

The Effects of the Supplementation of EPA on the Serum Profile of Antiatherogenic Lipoprotein, Its Subfractions, Non-HDL-c, and Atherogenic/Antiatherogenic Lipoproteins Ratios in the Patients with Non-Insulin Dependent Diabetes Mellitus

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Abstract: Background: HDL2-c, Non-HDL-c and lipoprotein ratios are as better risk indicators for CVD. EPA has the antioxidant, antiinflammatory, antithrombogenic, and antiarteriosclerotic properties. Therefore, we investigated the effect of EPA supplementation on the serum profile of antiatherogenic lipoprotein, its subfractions, non-HDL-c, and lipoprotein ratios 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. Blood sample for measurement of the serum levels of lipids and their ratios, 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 in the serum levels of HDL-c and HDL2-c, and a significant decrease in the serum levels of HDL3-c, non-HDL-c and lipoproteins ratios, as well as a slight reduce of TC, LDL-c, TG and FBS in the supplement group. **Conclusions:** EPA is atheroprotective via increase in the serum levels of HDL-c and HDL2-c, and decrease in the serum levels of HDL3-c, non-HDL-c and lipoprotein ratios, as well as change in the serum levels of other lipids, and FBS. [Mohammad Hassan Golzari, Saeed Hosseini, Fariba Koohdani, Seyde Ali Keshavarz, Mahmoud Djalali. **The Effects of the Supplementation of EPA on the Serum Profile of Antiatherogenic Lipoprotein, Its Subfractions, Non-HDL-c, and Atherogenic/Antiatherogenic Lipoproteins Ratios in the Patients with Non-Insulin Dependent Diabetes Mellitus.** *Cancer Biology* 2017;7(2):55-64]. ISSN: 2150-1041 (print); ISSN: 2150-105X (online). <http://www.cancerbio.net>. 8. doi:[10.7537/marscbj070217.08](https://doi.org/10.7537/marscbj070217.08).

Key Words: Eicosapentaenoic acid, HDL-c, HDL2-c, HDL3-c, non-HDL-c, TC/HDL-c, LDL-c/HDL-c, Type 2 Diabetes Mellitus.

Introduction

Type 2 diabetes mellitus is recognized as a major public health problem all over the world [1], and its prevalence has reached epidemic proportions worldwide [2]. This disease and its chronic complications impose a substantial economic burden on individuals, families, society, and the healthcare system of the country and make it as a public health challenge [1]. It is anticipated that in the year 2025, the healthcare expenditures of diabetes will be between 7% and 13% of the healthcare budget of worldwide [3]. The risk of developing cardiovascular disease (CVD) is two to fourfold higher in people with diabetes than in those without diabetes and the general population [4], and includes approximately 80% of all mortality in people with diabetes [5].

Lipoproteins transfer lipids from the liver and gut to the tissues where they are utilized for the production of energy, storage, the aggregation on membrane or the synthesis of hormone [6]. Although the atherogenesis process is multifactorial, but one of the main factors to contribute to the formation of atherosclerotic plaque is abnormalities in the lipoprotein metabolism [7].

The high density lipoprotein cholesterol (HDL-c) particles are a highly heterogenous class of particles to differ in apolipoprotein (apo) and lipid composition, density, size, and charge [8]. They are with a density >1.063 g /mL [9] and can differentiate based on the density in HDL2-c larger particles, and HDL3-c less dense subpopulation [10]. The HDL-c and each of the subfractions are regarded as one of the most important

independent protective and modifiable factors against arteriosclerosis, which the current therapies for their improvement are inadequate [11]. Larger subfractions of HDL-c may protect against atherosclerosis, whereas the smaller subfractions are more atherogenic [12]. These subclasses of HDL-c can also subdivide according to their apolipoprotein composition in the HDL-c particles that only include apo A-I (LpA-I) and the particles that contain both apoAI and apoA-II (LpA-I/A-II) [13].

The lipoprotein ratios [Total cholesterol (TC)/HDL-c and LDL-c/HDL-c], which indicate the proportion between the atherogenic and antiatherogenic lipoproteins, can be clinically useful and are as better risk indicators and predictors for early stage atherosclerosis, carotid intima-media thickness (IMT) and CVD than each lipid parameter used independently, even if their classic lipid parameters are within optimum range [7]. They are also good predictors of the efficacy and being beneficial value from lipid lowering interventions [14].

There is increasing evidence to suggest the non-HDL-c is a better predictor and with greater power for the assessment risk of early stage atherosclerosis, and CVD than the serum levels of LDL-c alone [15]. This parameter is defined as difference between TC and HDL-c to contain all atherogenic lipoproteins containing apoB i.e. LDL-c, lipoprotein (a) [LP (a)], VLDL-c and intermediate density lipoprotein (IDL) [16], and the Adult Treatment Panel III is also recommended that non-HDL-c is an admissible substitute for apoB, because there is a strong correlation between apoB and non-HDL-c [17].

Eicosapentaenoic acid (EPA) is one of ω -3 PUFAs which are present at the great amounts in the fish oil [18]. The findings of several studies have shown that EPA has the antioxidant [19], antiinflammatory [20], antithrombogenic [21], and antiarteriosclerotic [22] properties. The aim of this study was to determine the effects of the supplementation of EPA on the serum profile of HDL-c, its subfractions, non-HDL-c, atherogenic/antiatherogenic lipoproteins ratios 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 [23] 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 \geq 126 mg/dl or 2hPG \geq 200 mg/dl (2-hour plasma glucose), $25 \leq$ BMI $<$ 30 kg/m², 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 center at 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 $\geq 130/85$ mmHg [24].

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 $3000 \times g$ 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 HDL2-c, HDL3-c, non-HDL-c, TC/HDL-c and LDL-c/HDL-c ratios, FBS (fasting blood sugar), HbA1c, the serum TC, TG, LDL-c and HDL-c]. The blood samples were collected only for this study.

1.2.3. Measurement of the Serum Levels of HDL2-c, HDL3-c, Non-HDL-c, Atherogenic/Antiatherogenic Lipoproteins Ratios:

The serum levels of HDL2-c and HDL3-c were measured using Enzyme-linked immune sorbent assay kits for Human HDL2 and HDL3 from SHANGHAI CRYSTAL DAY BIOTECH CO., LTD, according to the manufacturer's instructions, Cat. No.: E2188Hu, E2189Hu, respectively, Size: 96 tests, FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. The sensitivity was 0.25 ng/mL for HDL2-c and 0.53 ng/mL for HDL3-c.

Non-HDL-c was determined as difference between TC and HDL-c. TC/HDL-c and LDL-c/HDL-

c ratios are calculated by using the division of atherogenic lipoproteins (TC and LDL-c) to HDL-c the antiatherogenic lipoprotein.

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 \pm 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 [25], 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 Lipids, the Atherogenic/Antiatherogenic Lipoproteins Ratios, and Serum Non-HDL-c:

The serum total cholesterol was 216.27 ± 38.73 mmol/L after receiving placebo and 192.93 ± 39.69 mmol/L after the supplementation with EPA. The serum triglycerides was 162.8 ± 128.81 mmol/L after receiving placebo and 176.48 ± 133.75 mmol/L after

the supplementation with EPA. The serum LDL-cholesterol was 95.73 ± 21.86 mmol/L after receiving placebo and 81.4 ± 24.63 mmol/L after the supplementation with EPA. The serum HDL-cholesterol was 76.50 ± 20.81 mmol/L after receiving placebo and 93.06 ± 25.53 mmol/L after the supplementation with EPA. The serum HDL2-cholesterol was 28.29 ± 11.37 ng/mL after receiving placebo and 38.27 ± 8.3 ng/mL after the supplementation with EPA. The serum HDL3-cholesterol was 95.19 ± 35.29 ng/mL after receiving placebo and 84.20 ± 29.3 ng/mL after the supplementation with EPA. The serum TC/HDL-c ratio was 2.82 ± 1.86 after receiving placebo and 2.07 ± 0.77 after the supplementation with EPA. The serum LDL-c/HDL-c ratio was 1.25 ± 1.05 after receiving placebo and 0.87 ± 0.4 after the supplementation with EPA. The serum Non-HDL-c was 139.77 ± 36.38 mmol/L after receiving placebo and 99.87 ± 24.25 mmol/L after the supplementation with EPA (Table 2).

Discussion:

The risk thresholds of lipid in the patients with diabetes mellitus than the general population are lower and their interactions with other risk factors of cardiovascular are more powerful [26].

1. HDL-c in Type 2 Diabetes Mellitus:

The low plasma levels of HDL-c and its subfractions, mainly a decrease in HDL2-c and to some extent in HDL3-c, are as the hallmark of diabetic dyslipidemia and common in the patients with type 2 diabetes mellitus [27], and reason decrease the plasma levels of HDL-c can mainly be of increment in the transfer of cholesterol from HDL-c to lipoproteins enriched in triglyceride by cholesteryl ester transfer protein (CETP) along with the transfer of triglycerides to HDL-c. Then hepatic lipase (HL) hydrolyzes these particles enriched in triglyceride and rapidly catabolized and clears [28]. Meanwhile, mean size of the HDL-c particle is decreased in insulin resistant and diabetic subjects [29]. Decrease in the plasma levels of HDL-c is also associated with the reduction of HDL2-c subfraction in type 2 diabetes mellitus [30], and there is a correlation between reduced in the plasma level of HDL2-c with both obesity and hypertriglyceridaemia in these patients [31]. Insulin resistance can lead to disability of insulin to increase in the production of apoA-I that this might contribute to the low levels of HDL-c [32]. Tumor necrosis factor (TNF)- α as a mediator in insulin resistance status related to obesity is known that lower the serum levels of HDL-c [33]. In the subjects with insulin resistance, the alteration in several key enzymes involved in the HDL-c metabolism is associated with the low plasma levels of HDL-c [34]. Moreover, in vivo kinetic studies by using radioisotopes [35] and stable isotopes

[36] have been demonstrated that the decrease in HDL-c noted in the type 2 diabetic patients is due to increase the catabolism rate of HDL-c particles. In insulin resistant status and the type 2 diabetes mellitus, the activity of HL augmented [37], and the HDL-c particles enriched in triglycerides are very good substrate for this enzyme, thereby whose catabolism increase [38]. Meanwhile, one of other mechanisms responsible for the reduction of the plasma levels of HDL-c in insulin resistant states and type 2 diabetes is the reduction in plasma levels of adiponectin [38].

The HDL-c and small, dense HDL3-c particles from the patients with type 2 diabetes mellitus than HDL3-c from control healthy subjects had significantly less capacity to the protection of LDL-c against oxidation, to destroying of oxidized phospholipids, and to the induction of cholesterol efflux from macrophage [39]. It has also been shown that the small, dense HDL-c particles in patients with type 2 diabetes result in decrease in the antioxidative activity [40].

2. Functions and Molecular Mechanisms of Action of EPA:

Several studies have shown that EPA has various effects, including preventing of the insulin resistance [41], increasing the insulin secretion [42], enhancing the size of LDL-c particle [43], reducing the serum levels of TG, lowering the blood viscosity, increasing the production of nitric oxide (NO), having the antiinflammatory and antithrombotic properties [44-46], 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 [47]. 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 phospholipase A2 (PLase A2) [48, 49].

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 [50].

The findings of an epidemiological study of Greenland Eskimos suggested that EPA could be has the antithrombogenic and antiarteriosclerotic properties [22]. It has been postulated that the mechanisms of these actions are including the

suppression of platelet aggregation and the improvement of blood rheologic properties [51].

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 [21]. These results suggest that the administration of EPA to the patients with type 2 diabetes may prevent of 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 HDL-c and Its Subfractions:

The data from some clinical trials show that the ω -3 PUFAs increase the plasma levels of HDL-c [52, 53] and HDL2-c [53, 54], whereas in other some indicated no effect on this antiatherogenic lipoprotein [55, 56] and HDL2-c subfraction [57]. Increase in the plasma levels of HDL2-c may be owing to the considerable reduction of plasma lipoproteins enriched in triglyceride, leading to a decrease in the transfer of cholesterol ester (CE) out of HDL-c [58], and thereby the plasma accumulation of HDL-c enriched in CE and catabolizing slow of HDL2-c particles [59]. In two human studies carried out about effect of EPA on the plasma levels of HDL-c, one of studies not significant an increase [60], and another no significant changes [61] in the plasma levels of this atherogenic lipoprotein reported. Furthermore, several clinical trials have reported that the ω -3 PUFAs decrease the plasma levels of HDL3-c [62, 63], but two studies no significant changes of HDL3-c demonstrated [57, 64]. 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 enhancement in the serum levels of HDL-c 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, respectively, significantly increase and decreases in the serum levels of HDL2-c and HDL3-c (Table 2). So that these results are compatible with the data of some human studies and different from some other of clinical trials previously reported with ω -3 PUFAs. Meanwhile, as yet, the effect of EPA on the serum levels of HDL-c subfractions in vitro and in vivo was not studied, and this is the first time that has been demonstrated EPA

can increase the serum levels of HDL2-c and reduce the serum levels of HDL3-c in vivo.

4. Effects of ω -3 PUFAs on the Atherogenic/Antiatherogenic Lipoproteins Ratios and Non-HDL-c:

The results of a number of previous studies have shown a decrease in the plasma ratio of LDL-c/HDL-c in the patients supplemented with ω -3 PUFAs [62, 65], while one study reported no significant changes in this plasma ratio [66].

As yet, the effect of EPA on the serum ratios of LDL-c/HDL-c and TC/HDL-c in vitro and in vivo was not studied, and this is the first time that has been demonstrated EPA can decrease these two serum ratios 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 ratios of LDL-c/HDL-c and TC/HDL-c than the placebo group (Table 2).

Meanwhile, in one study performed on patients with type 2 diabetes mellitus and hypertriglyceridemia is observed that ω -3 PUFA can reduce the serum non-HDL-c [53]. But the effect of EPA on the serum non-HDL-c in vitro and in vivo was not yet studied, and this is the first time that has been demonstrated EPA can decrease non-HDL-c in vivo (Table 2). Therefore, our finding is in agreement with that of the interventional study performed in this regard with the ω -3 PUFA supplementation on the patients with type 2 diabetes mellitus and hypertriglyceridemia.

Thus, it is significant to point out that our data provide evidence compatible with the hypothesis that EPA influences the serum levels of HDL-c, its subfractions, the serum ratios of LDL-c/HDL-c and TC/HDL-c, and non-HDL-c 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 [67], reducing the serum levels of TG [68], increasing the plasma levels of HDL-c and HDL2-c [68, 69], and decreasing the plasma levels of HDL3-c [68]. 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 [68, 69], 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 HDL-c and its subfractions 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 HDL-c and its subfractions. 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

apoB/apoA-I serum ratio, and 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, additional studies will be necessary to determine the general applicability of our study results.

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

Group	Variable	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/m ²)	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 and ratios of lipids, and serum non-HDL-c at baseline and after of 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	216.27 ± 38.73	0.396	208.44 ± 43.57	192.93 ± 39.69	0.272
Triglyceride (mmol/L)	221.50 ± 91.49	162.8 ± 128.81	0.124	218.61 ± 94.52	176.48 ± 133.75	0.282
LDL-cholesterol (mmol/L)	92.61 ± 25.92	95.73 ± 21.86	0.698	96.33 ± 26.13	81.4 ± 24.63	0.086
HDL-cholesterol (mmol/L)	76.22 ± 32.85	76.50 ± 20.81	0.975	77.72 ± 14.92	93.06 ± 25.53	0.034
HDL2-cholesterol (ng/mL)	28.35 ± 11.54	28.29 ± 11.37	0.987	31.99 ± 9.1	38.27 ± 8.3	0.037
HDL3-cholesterol (ng/mL)	94.17 ± 61.46	95.19 ± 35.29	0.951	103.67 ± 25.4	84.20 ± 29.3	0.040
TC/HDL-c (ratio)	2.68 ± 1.33	2.82 ± 1.86	0.796	2.68 ± 0.89	2.07 ± 0.77	0.034
LDL-c/HDL-c (ratio)	1.22 ± 0.79	1.25 ± 1.05	0.923	1.24 ± 0.49	0.87 ± 0.4	0.018
Non-HDL-c (mmol/L)	128.22 ± 42.81	139.77 ± 36.38	0.389	130.72 ± 21.06	99.87 ± 24.25	< 0.001

Conclusion:

Considering our results, we concluded that EPA has a beneficial effect on antiatherogenic lipoprotein, its subfractions and thereby on 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.

Acknowledgements:

This study was supported by a grant from the Research Deputy of Tehran University of Medical Sciences (project number 15202). We thank from the staff of Iran Diabetes Association for helping in recruiting of the patients, and from several colleagues from School of Nutrition Sciences and Dietetics, the Tehran University of Medical Sciences for their technical assistance.

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