Efficacy of Eicosapentaenoic acid Supplementation on the Serum Profile of Lp (a) in the Patients with Type 2 Diabetes Mellitus

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Abstract: Background: The serum levels of Lp (a) is considered as a risk factor and a predictor of atherosclerotic vascular disease. The patients with type 2 diabetes mellitus have an increased in the serum levels of Lp (a), and endothelial dysfunction. EPA has the antioxidant, antiinflammatory, antithrombogenic, and antiarteriosclerotic properties. Therefore, we investigated the efficacy of Eicosapentaenoic acid supplementation on the serum profile of Lp (a) 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 Lp (a), 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 Lp (a), as well as a slight reduce of TC, LDL-c, TG and FBS in the supplement group. **Conclusions:** EPA is atheroprotective via decrease in the serum levels of Lp (a), as well as change in the serum levels of lipids, and FBS.
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Introduction

Type 2 diabetes is one of the most common endocrine disorders [1]. In the present century, this disease is recognized as a major public health problem all over the world [2]. The prevalence of diabetes in the adults all over the world was estimated 4% in 1995 [3], and is currently estimated to be about 6.4% worldwide [4]. This disease is one of the most costly diseases to manage [5]. Approximately 5% of all deaths all over the world each year is due to diabetes [6], and the risk of developing cardiovascular disease (CVD) is two to fourfold higher in people with diabetes than in those without diabetes [7].

Lipoprotein (a) [Lp (a)] is composed of a lipoprotein enriched with cholesterol, and with the structural similarities to low-density lipoprotein cholesterol (LDL-c). An apolipoprotein (apo) (a); i.e. a large, highly glycosylated, hydrophilic protein with sequence homology to plasminogen; which covalently bound to apoB-100 is linked on Lp (a) via a disulphide linkage. This type of lipoprotein secrets from hepatocytes [8], but it does not participate in lipid transport in the blood circulation, and its metabolic fate seems different from that of other lipoproteins containing apoB [9]. The plasma concentration of Lp (a) is very different between subjects [10], and its density (size) is significantly related to the size of HDL-c and LDL-c particles, which are diminished in response to insulin resistance [11].

The physiological role of Lp (a) is not well-known. However, Lp (a) is as a recognized highly atherogenic independent risk factor for atherosclerotic vascular disease, such as CVD, cerebrovascular disease, ischemic stroke, and peripheral vascular disease [8]. Therefore, serum levels of Lp (a) is considered as a predictor of atherosclerosis and vascular disease [12]. Furthermore, other biological functions of it are including a potential role in the

thrombogenesis process via the intervention in several steps of the fibrinolytic pathway [13], the activation of various cells types to have important roles in the atherogenesis process [14]. These biological effects of it can be associated with either to apo (a) or to constituents of particle with the structural similarities to LDL-c, among the enzyme platelet-activating factor acetylhydrolase that mainly exhibits a phospholipase A2 activity and is attached to lipoproteins in the blood circulation, thereby, as lipoprotein-associated phospholipase A2 (Lp-PLase A2) is termed [15]. The proinflammatory phospholipid platelet activating factor (PAF), and oxidized phospholipids (Ox-PLs) are substrates for Lp-PLase A2 [16]. It is thought to such phospholipids have key roles in the inflammatory responses and especially in vascular inflammation and atherosclerosis [17]. In addition, lipid esters partly soluble in the aqueous phase are as other substrates for Lp-PLase A2 [18]. They are including short-chain diacylglycerols, triacylglycerols, and acetylated alkanols [19]. In addition to the lipase and esterase activities, this enzyme also exhibits a transacetylase activity [16]. This activity exists on LDL-c and play a role in the transfer of short-chain fatty acids (SCFAs) and acetate from PAF and its ether/ester-linked analogues to ether/ester-linked lysophospholipids [20].

In the patients with diabetes mellitus, the plasma levels of Lp (a) are often higher than in the control healthy subjects [21], and are independently associated with some complications of this disease [22].

Eicosapentaenoic acid (EPA) is one of ω-3 PUFAs which are present at the great amounts in the fish oil [23]. The findings of several studies have shown that EPA has the antioxidant [24], antiinflammatory [25], antithrombogenic [26], and antiarteriosclerotic [27] properties. The aim of this study was to determine the efficacy of the supplementation of Eicosapentaenoic acid on the serum profile of Lp (a) 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 [28] 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 [29].

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 Lp (a), 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 Lp (a):

The serum levels of Lp (a) were measured using Enzyme-linked immune sorbent assay kit for Human Lipoprotein α (Lpα) from SHANGHAI CRYSTAL DAY BIOTECH CO., LTD, according to the manufacturer's instructions, Cat. No.: E1521Hu, Size: 96 tests, FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. The sensitivity was 0.23 ng/mL.

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 [30], 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 Lp (a):

There were no significant differences in the serum levels of Lp (a) between the two groups of patients at the baseline (Table 2), whereas as shown in Table 2, the serum levels of Lp (a) reduced significantly ($p < 0.05$) in the EPA receiving patients compared with the placebo receiving patients.

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).

Table 1. The baseline and after characteristics of the two groups of patients

Variable / Group	Placebo [n (Female/Male)=18]		P-value	EPA [n (Female/Male)=18]		P-value
	Baseline	After		Baseline	After	
Age (years)	44.72 ± 4.69			44.44 ± 3.79		> 0.05
Duration of DM (years)	6.61 ± 3.68			6.44 ± 2.83		> 0.05
Weight (kg)	78.30 ± 12.34	78.24 ± 13.39	> 0.05	78.03 ± 12.68	77.15 ± 12.68	> 0.05
Height (cm)	165.11 ± 8.85			165.39 ± 8.12		> 0.05
Body mass index (kg/m²)	28.92 ± 5.39	28.87 ± 5.61	> 0.05	28.49 ± 3.95	28.17 ± 3.94	> 0.05
Waist circumference (cm)	97.47 ± 10.93	97.08 ± 11.73	> 0.05	97.55 ± 9.65	96.44 ± 10.16	> 0.05
Hip circumference (cm)	106.00 ± 11.82	105.61 ± 12.32	> 0.05	105.33 ± 6.70	104.61 ± 7.59	> 0.05
Waist/hip (ratio)	0.92 ± 0.08	0.92 ± 0.07	> 0.05	0.92 ± 0.05	0.92 ± 0.06	> 0.05
Systolic blood pressure (SBP) (mmHg)	124.11 ± 15.32	124.89 ± 18.08	> 0.05	124.00 ± 16.25	123.06 ± 18.78	> 0.05
Diastolic blood pressure (DBP) (mmHg)	80.00 ± 6.69	80.00 ± 7.22	> 0.05	79.78 ± 13.40	79.44 ± 11.83	> 0.05
Mean blood pressure (MBP) (mmHg)	94.70 ± 7.87	94.96 ± 8.98	> 0.05	94.52 ± 13.69	93.98 ± 13.41	> 0.05
Pulse Pressure (PP) (mmHg)	44.11 ± 14.42	44.89 ± 16.83	> 0.05	44.22 ± 9.59	43.62 ± 11.84	> 0.05
Heart rate (HR) (beat/minute)	89.44 ± 12.49	89.33 ± 11.73	> 0.05	89.67 ± 10.50	89.33 ± 10.91	> 0.05
FBS (mg/dL)	138.06 ± 49.13	142.06 ± 52.34	> 0.05	143.72 ± 53.53	137.94 ± 23.566	> 0.05
HbA1C (%)	7.47 ± 1.67	7.77 ± 1.42	0.022	7.89 ± 1.75	7.86 ± 1.58	> 0.05
Total energy intake (kcal)	1953.94 ± 297.12	1961.56 ± 232.21	> 0.05	1955.94 ± 279.49	274.36 ± 1973.61	> 0.05
Carbohydrates intake (g/d)	260.32 ± 35.44	37.22 ± 265.08	> 0.05	260.85 ± 41.78	42.89 ± 260.82	> 0.05
Proteins intake (g/d)	63.19 ± 14.78	11.97 ± 70.09	0.041	14.34 ± 63.83	63.92 ± 14.06	> 0.05
Lipids intake (g/d)	22.68 ± 76.11	76.39± 16.56	> 0.05	16.78 ± 73.82	20.13 ± 76.86	> 0.05
Fibers intake (g/d)	14.75 ± 4.64	2.28 ± 14.64	> 0.05	16.66 ± 4.99	16.84 ± 3.82	> 0.05

Data are shown as mean ± SD. Statistical analysis was performed using paired t-test and Independent t-test.

Table 2. Serum levels of Lp (a) at baseline and after of the supplementation with EPA or placebo

Group / Variable	Placebo		P-value	EPA		P-value
	Baseline	After		Baseline	After	
Lp (a) (ng/mL)	**44.19 ± 35.99**	**43.84 ± 38.05**	**0.872**	**67.07 ± 62.02**	**44.29 ± 41.47**	**0.045**

Data are shown as mean ± SD. Statistical analysis was performed using paired t-test.

Table 3. Serum levels of lipids (mmol/L) at baseline and after the supplementation with EPA or placebo

Group / Variable	Placebo		P-value	EPA		P-value
	Baseline	After		Baseline	After	
Total cholesterol (mmol/L)	**204.44 ± 43.91**	**226.27 ± 38.73**	**> 0.05**	**211.22 ± 43.57**	**207.16 ± 39.69**	**> 0.05**
LDL-cholesterol (mmol/L)	**92.61 ± 35.92**	**95.73 ± 29.86**	**> 0.05**	**96.33 ± 38.13**	**81.4 ± 32.63**	**> 0.05**
HDL-cholesterol (mmol/L)	**31.11 ± 4.24**	**31.38 ± 4.76**	**> 0.05**	**29.72 ± 5.31**	**37.11 ± 5.97**	**< 0.05**
Triglycerides (mmol/L)	**221.50 ± 121.49**	**162. 8± 158.81**	**> 0.05**	**218.61 ± 94.52**	**176.48 ± 133.75**	**> 0.05**

Data are shown as mean ± SD. Statistical analysis was performed using paired t-test.

Discussion:

It is well-known that dyslipidemia is associated with the poor metabolic control in diabetes mellitus [31]. Most studies have not shown increase in the plasma levels of Lp (a) in the patients with type 2 diabetes mellitus [32]. This atherogenic lipoprotein is also considered as an independent risk factor for CVD in type 2 diabetes mellitus with the poor metabolic control [33].

1. Genetic and Lp (a):

The plasma levels of Lp (a) is strongly under the genetic control [34]. So that the its plasma range is

widely within various ethnic groups and within members of the same family, whereas the individual range of the plasma concentration of Lp (a) is conversely limited. This is reflecting the important role of genetic in the regulation of Lp (a) synthesis by the apo (a) gene [21].

2. Role of Lp (a) in Atherogenesis:

Lp (a) is as an atherogenic lipoprotein and exists in the atherosclerotic plaques but not in the normal vessel walls [35]. In the early plaque, the majority of this type of lipoprotein is located within the endothelial cells and considerably influences their function [10]. Whereas, in the advanced lesions, Lp (a) is found principally in the intima, where it is predominantly accumulate with foam cells [36]. Since it can be oxidized, aggregated, or subjected to the modification of PLase A2, therefore, Lp (a) helps to the formation of foam cells, and then these foam cells take up by the scavenger receptors of macrophages [16]. Moreover, Lp (a) affects the function of cells that have important roles in the atherogenesis process, such as the endothelial cells and monocytes-macrophages [37, 38].

3. Functions and Molecular Mechanisms of Action of EPA:

Several studies have shown that EPA has various effects, including preventing of the insulin resistance [39], increasing the insulin secretion [40], enhancing the size of LDL-c particle [41], reducing the serum levels of TG, lowering the blood viscosity, increasing the production of nitric oxide (NO), having the antiinflammatory and antithrombotic properties [42-44], and decreasing the blood pressure [45].

It has been demonstrated that EPA is more effective than docosahexaenoic acid (DHA) in the suppression of inflammatory response [46]. 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 [47, 48].

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 [49].

The findings of an epidemiological study of Greenland Eskimos suggested that EPA could be has the antithrombogenic and antiarteriosclerotic properties [27]. It has been postulated that the mechanisms of these actions are including the suppression of platelet aggregation and the improvement of blood rheologic properties [50].

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 [26]. 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.

4. ω-3 PUFAs and Lipoprotein (a):

In a human study on the elderly, ω-3 PUFAs did not decrease the serum levels of LP (a) [51].

As yet, the effect of EPA on the serum levels of LP (a) in vitro and in vivo was not studied, and this is the first time that has been demonstrated EPA can decrease the serum levels of LP (a) in vivo. 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 LP (a) than the placebo group (Table 2).

Thus, it is significant to point out that our data provide evidence compatible with the hypothesis that EPA influences the serum levels of LP (a) in the patients with type 2 diabetes mellitus.

5. 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 [52], reducing the serum levels of TG [53], increasing the plasma levels of HDL-c and HDL2-c [53, 54], and decreasing the plasma levels of HDL3-c [53]. 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 [53, 54], but did not significantly affect the other serum levels of lipids.

6. 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 LP (a) have not been clarified, and further work is necessary to delineate the molecular mechanism of action of EPA on the regulation of serum levels of LP (a). 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 Lp-

PLase A2, 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, 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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