Effect of Eicosapentaenoic acid Supplementation on the Serum Levels of sE-selectin and sVCAM-1 in the Patients with Non Insulin-Dependent Diabetes

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Abstract: Background: An increased in the serum levels of sE-selectin and sVCAM-1, and the endothelial dysfunction are of characteristics associated with the patients with type 2 diabetes mellitus. EPA has the antioxidant, antiinflammatory, antithrombogenic, and antiarteriosclerotic properties. Therefore, we investigated the effect of Eicosapentaenoic acid supplementation on the serum levels of sE-selectin and sVCAM-1 in the diabetic patients. **Methods:** This study was designed as a randomized, double-blind, and placebo-controlled clinical trial. Thirty six patients with type 2 diabetes were given written; informed consent, randomly were classified into 2 groups. They were supplemented with 2 g/day of the capsules of EPA or placebo. At the start and the end of the intervention, blood sample for measurement of the serum levels of sE-selectin, sVCAM-1, and lipids, as well as FBS and HbA1c were given. **Results:** There were no significant differences between the two groups regarding any demographic, clinical or biochemical data, total energy intake, and macronutrient intake at the baseline, and during the intervention, except for a significant increase of protein intake and the levels of HbA1c in the placebo group, and a significant increase of HDL-c, and a significant decrease in the serum levels of sE-selectin and sVCAM-1, as well as a slight reduce of total cholesterol, LDL-c, TG and FBS in the supplement group. **Conclusions:** EPA is atheroprotective via decrease in the serum levels of sE-selectin and sVCAM-1, as well as change in the serum levels of lipids, and FBS.

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Introduction

Type 2 diabetes is as a serious chronic metabolic disease resulting from defect in the insulin secretion, the insulin resistance, or both [1]. Over the last two decades, the prevalence of diabetes, in particular type 2 diabetes, has increased rapidly worldwide [2]. Approximately 5% of all deaths all over the world each year is due to diabetes [3], and the risk of developing cardiovascular disease (CVD) is two to fourfold higher in people with diabetes than in those without diabetes [4].

Endothelial cells (ECs) have a main role in carrying of metabolic substrates and cells between the blood circulation and the interstitial space [5]. ECs function may be measured by using the measurement of blood flow [6], or indirectly, through the determination of blood levels of compounds derived from endothelial, such as endothelial adhesion molecules, von Willebrand factor (vWF), tissue plasminogen activator and its inhibitor, PAI-1 [7]. In general, the interaction of subsets of monocytes and lymphocytes with each other and the activation of endothelial cells or likely the stimulation of this type of cells by oxidized LDL (Ox-LDL) result in increase in the expression of endothelial adhesion molecules and as a consequence of the promotion of vascular damage [8].

It seems that the expression of serum selectins molecules (sP-selectin and sE-selectin) and soluble cell adhesion molecules (intracellular cell adhesion molecule, sICAM-1; vascular cell adhesion molecule, sVCAM-1) regulate at the transcription level [9]. Although adhesion molecules are quite requirement and play a key role in the normal development and function of cardiovascular system [10], but the plasma measurement of adhesion molecules are considered as markers of the endothelial dysfunction, and predictors of early atherothrombotic and atherosclerosis processes and vascular disease [5].

E-selectin and VCAM-1:

E-selectin, which is previously called the endothelial leukocyte adhesion molecule-1, is a transmembrane glycoprotein produced by the endothelial cells in response to vascular endothelial growth factor (VEGF) [11]. The adhesion of monocytes, neutrophils, and memory T cells to the endothelium mediate by E-selectin. This protein is

expressed by the endothelial cells at the sites of atherosclerotic lesions [12], and may also be implicate in metastasis [13].

VCAM-1 is also, like E-selectin, a transmembrane glycoprotein produced by activated vascular endothelial cells in response to VEGF Moreover, it is expressed on proximal renal tubule cells, dendritic cells [14], and smooth muscle cells surrounding the larger arteries of the retina [15]. Interestingly, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) cytokines have been indicated to induce VCAM-1 expression [16]. Under conditions of inflammation, this adhesion molecule plays a main role in the adhesion of leukocytes to the endothelium. It may bind melanoma cell lines and act, like E-selectin, as an adhesion molecule in the facility of metastasis [14].

Eicosapentaenoic acid (EPA) is one of ω-3 PUFAs which are present at the great amounts in the fish oil [17]. The findings of several studies have shown that EPA has the antioxidant [18], anti-inflammatory [19], antithrombogenic [8], and antiarteriosclerotic [20] properties. The aim of this study was to determine the effects of the supplementation of Eicosapentaenoic acid on the serum profile of sE-selectin and sVCAM-1 in the patients with type 2 diabetes mellitus.

Material and Methods

1. Patients and Study Design:

1. 1. Patients:

The study subjects were 36 patients with type 2 diabetes mellitus who were selected from Iran Diabetes Association (Tehran, Iran). Only patients with a previous clinical diagnosis of type 2 diabetes mellitus according to the criteria for the diagnosis of diabetes as recommended by American Diabetes Association [21] were recruited.

1.1.1. Inclusion/Exclusion Criteria:

Inclusion criteria for the participation in the study were, willingness to collaborate in the study, aged 35-50 years, having a history of at least 1 year of the diagnosis of type 2 diabetes mellitus before the participation in the study based on FBS ≥126 mg/dl or 2hPG ≥200 mg/dl (2-hour plasma glucose), 25≤BMI<30 kg/m^2, identified and maintaining of the antidiabetic's drug (s) dose from 3 months ago.

Participants were excluded from the study if they had, unwillingness to continue the cooperation in the study, need to take insulin, change in the dose (s) and type of medication to the treatment of diabetes, change in the levels of physical activity, do not use (noncompliance) supplements (<10%), affected to the acute inflammatory diseases; according to the consultant physician endocrinologist.

1.2. Study Design:

The study protocol was designed as a randomized, double-blind, and placebo-controlled clinical trial. At the first, the study protocol was approved by the ethics committee of Tehran University of Medical Sciences, and all participants gave written, informed consent before the participation in the study.

The patients were randomly classified into 2 groups to the supplementation with 2 g/day of the softgels of EPA or placebo (supplied as 1-g softgels), the two groups were randomly allocated to the supplement and placebo groups by balanced permuted block on the sex. The softgels containing Eicosapentaenoic acid ethyl ester (75%) [EPA, Mino Pharmaceutical Co. Iran], or edible paraffin were provided by Mino Pharmaceutical Co., Iran. They were strictly advised to maintain their usual diets and nutritional habits, level of physical activity, and not to change their medication dose (s) during the study, as well as were asked to record and report any side effect of taking capsules gave to them.

Compliance with the supplementation was assessed by counting the number of softgels had used and the number of softgels returned to the study centerat the time of specified visits. The patients were followed up by telephone each week.

1.2.1. Nutritional Assessment:

At the beginning and at the end of the intervention, nutrients intakes were estimated using a 24-hour diet recall questionnaire for 3 days.

1.2.2. Questionnaires, Anthropometric and Biometric Measurements:

At the start and at the end of the study, each participant was evaluated with the physical examination and a general questionnaire containing questions regarding demographic variables (age, sex), anthropometric data (weight, height, waist and hip circumference, heart rate, and measurements of systolic, diastolic and mean blood pressure (SBP, DBP and MBP), and pulse pressure (PP)), family history of diseases (diabetes, hyperlipidemia and hypertension, cardiovascular, etc), age at the diagnosis of type 2 diabetes, type of the treatment and medication used, and lifestyle habits (including the history of smoking, alcohol consumption). The average of type and duration of all physical activities were measured using the International Physical Activity Questionnaire (IPAQ), at the beginning and at the end of the intervention.

Anthropometric measurements, including weight, height, as well as waist and hip circumference, and blood pressure were measured at the start and at the end of the study. Weight, changes in the level of

physical activity, and any disease were recorded at the baseline and during weeks 2, 4, 6, and 8 of the intervention.

Subjects were weighed without shoes, in light indoor clothes by a Seca scale with an accuracy of ±100 g. Standing height was measured without shoes to the nearest 0.5 cm using a commercial stadiometer. Body mass index (BMI) was calculated as weight/height² (kg/m²). According to the recommendation of International Diabetes Federation, hypertension was defined as blood pressure ≥130/85 mmHg [22].

Each participant gave a blood sample in the early morning after an overnight fast for 10–12 hours and before taking any oral hypoglycemic agent (s) at the beginning and at the end of intervention (8th week). Samples were drawn from the antecubital vein, and were collected into blood tubes containing EDTA or heparin. After at least 30 minutes, plasma and serum were separated by centrifugation at 3000 ×g for 10 minutes at 4 °C. Serum and plasma aliquots of each sample stored at −80 °C, for analysis of biochemical parameters [Serum levels of sE-selectin, sVCAM-1, FBS (fasting blood sugar), HbA1c, the serum total cholesterol (TC), triglyceride (TG), LDL-c and HDL-c]. The blood samples were collected only for this study.

1.2.3. Measurement of the Serum Levels of sE-selectin and sVCAM-1:

The serum levels of sE-selectin and sVCAM-1 were measured using Enzyme-linked immune sorbent assay kits for Human sE-selectin and sVCAM-1from SHANGHAI CRYSTAL DAY BIOTECH CO., LTD, according to the manufacturer's instructions, Cat. No.: E0262Hu, E0264Ra, respectively, Size: 96 tests, FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. The sensitivity was 0.56 ng/mL for sE-selectin and 0.05 ng/mL for sVCAM-1.

1.2.4. Other Laboratory Analyses:

Serum was used for the determination of lipids and glucose. Glucose and HbA1c were measured by enzymatic methods. Serum lipid (serum total cholesterol, HDL-cholesterol, triglyceride and LDL-cholesterol) analyses were performed by spectrophotometric method (Pars azmoon, Iran).

1.2.5. Statistical Analyses:

The data were analysed using SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA), and the results are expressed as mean ± SD. The Independent t-test was used for the comparison of variables between two groups. 24-hour diet recalls analysed using Food processor II software [23], and the comparison of means in different intervals of 24-hour diet recalls was performed using Independent t-test. Values of $p < 0.05$ were considered statistically significant.

Results

1. Patient Characteristics:

The baseline characteristics of the two groups of patients are shown in Table 1. There were no significant differences in age, sex, duration of diabetes, weight, height, body mass index (BMI), waist circumference, hip circumference, waist/hip ratio, measurements of systolic, diastolic and mean blood pressure (SBP, DBP and MBP), pulse pressure, heart rate and biochemical data between the two groups at the baseline.

2. Dietary Intake and Lifestyle:

There were no significant differences in total energy intake, macronutrient intake, and body weight between the two groups of patients at the baseline (Table 1), and no significant changes observed during the intervention (data not shown). Medication dose (s), and the levels of physical activity from both groups had no significant difference at the baseline, and remained constant during the intervention period (data not shown).

3. Compliance and Side Effect:

All patients were fulfilled the intervention program, and were well tolerated intervention with study capsules for 8 weeks. Also, they were reported no side effects throughout the study.

4. The Serum Levels of sE-selectin and sVCAM-1:

There were no significant differences in the serum levels of sE-selectin between the two groups of patients at the baseline (Table 2), whereas as shown in Table 2, the serum levels of sE-selectin reduced significantly ($p < 0.001$) in the EPA receiving patients compared with the placebo receiving patients.

As shown in Table 2, no statistically significant differences were observed between the two groups of patients at the baseline with regard to the serum levels of sVCAM-1, whereas the serum levels of sVCAM-1 in the EPA receiving patients compared with the placebo receiving patients decreased significantly ($p < 0.05$).

5. The Serum Levels of Lipids:

The serum total cholesterol was 226.27 ± 38.73 mmol/L after receiving placebo and 207.16 ± 39.69 mmol/L after the supplementation with EPA. The serum LDL-cholesterol was 95.73 ± 29.86 mmol/L after receiving placebo and 81.4 ± 32.63 mmol/L after the supplementation with EPA. The serum HDL-

cholesterol was 31.38 ± 4.76 mmol/L after receiving placebo and 37.11 ± 5.97 mmol/L after the supplementation with EPA. The serum triglycerides was 162.8 ± 158.81 mmol/L after receiving placebo and 176.48 ± 133.75 mmol/L after the supplementation with EPA (Table 3).

Discussion:

The modification of LDL-c by the oxidation can lead to the transfer of cholesterol into macrophages, whereas unmodified LDL-c cannot [24]. Ox-LDL is considered as a component atherogenic and has several biological activities, such as increased the accumulation of lipids in macrophages [25], the stimulation of chemotaxis of circulating monocytes [26], the modulation in the expression of adhesion molecules, various growth factors, and cytokines [27], the induction of endothelial dysfunction and inflammatory responses, and the deposition of lipids in the arterial wall [28].

The expression of cell adhesion molecules are enhanced in both types of diabetes mellitus [29], thereby, serum levels of soluble E-selectin (sE-selectin) [30], and soluble vascular cell adhesion molecule 1 (sVCAM-1) [31] are increased in the patients with diabetes mellitus, and these molecules have been involved in the microvascular complications of diabetes. These findings support the theory that various atherosclerotic processes are promoted in the diabetes mellitus.

1. The Potential Mechanisms for the Initiation of Endothelial Dysfunction in Type 2 Diabetes Mellitus:

Vascular endothelial dysfunction indicates an early and reversible event in the development of atherosclerosis which is related to specific diseases in type 2 diabetes mellitus. The pathogenesis of endothelial dysfunction is not well known, particularly in relation to type 2 diabetes. Endothelial dysfunction has been demonstrated in apparently healthy individuals with cardiovascular risk factors [32], including type 2 diabetes [33]. A number of potential mechanisms for the onset of endothelial dysfunction in type 2 diabetes mellitus have been explained, such as the effects of hyperglycaemia, dyslipidaemia and advanced glycation end-products (AGEs), the accumulation of sorbitol [34], impairement to synthesis or release of nitric oxide (NO) by the endothelial cells [35], an enhancement in degradation or decreased sensitivity to NO [35, 36], and the up-regulation of the renin-angiotensisn system and VEGF [34].

2. Functions and Molecular Mechanisms of Action of EPA:

Several studies have shown that EPA has various effects, including preventing of the insulin resistance [37], increasing the insulin secretion [38], enhancing the size of LDL-c particle [39], reducing the serum levels of TG, lowering the blood viscosity, increasing the production of NO, having the antiinflammatory and antithrombotic properties [40-42], and decreasing the blood pressure [43].

It has been demonstrated that EPA is more effective than docosahexaenoic acid (DHA) in the suppression of inflammatory response [44]. EPA plays as a substrate to decreases the production of inflammatory eicosanoids from arachidonic acid, via competing for the cyclooxygenase-2 and lipooxygenase (COX-2/LOX) enzymes. These alternative eicosanoids, which are termed E-series resolvins, have identified as a group of mediators to exert the antiinflammatory functions. Moreover, both DHA and EPA reduce the release of arachidonic acid via the inhibition of PLase A2 [45-47].

Also, EPA has an inhibitory role on the endotoxin-induced expression of adhesion molecules upon the endothelial cells (ECs) of human vein, and results in the excessive reduction of monocytes attached to the arterial endothelium [48].

The findings of an epidemiological study of Greenland Eskimos suggested that EPA could be has the antithrombogenic and antiarteriosclerotic properties [20]. It has been postulated that the mechanisms of these actions are including the suppression of platelet aggregation and the improvement of blood rheologic properties [49].

It has also been reported that EPA has the beneficial effects on the serum levels of lipids to is suggesting that EPA may be useful as a supplement for the prevention and treatment of arteriosclerotic disease [8]. These results suggest that the administration of EPA to the patients with type 2 diabetes may prevent the development of cardiovascular complications caused by some different risk factors. It seems that a combination of these actions and mechanisms explained above are responsible for the antiinflammatory, antiatherosclerotic, and antithrombotic effects caused by EPA.

3. Effects of ω-3 PUFAs on Serum sE-selectin and sVCAM-1:

Studies based on cell cultures and in vitro models have demonstrated the beneficial effects of ω-3 PUFAs on the cellular adhesion molecules [50]. Previous human studies have shown that the effects of EPA and DHA on the serum levels of VCAM and E-selectin were inconclusive and contradictory [51-53].

These inconsistencies can be due to several factors, such as discrepancies in the population studied, the duration of study, the content of EPA and DHA in the supplement or the history of diet. Moreover, the concentrations of EPA and DHA are often only estimated from reported intake instead of analysis in the percentage of EPA and DHA in the membrane of RBC, which might describe contradicting results on the endothelial function [54].

In a human study performed in this regard, Nomura et al. observed that the administration of EPA was significantly decreased the serum levels of sE-selectin in the hyperlipidemic patients with type 2 diabetes [8]. Our present study clearly shows that the supplementation of EPA for 8 weeks in the patients with type 2 diabetes mellitus leads to a significant reduction in the serum levels of sE-selectin than the placebo group (Table 2), and this finding is in accordance with that of the interventional study performed in this regard with the EPA supplementation on the hyperlipidemic patients with type 2 diabetes.

On the other hand, our findings clearly show that the supplementation of EPA for 8 weeks in the patients with type 2 diabetes mellitus significantly decreases the serum levels of sVCAM-1 (Table 2). As yet, the effect of EPA on the serum levels of sVCAM-1 in vitro and in vivo was not studied, and this is the first time that has been demonstrated EPA can reduce the serum levels of sVCAM-1 in vivo.

Thus, it is significant to point out that our data provide evidence compatible with the hypothesis that EPA influences the serum levels of sE-selectin and sVCAM-1 in the patients with type 2 diabetes mellitus.

4. ω-3 PUFAs and the lipid profile

Meanwhile, several studies have shown that the ω-3 PUFAs have various effects on the lipid profile in type 2 diabetic patients, including enhancing the size of LDL-c particle [55], reducing the serum levels of TG [56], increasing the plasma levels of HDL-c and HDL2-c [56, 57], and decreasing the plasma levels of HDL3-c [56]. This study demonstrated that EPA can significantly increase the serum levels of HDL-c which is compatible with the results in the other studies with ω-3 PUFAs [56, 57], but did not significantly affect the other serum levels of lipids.

5. The Study Limitations:

There were several limitations for our study. First, a relatively small sample size of patients, therefore, it should point out that the results of our study are preliminary and need to be confirmed in a larger sample size of patients. Second, the exact mechanisms by which EPA decrease the serum levels of sE-selectin and sVCAM-1 have not been clarified, and further work is necessary to delineate the molecular mechanism of action of EPA on the regulation of serum levels sE-selectin and sVCAM-1. Third, the supplementation with EPA for more long term should be studied for possible increases in more susceptible to oxidation of lipoproteins. Thus, it is better and important that the serum levels of CPR, and inflammatory cytokines, as well as the percentage of EPA in the membrane of RBC measure in the further studies. For these reasons, the additional studies will be necessary to determine the general applicability of our study results.

Conclusion:

Considering our results in conjunction with epidemiologic data, we concluded that EPA has a beneficial effect on the endothelial function, and this effect may vanquish the high oxidative susceptibility of plasma lipoproteins. Therefore, EPA can reduce the oxidative stress and endothelial dysfunction as a main initiating step in the development of atherosclerosis, thereby, it may be useful as a primary prevention therapy for atherothrombosis and vascular complications in the patients with type 2 diabetes mellitus.

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