluated before and after the 8 weeks of intervention. Statistical analyses were performed using SPSS version 22.

Results: Two participants from the placebo group withdrew from the study. Prior to the intervention, no significant differences were present between the two groups in age, body mass index, body composition, dietary intakes, lipid profiles and blood pressure. Compared to placebo, the intake of omega-3 fatty acids increased serum apelin levels ($p= 0.018$), decreased the levels of LDL cholesterol, and decreased serum hs-CRP concentrations ($p= 0.007$, $p= 0.011$ respectively). Additionally, the concentrations of VLDL, TG and hs-CRP ($p= 0.037$, $p= 0.037$ and $p= 0.016$ respectively) declined compared to baseline and final values in the omega-3 fatty acids group.

Conclusions: Omega-3 fatty acid supplementation increases serum apelin and HDL concentrations, while decreasing serum LDL-C and hs-CRP levels.

Keywords: Adipokines, Apelin, Cardiovascular disease, Omega-3 fatty acids.

Introduction

Worldwide, cardiovascular disease (CVD) ranks as the main cause of death (1), inflicting a substantial economic burden on society (2). Atherosclerosis is a chronic inflammatory disease of the artery walls characterized by the accumulation of macrophages and T cells. Atherosclerotic plaque can begin early on in life and gradually build and progress with age (3). The global prevalence of carotid atherosclerosis is 25.4% and 26.4% in men and women, respectively (4). According to the 2015 world health organization (WHO) report, CVD accounted for 31% of all deaths (5).

Omega-3 fatty acids have the potential to delay CVD and decrease the incidence of obesity and type

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2 diabetes. Additionally, omega-3 fatty acids have been observed to display anti-atherogenic, anti-inflammatory and lipid lowering effects (6). The fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) –which are effective component of omega-3 fatty acids- have been shown to have an important role in decreasing the incidence of CVD. Therefore, EPA and DHA represent a potential preventive and therapeutic approach to atherosclerosis and myocardium infarction (7). Various studies have shown omega-3 fatty acids to have an influence on adipokine expression and production (6). G-protein coupled receptors and their ligands have been shown to hold essential roles in proper cardiovascular functioning (8). One such receptor-ligand pair involves the APJ receptor and the apelin ligand. The apelin-APJ pathway has been shown to be a vital regulatory factor of cardiovascular functioning (9, 10).

The apelin protein originates from a 77 amino acid preproprotein, which is cleaved through means of peptidase activity into mature apelin peptides of varying lengths (11). The thirteen amino acid peptide (apelin-13) is the prominent variant of apelin within the blood stream and myocardium. This variant of apelin has the highest affinity for the APJ receptor (11, 12). The presence of apelin has been shown to slow the development of atherosclerosis and decreases the incidence of CVD (9, 10).

Apelin has been shown to have a diverse range of protective effects on the cardiovascular system including, lowering blood pressure (13), promoting artery relaxation (14), angiogenesis (15), diminishing systematic vein tone (16), providing protection against vascular injuries and decreasing left ventricular pre and after loads (17). The aim of this study was to examine the how omega-3 fatty acid supplementation may influence the levels of apelin levels among male patients with CVD.

Materials and methods

Subjects and study design

Forty-six male patients with CVD, who were referred to Tehran heart center, ages 45-55 and with a BMI less than 30 kg /m2, were enrolled in the study. The IRCT registry code is: NCT02117960.

The selected patients had at least 50% occlusion in one coronary artery as defined by angiography a month prior to beginning the study. Participants were informed about the purpose and goal of the study before deciding to participate. Patients with diabetes, thyroid, liver, kidney and gastrointestinal diseases were excluded from the study. Individuals having previously consumed warfarin or omega-3 fatty acid supplements 3 months before the study, as well as any other vitamin or dietary supplement during the last 4-6 weeks were excluded. Throughout the duration of the trial patients were to refrain from consuming any additional supplements. Individuals included in the study had no history of coronary artery surgery, did not exceed 5 cigarettes per day 6 months prior to the study, and did not partake in strenuous physical activity. Patients allergic to omega-3 fatty acids were excluded. Subjects with an unwillingness to continue with the project, those that failed to comply, changed the dose or kind of medication, suffered from an inflammatory disease, or the long term use of anti-inflammatory drugs were not included.

Patients were randomly distributed in two groups receiving either the omega-3 fatty acid supplement (n=23) or a placebo (n=23), as determined via permuted block randomization method. Participants received 4 mg of omega-3 (EPA: 720 mg, DHA: 480 mg) or placebo (edible paraffin) for 8 weeks. They consumed two soft gels with lunch and dinner. Both omega-3 fatty acid and placebo capsules had a similar shape, size, and color. Furthermore, capsules were packaged in similar boxes. The study was conducted in a double-blind manner, in which both researchers and patients were unaware of the type of supplements each group received. Omega-3 fatty acid and placebo capsules were obtained from Minoo Pharmaceutical, Cosmetic & Hygienic Company, Tehran-Iran.

Height, weight, waist and hip circumferences were measured at baseline and at the endpoint of the study. Weight was measured while patients were in a state of fasting with a digital scale (Seca, clara803, Germany). Height was assessed by stadiometer (Seca, Germany), following the removal of shoes. Waist and hip circumferences were assessed in a standing state with a non-elastic tape measure according to the WHO recommendations. Waist to hip ratio was estimated by dividing waist circumference by hip measurements, each of which were measured in centimeters. Fat and lean masses were quantified via the use of a body composition

analyzer (BC-418, Tanita, Japan). Systolic and diastolic blood pressure was measured by digital sphygmomanometer (Zyklusmed, Germany) in sitting position, after 10 minutes' rest, twice every visit. The patients' dietary intakes were estimated through 24-hour dietary recall.

Biochemical analysis

Fasting blood samples were drawn at weeks 0 and 8. Serum was isolated and stored at -70 C until the time of analysis. Serum concentrations of the lipid profiles were measured colorimetrically. For the lipid profile, total cholesterol, LDL, and HDL cholesterols, VLDL and triglyceride levels were examined. Serum apelin-13 levels were measured by ELISA kit (Cristal day E1273Hu, China) according to the manufacturer instructions. Serum hs-CRP concentration was measured by ELISA kit (LDN, Germany).

Statistics

Statistical analyses were performed with SPSS version 22. All data reported as mean ± SEMs. The normal distribution was assessed via Kolmogorov-Smirnov test. The contrasts between baseline and endpoint values inside each group were evaluated by means of a Paired t-test. Mean differences between two groups were evaluated using an Independent Sample t-test. For removing the confounding effect of basal values analysis covariance (ANCOVA) was applied. Comparisons of qualitative values were assessed through chi-square tests. In this study, a P value of <0.05 was considered statistically significant.

The sample size was determined accounting for all dependent variables. The largest sample size was calculated through measuring serum apelin levels. To obtain the largest sample size, it was determined that the serum apelin mean between the placebo and omega-3 fatty acid group should differ by 0.45 ng/ml. A total of 40 patients, 20 per group, was considered to be a sufficient sample size. However, due to the possibility of sample loss, 44 patients were recruited. This trial was registered at clinical trials.gov as NTC02382471.

Results

This study was a double blind clinical trial that examined the effect of omega-3 fatty acid supplementation on serum apelin concentrations in patients with CVD. Each group, the omega-3 fatty acid group and the placebo group, contained 23 male CVD patients. During the study, two patients in the placebo group discontinued the intervention owing to heart surgery (Fig. 1).

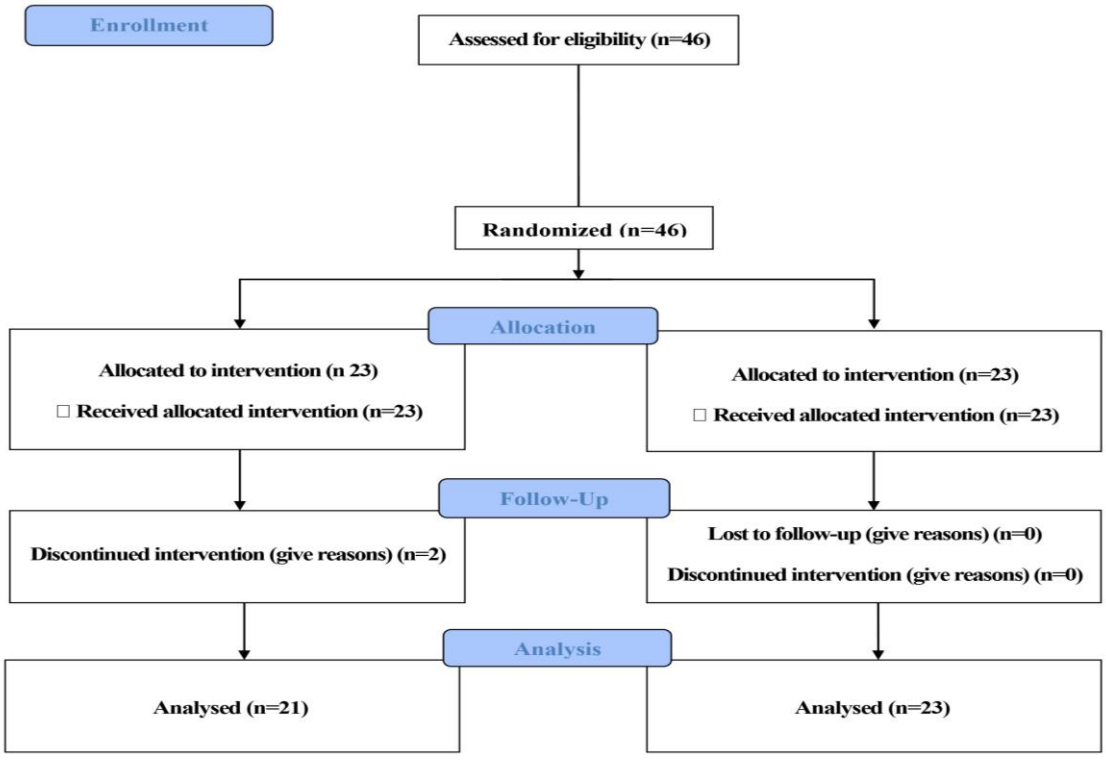

Fig. 1. The flowchart of subjects and study design

Apelin concentration

Table 1 shows the serum apelin levels. The placebo and omega-3 fatty acid groups were not significantly different when examining basal serum apelin concentrations. Consumption of 4 g/day of omega-3 fatty acids for two months resulted in a significant increase in serum apelin levels in the omega-3 group (p= 0.017). The serum apelin levels before and after the intervention were significantly different between the groups (p= 0.018).

Hs-CRP levels

Omega-3 fatty acid supplementation caused a significant decrease in hs-CRP levels in the omega-3 group (p= 0.016), as it shown in Table 1. Following intervention, hs-CRP levels were significantly different between the two groups (p= 0.028). During the intervention the hs-CRP serum concentrations became significantly different between the two groups (p= 0.01).

Lipid profiles

The effect of omega-3 fatty acids on lipid profiles are summarized in Table 1. Omega-3 fatty acid supplementation resulted in a significant decrease in TG and VLDL levels (p= 0.03). The HDL levels increased in both groups (p= 0.04 and p= 0.001), however the values between groups were not significantly different. Serum total cholesterol did not change throughout the intervention in both groups (p= 0.87). The serum LDL C. concentration was significantly different between groups (p= 0.04). Unexpectedly, the LDL C. did not change significantly in each group. Surprisingly, at the end of the intervention after adjusting for basal values, serum LDL C. concentrations were significantly different between the two groups (p= 0.014, ANCOVA).

Table 1. Biochemical values of patients before and after intervention

	Omega-3 group	Placebo group	p-value
Apelin (ng/l)	11.23±4.37	-0.35±1.36	0.018
Hs-CRP (mg/l)	-1.17±0.43	0.68±0.41	0.011
TC (mg/dl)	-11.85±9.07	-13.45±10.3	0.81
TG (mg/dl)	32.06±12.3-	-21.59±14.9	0.58
HDL (mg/dl)	4.26±1.7	3.81±0.7	0.87
LDL (mg/dl)	-6.44±3.4	9.43±5.3	0.007
VLDL (mg/dl)	-6.41±2.4	-4.34±2.9	0.58

Dietary intakes

No significant differences were found between groups with regards to energy and macronutrient intake before or after the intervention (Table 2).

Table 2. Macronutrients intakes of patients before and after intervention

	Before	After	Omga-3 group	Placebo group
Energy (kcal/d)	0.95	0.26	0.38	0.65
Carbohydrate (g/d)	0.65	0.11	0.10	0.57
Protein (g/d)	0.94	0.64	0.62	0.76
Fat (g/d)	0.43	0.38	0.21	0.68
Omega-3 fatty acids (g/d)	0.31	0.46	0.57	0.62
Omega-6 fatty acids (g/d)	0.25	0.67	0.16	0.31
Saturated fatty acids (g/d)	0.75	0.66	0.88	0.74

Anthropometric indices and body composition

Anthropometric indices (weight, waist circumference, hip circumference, waist to hip ratio) and body composition were not significantly different within or between groups. (Table 3).

Table 3. Anthropometric indices and body composition of patients before and after intervention

	Omega-3 group	Placebo group	p-value
Weight (kg)	- 0.05±0.30	0.68±0.32	0.10
BMI (kg/m2)	-0.02±0.10	0.22±0.11	0.10
Waist circumference (cm)	-0.01±0.39	0.34±0.34	0.53
Hip circumference (cm)	-0.31±0.44	0.19±0.34	0.37
WHR (cm)	0.002±0.004	0.0002±0.003	0.67
Fat mass (kg)	-0.41±0.57	0.68±0.52	0.17
Lean mass (kg)	0.88±0.49	0.17±0.42	0.30

Blood pressure

Table 4 displays blood pressures. Systolic and diastolic blood pressures decreased, although insignificantly, during the intervention in the omega-3 fatty acid group (p= 0.08 and p= 0.20, respectively).

Table 4. Blood pressures of patients before and after intervention

	Omega-3 group	Placebo group	p-value
Systolic blood pressure (mmHg)	-4.92±2.9	-0.29±2.5	0.30
Diastolic blood pressure (mmHg)	-3.65±2.5	0.24±1.7	0.31

Discussion

The main findings of this study show that supplementation with omega-3 fatty acids results in a significant increase in serum apelin levels.

Recently, the protective role of apelin in the pathogenesis of many diseases has been reported. Lower serum apelin levels have been observed to be present in several chronic diseases including diabetes, lupus erythematosus, rheumatoid arthritis, multiple sclerosis, psoriasis, autoimmune disorders and infectious diseases (18). Apelin has been shown to have protective effects on the cardiovascular system. Accordingly, decreased levels of apelin have been demonstrated be a contributing factor in cardiovascular disease (19).

Previous work examining different omega-3 fatty acids have shown conflicting data. Huerta et al. (20) assessed the effect of α-lipoic acid (LA) (0.3 g/d) and EPA (1.3 g/d) supplementation in obese or overweight women for a period of10 weeks. The results of the study showed a decrease in the levels of LA induced inflammatory indices, independent of weight loss. Apelin concentrations were observed to decrease less in the group that received LA. Supplementation of EPA showed a regulation in inflammation-related gene expression.

In a separate study, Peres-Eccarri et al. (21) reported that EPA significantly increased apelin gene expression and serum levels. Within the context of insulin sensitivity, the effect of supplementing with 1mg/kg of EPA ethyl ester was examined in an *in vivo* rat model. EPA was observed to decrease the baseline insulin concentrations, HOMA-IR, and TNF-α mRNA of fat mass. within line with these results, Lorento-Cebrian et al. (22) found EPA (100-200 µmol) supplementation to up-regulate apelin gene expression and production in 3T3-L1 mature adipocytes. The results of this study showed that the significant increase in basal and insulin-stimulated apelin gene expression and production by EPA is mediated by Akt phosphorylation. Moreover, Bertrand et al. (6) measured the effect of 36 g/kg wt EPA on metabolic disorders in mice induced by consumption of a fatty diet. The apelin-APJ system was shown to result in an increase in skeletal muscle, improved fat consumption, decreased fat mass and steatosis. In response to EPA, the APJ receptor expression, as well as apelin expression and secretion were significantly increased in the muscles of mice. The signaling

pathway in this study was shown to be dependent on ERK1/2 activation. Moreover, a study on the fetal mouse fibroblast 3T3-L1 cell line showed that treatment with 100 µmol EPA and 50 µm DHA caused the upregulation of the apelin gene in younger cells (23).

For the first time, our study demonstrates that supplementation with omega-3 fatty acids for two months, can increase serum apelin concentration in patients with CVD. In addition, omega-3 fatty acid supplementation was observed to decrease hs-CRP levels. Hs-CRP was assessed as a cardiovascular predicting factor. Different studies have shown contradictory results when examining the effects of omega-3 fatty acids on the lipid profile. In agreement with our study, Saifullah et al. (24) found that supplementation with omega-3 fatty acids for 12 weeks decreased hs-CRP levels in the hemodialysis patients. Conversely, Mahmoudi et al. (25) found no changes in hs-CRP levels following a 6-month trial in elderly patients with metabolic syndrome. Furthermore, Chan et al. (26) did not observe any significant changes in hs-CRP levels in obese individuals after omega-3 fatty acid supplementation for 6 weeks. Our current trial demonstrates that omega-3 fatty acid supplementation can induce a significant decrease in TG and VLDL levels. Furthermore, LDL C. levels significantly decreased after intervention. In agreement with our study, Pie et al. (27) found that omega-3 fatty acids decreased TG levels, although no changes the serum Total C., LDL and HDL levels were observed in patients with ESRD (End Stage Renal Disease). Phelan et al. (28) observed supplementation with omega-3 fatty acids to modulate TG, LDL and HDL in women with the poly cystic syndrome. A separate study by Emami Naini et al. (29) found no significant decreases in total C. and LDL levels and significant increases in HDL C. levels when supplementing with omega-3 fatty acids. Following eight weeks of supplementation, no change was observed in anthropometric indices, body composition or blood pressure.

In summary, our study shows that dietary supplementation with omega-3 fatty acids can increase the serum apelin levels in the blood of patients with CVD. Given that apelin has been shown to be involved in the improvement of cardiovascular system function, increasing the levels of this adipokine could prevent the development of CVD in affected patients. Additionally, measuring the levels of serum apelin could also provide a biomarker predicting CVD risk.

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