

Effects of EPA and Vitamin E on Serum Enzymatic Antioxidants and Peroxidation Indices in Patients with Type II Diabetes Mellitus

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Abstract

Background: Diabetes mellitus is associated with chronic changes in peripheral arteries because of oxidative stress and insufficient antioxidative defense mechanism. Omega-3 fatty acid supplementation could be effective in some diabetes complications; however, polyunsaturated fatty acids may increase lipid peroxidation. This study aimed to determine whether eicosapentaenoic acid alone or in conjunction with vitamin E had differential effects on serum antioxidants and peroxidation indices.

Methods: This double-blind, placebo-controlled trial was carried out on 136 patients with type II diabetic mellitus (age 48.8±4.4 yr, BMI 27.8±1.7 kg/m²). The four groups of the study either received two grams of omega-3 fatty acids, 400 IU of vitamin E, a combination of the two or placebo for three months. Their serum total antioxidant capacity, enzymatic antioxidants and peroxidation indices were assessed.

Result: Fasting serum TAC increased in EPA+E (10.7%, $P < 0.001$) and E groups (7.5%, $P < 0.05$). SOD, G-PX and G-RD increased in EPA group (7.3%, 5.1%, and 8.4%, $P < 0.05$, respectively). MDA and protein carbonyl decreased in EPA and E groups (respectively, 12.5%, 7.6% $P < 0.05$, $P < 0.05$; 13%, 15.3% $P < 0.001$, $P < 0.05$). After adjustment for baseline values, age, sex, BMI and duration of diagnosed diabetes, protein carbonyl decreased in EPA+E and E group (30.7%, 15.3%; $P < 0.05$ respectively) relative to the placebo group.

Conclusion: EPA, by itself has a statistically significant effect on serum total antioxidant capacity, enzymatic antioxidants and peroxidation indices in diabetic patients compared to EPA+E or E alone.

Trial registration: ClinicalTrials.gov NCT00817622

Keywords: Diabetes mellitus, Eicosapentaenoic acid, EPA, Total antioxidant capacity, Vitamin E

Introduction

Diabetes mellitus is one of the most prevalent non-communicable chronic diseases around the world which is spreading rapidly in epidemic proportions (1). There are reports that patients with diabetes are faced with increasing oxidative stress, elevated production of oxygen free radicals (OFRs) and insufficient antioxidant defense mechanism (2-4). On the other hand, hyperlipidemia is one of the causative factors of increased lipid peroxidation due to metabolic imbalances in diabetes (2).

Different studies have demonstrated that the lipid composition of atheroma in atherosclerotic plaques is similar to that of blood lipids (5) and a close relationship exists between dietary lipids and atheromatous plaques in coronary artery disease (4). In addition, the type of dietary lipids has been more important than the total consumed lipids (5). Therefore, consumption of omega-3 fatty acids present in deep sea and cold water fish in wild vegetables with dark green color leaves or in animals grazing freely in the wild have been recommended (3, 6-8).

Omega-3 fatty acids (DHA, EPA) are essential for cell membrane structure and function (9).

Some studies have reported the dual effects of antioxidative defense mechanisms in diabetes where increased or decreased antioxidant levels are observed (10). Conversely, unpredictable effects of materials containing omega-3 predisposing the subjects to lipid peroxidation in aerobic conditions have been reported too (11). These instances normally occur in aerobic conditions in the absence of antioxidants (9). Recent studies have shown that polyunsaturated fatty acids can act as an antioxidant in-vivo (12, 13) if they are consumed with adequate amounts of vitamin E to act as a scavenger for reactive oxygen species as do omega 3 and 6 in similar in-vivo conditions (14). Therefore, this study was undertaken in patients with diabetes type II with a BMI of ≥ 25 -30 to evaluate the effects of pure eicosapentaenoic acid (EPA) with and without vitamin E on lipid peroxidation indices, enzymatic antioxidant activity, and total serum antioxidant capacity to find an appropriate approach for dietary modifications and prevent related complications in these patients.

Materials and Methods

Study population

The study population consisted of 136 patients with type II diabetes mellitus (an individual being treated by oral hypoglycemic agents or having a fasting plasma glucose concentration >7.0 mmol/L) (15).

The inclusion criteria included being 35-50 yr old, male or female, with a BMI ≥ 25 -30, having been diagnosed with diabetes for 2-15 yr, no recent (past year) digestive, renal, microalbuminuria (16), hepatic, cardiovascular, thyroid or respiratory diseases or a diagnosed cancer. Additionally, being non-alcoholic, non-smoker, with no history of drug abuse, ongoing pregnancy or breast-feeding were the other criteria for inclusion in the study.

Study design

In this placebo-controlled, double blinded study with parallel designs, subjects were randomly assigned to one of four groups and for duration of

diagnosed diabetes, after signing an informed consent form. The individuals filled a demographic form and reported a 24 h dietary recall, as they did two more times during the study. The four groups included:

(P)= The placebo or the group receiving four grams of corn oil,

(EPA)= The EPA group receiving two grams of EPA supplement + placebo

(E)= The vitamin E group receiving 400 IU of vitamin E supplement + placebo

(EPA+E)= The EPA plus vitamin E group receiving two grams of EPA supplement + 400 IU of vitamin E

The individuals were told to take the supplements postprandially (after lunch) without drinking hot fluids. The regimen continued for 12 wk, while they took their antidiabetic agents as before. The subjects were also recommended not to change their diet or their physical activity until the end of the study.

The subjects' weight, BMI and blood pressure were measured and they were referred to laboratory at the beginning and at end of the 12th wk.

All the subjects were closely followed up every two weeks for weight, BP and BMI measurements and the way they were taking the supplement compliance, their diet and physical activity and by phone for making sure they were following the recommendations.

For blood pressure measurements, the individuals' right arm systolic pressure was measured in sitting position after five minutes of rest. The mean arterial pressure (MAP) and plus pressure (PP) were measured by the following formula (17):

$$\text{MAP} = (2\text{DBP} + \text{SBP})/3 \quad \text{PP} = \text{SBP} - \text{DBP}$$

The patients' weight and height were measured by a Seca height and weight measuring scale (Seca 725 gmbh & co. kg., Germany) and BMI was calculated by using weight/height²m formula.

The Ethics Committee of Tehran University of Medical Sciences approved the study proposal.

An individual outside the study was asked to help allocate the medications to the groups.

Supplement Characteristics

Plus EPA 500 mg pearls containing pure EPA (95%) from Minami, Nutrition, Belgium and Vitamin E pearls containing 400 IU of α -Tocopherol from (Dana Pharmaceutical Company, Tabriz, Iran) were used.

Blood collection

Venous blood samples were drawn in empty and in EDTA vacuum tubes after an overnight fast. Plasma and erythrocytes (RBC) were separated by centrifugation at 3000 rpm for 10 min at room temperature. RBCs were washed three times with 0.9% NaCl solution, and then hemolyzed with four volumes of cold distilled water. Plasma samples and erythrocyte lysates were stored at -70°C until assayed. Samples were protected from the natural light.

Serum Total Antioxidant Capacity, Enzymatic Antioxidants, Malondialdehyde and Protein Carbonyl

Fasting serum Total Antioxidant Capacity (TAC) was measured at baseline and at the end of the intervention by 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid (ABTS) (18). Erythrocyte activity of Glutathione Peroxidase (G-PX) and Glutathione Reductase (G-RD) were assessed in the hemolysates by RANDOX kit (RANDOX Laboratories Ltd, United Kingdom) (19). The coefficient of variability between assays was 4%. The activity of SOD was measured at 500 nm by testing the inhibition degree of tetrazolium salt oxidation reaction (RANDOX kit) (20). The coefficient of variability between assays was 4.2%.

Fasting serum Catalase (CAT) activity was measured according to Hego Aebi's method (21) and serum MDA activities were determined using the thiobarbituric acid method (22).

Protein oxidation was determined by measuring the carbonyl groups on the amino acids of plasma proteins. With this method, the carbonyl groups react with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone derivatives, which can be measured spectrophotometrically (23).

Samples obtained at baseline and at the end of the intervention were measured in a single assay to minimize inter-assay variations.

Dietary survey

The subjects' food intake was assessed by two dietitians using an open-ended, interview-administered diet history. Subjects were asked on their daily diet over a week period (24 h recall report, 3 times: Saturday, Monday and Thursday). Energy and nutrient intakes were calculated by using the Food Processor (version 2, Esha Research, USA) software.

Statistical analysis

The data were analyzed using SPSS (version 16.0 for Windows, Polar Engineering and Consulting, USA) by general linear models to assess the effects of EPA, E, or EPA+E relative to the placebo. All variables were tested for normality by using histograms and Kolmogorov-Smirnov statistics. If the data distribution was not normal, either before or after the intervention, they were log transformed for statistical analysis.

Significance levels were adjusted for multiple comparisons by using Bonferroni method. Differences between the four groups were considered significant when $P < 0.05$ (after Bonferroni adjustment). All values were reported as means \pm SEMs except for the demographic data of the patients at baseline, which are reported as means \pm SDs or as percentage (%).

Results

Study population

At the beginning of the study, 136 patients were recruited and 127 patients finished the study successfully (without any changes or fluctuations in taking the supplements, physical activity or diet). Two subjects withdrew because of digestive intolerance to the supplements, two for unplanned journeys, two for incompliance to the program, one for appendectomy, one for immigration while one subject withdrew because he could not attend the center for blood measurement in the final stage. Baseline characteristics of the four groups confirmed that they had been well-matched for the entry criteria (Table 1 and 2). There were no significant differences between the groups in either the

type or number of oral hypoglycemic medications. The subjects also took oral hypoglycemic agents in the form of biguanides with sulfonylureas (76%), sulfonylureas (16%) or biguanides agents (8%).

Energy and macronutrient intakes

Evidence of adherence to the diets was confirmed by analyzing the diet recall records and supplement counts.

Analysis of diet records indicated that total energy and major macronutrient and micronutrient intakes were not significantly different between the groups at baseline (Table 2) and did not change significantly in any of the groups during the intervention. Antidiabetic agents were unchanged during the intervention in all groups too.

Serum Antioxidants

There were no significant differences between the groups regarding fasting serum total antioxidant capacity, enzymatic antioxidants, malondialdehyde and protein carbonyl at baseline (Table 3). Changes in fasting serum total antioxidant capacity, enzymatic antioxidants, malondialdehyde and protein carbonyl are shown in (Table 3).

Fasting serum TAC was significantly higher at the end of the intervention than the baseline in the EPA+E and E groups (10.7%, $P < 0.05$ and 7.5%, $P < 0.05$; respectively).

Fasting serum SOD was significantly higher at the end of the intervention, compared to baseline values, in EPA (7.3%, $P < 0.05$), EPA+E and E groups (5.7% and 4.8%; NS) but it decreased in the placebo group (3%, NS).

A significant increase was seen in catalase activity in EPA+E groups (7.5%, $P < 0.04$) (Table 3). Serum G-PX activity had a significant increase in EPA (5.1%, $P < 0.05$), and non-significant increases were found in EPA+E and E groups (2% and 4.2%, respectively), while the placebo group underwent a non-significant decrease (NS).

Serum G-RD activity underwent a significant increase in EPA (by 8.4% $P < 0.05$).

Fasting serum MDA decreased in all groups except placebo, but it was statistically significant in EPA (12.5%, $P < 0.05$) and E groups (13%, $P < 0.05$).

Fasting serum protein carbonyl decreased in E, EPA and EPA+E groups (15.3%, $P < 0.05$; 7.6%, $P < 0.05$ and 6%, NS).

After adjustment for baseline values, age, sex, BMI and duration of diagnosed diabetes, dietary ingredients, all enzymatic antioxidant, TAC and MDA did not have any significant differences among all groups, but fasting serum protein carbonyl in EPA+E and E groups had a significant decrease relative to the placebo (30.7%, $P < 0.05$; 15.3%, $P < 0.05$; respectively) (Table 3).

Table 1: Characteristics of participants in four groups at baseline¹

Treatment group	EPA+E (n=34)	EPA (n=34)	E (n=32)	P (n=36)
Age (Y)	45 ± 4.3	44 ± 5.0	44 ± 4.4	45 ± 4.1
Gender (M/F) (%)	67/32	58/41	53/46	63/36
Weight (kg)	72.2 ± 8.1	74.3 ± 8.4	72.4 ± 9.6	70.5 ± 8.3
BMI (kg/m ²)	27.8 ± 2.4	27.9 ± 1.7	27.8 ± 1.6	27.8 ± 1.6
Duration of diagnosed diabetes (y)	7.5 ± 4.2	7.4 ± 3.9	7.1 ± 3.5	8.3 ± 3.9
Mean Blood Pressure (mmHg)	90 ± 6.1	89 ± 6.3	86 ± 6.2	90 ± 7.1
Pulse pressure (mmHg)	41 ± 10	41 ± 7	42 ± 10	48 ± 12

1- X-bar ± SD or %. There were no significant differences between the four groups at baseline (by UNIANOVA).

Chi-square test was used to detect differences in gender profile between the groups. [EPA= eicosapentaenoic acid; E= Vitamin E].

Table 2: Total energy and macro and micronutrient intakes at baseline and postintervention after 3 months in four groups¹

Treatment group		EPA+E (n=34)	E (n=32)	EPA (n=34)	P (n=36)
Total energy intake (kcal/d)	Baseline	1656 ± 372	1764 ± 403	1616±336	1793±395
	Postintervention	1635 ± 292	1742 ± 428	1653±373	1838± 409
Total fat (g/d)	Baseline	64± 15	70± 18	63± 19	70±18
	Postintervention	68± 20	70± 21	62±18	68±21
Saturated fat (g/d)	Baseline	21±7	22 ±9	20±7	22±9
	Postintervention	21±6	23 ±8	21±6	22± 7
Monounsaturated fat (g/d)	Baseline	19±5	21±5	18± 6	21±5
	Postintervention	20±6	21±7	18±5	21± 7
Polyunsaturated fat (g/d)	Baseline	19±5	23±8	19± 8	23±8
	Postintervention	21±9	21±7	19±9	20±8
Cholesterol (g/d)	Baseline	206±106	216±123	195±113	200±112
	Postintervention	232±111	213±124	184±78	192±105
Protein (g/d)	Baseline	60±15	60±18	56±13	62±18
	Postintervention	64±13	66±19	56±11	64±17
Carbohydrate (g/d)	Baseline	220±66	241± 73	215± 47	239±67
	Postintervention	229±55	239±64	218±50	243±60
Fiber (g/d)	Baseline	15± 5	17±6	16±6	17±6
	Postintervention	17±6	17±7	15±5	18±6
Vit A (mcg/d)	Baseline	1073± 1365	1165±1364	844 ±587	890±637
	Postintervention	981± 652	1100 ± 679	809± 545	974±624
Vit E (mg/d)	Baseline	13±5	17 ± 9	14± 6	17 ± 9
	Postintervention	15±8	17± 8	14± 9	16 ± 9
Vit C (mg/d)	Baseline	99±58	86± 66	58± 38	82± 57
	Postintervention	97±53	84 ±68	68±51	83± 64
Selenium (mcg/d)	Baseline	121±43	128± 46	120± 41	131±42
	Postintervention	132±34	125±40	114± 43	123±34
Fe (mg/d)	Baseline	11±2	11±2	10 ±2	12±3
	Postintervention	11±2	10±2	10 ±2	11±3
Zinc (mg/d)	Baseline	8±3	8±2	8±2	8±2
	Postintervention	9±2	9± 2	8±2	8±2
Mg (mg/d)	Baseline	123±31	124 ±36	118± 32	124±41
	Postintervention	111±27	122±36	117±32	122±31

1- X-bar±SD. Baseline and Postintervention measures were compared by paired t-test (within groups) and between groups by UNIANOVA. There were no significant differences between the groups in any of the dietary nutrients at baseline and no significant changes during the intervention. [EPA= eicosapentaenoic acid; E = Vitamin E].

Table 3: Fasting serum TAC, SOD, CAT, G-PX, G-RD, MDA and Pro-Carbonyl at baseline and Postintervention in four groups¹

Treatment group		EPA+E (n=34)	EPA (n=34)	E (n=32)	P (n=36)	Treatment effect (p) ²		
						EPA	EPA+E	E
TAC (mg/dl)	Baseline	2.8 ± 0.4	3.0 ± 0.3	2.8 ± 0.4	2.9 ± 1.2			
	Postintervention	3.1 ± 0.3*	3.1 ± 0.3	3.1 ± 0.3*	2.9 ± 0.3	+0.2 ± 0.3	+0.3 ± 0.3	+0.0 ± 0.3
SOD (U/gHb)	Baseline	1068.9±350.4	1061.7±241.1	990.1±243.1	1071.4±250.9			
	Postintervention	1130.9± 257.8	1139.5±238.1*	1038.0±237.3	1039.1±228.6	+134.7±71.7	+4.5±78.3	+20.7±72.9
CAT (U/gHb)	Baseline	202.1±34.6	205.0± 30.9	203.4± 32.0	201.8± 39.2			
	Postintervention	217.4±48.3*	221.7±58.8	214.7± 53.8	200.5± 48.0	+23.6±14.9	+6.3±16.2	+5.0 ±15.1
G-PX (U/gHb)	Baseline	43.8± 6.7	40.9±5.8	44.5 ± 8.1	42.5± 5.7			
	Postintervention	44.8± 6.2	43.0±8.0*	46.4 ± 7.1	42.3± 4.6	+0.6± 1.9	+1.4 ± 2.0	+2.6 ± 1.9
G-RD (U/gHb)	Baseline	5.4 ±1.8	4.4 ±1.4	4.4 ±2.3	4.6± 1.4			
	Postintervention	5.7±1.7	4.8 ±1.7*	5.0 ± 2.2	4.6 ± 1.4	- 0.1± 0.5	+0.9± 0.5	+0.3± 0.5
MDA (ng/ml)	Baseline	2.1± 0.4	2.4 ± 0.6	2.3 ± 0.6	2.3± 0.6			
	Postintervention	1.9 ± 0.5	2.1 ±0.3*	2.0 ± 0.6*	2.1± 0.4	- 0.2± 0.1	- 0.3± 0.1	-0.3± 0.1
Pro-Carbonyl (nmol/mg protein)	Baseline	1.0 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.3± 0.3			
	Postintervention	0.9 ± 0.2	1.2 ±0.2*	1.2 ± 0.2*	1.2± 0.5	- 0.2± 0.0	- 0.3± 0.1‡	-0.2 ± 0.0‡

1- $\bar{x} \pm SD$. There were no significant differences between the four groups at baseline (by UNIANOVA).

*Significantly different from before and after treatment (by paired t-test) within groups: $P < 0.01$. ² $\bar{x} \pm SEM$, A general linear model (ANCOVA) was used to assess treatment effects on Postintervention values adjustment for age, sex, BMI, duration of diagnosed diabetes and baseline value. ‡ Significantly changes for each of the three treatment groups relative to the corn oil group. [EPA= eicosapentaenoic acid; E= Vitamin E; TAC= Total Capacity of Antioxidant; CAT= Catalase; SOD= Superoxide Dismutase; G-PX= Glutathione Peroxidase; G-RD= Glutathione Reductase; MDA= Malondialdehyde; Pro-Carbonyl = Protein Carbonyl].

Discussion

In this study, purified EPA +E had a significant effect on TAC and CAT activities in patients with diabetes type II. EPA alone had significant effects on serum enzymatic antioxidants as measured by SOD, G-PX and G-RD, except CAT and peroxidation indices as measured by MDA and protein carbonyl.

Although worries about the consumption of n3-PUFA and increased lipid peroxidation have lingered about for some time, but in the present study the index for lipid peroxidation (MDA) was not

only decreased about 12.5% ($P < 0.05$) but the protein peroxidation index (protein carbonyl) was also decreased about 7.6% ($P < 0.05$) in diabetic patients receiving two grams of daily EPA for three months. However, in patients on EPA+E, lipid and protein peroxidation indices underwent statistically insignificant decreases MDA 4.7% and protein carbonyl 6%. These worries have also been reported on the use of n3-PUFA by Mori et al. (24, 25) and Trevor et al. (26, 27). They found that in vivo consumption of fish, providing four grams of omega-3, in diabetes type II patients did

not confirm the theorem of increased lipid and protein peroxidations after n3-PUFA consumption and instead it decreased the oxidations shown by decreases in urinary F2-isoprostan (27).

By this finding Mori et al had once again proven their previous studies carried out in 2000 in which consumption of EPA and pure DHA in comparison with olive oil in overweight people with hyperlipidemia had resulted in a 27% and 26% decrease in urinary F2-isoprostan (28, 29). They believed that decrease in oxidative stress had been due to the anti-inflammatory effects of omega-3 fatty acids executed by reduction of free radical formation in white blood cells (27).

On the other hand, studies such as the one by Ramirez have demonstrated that MDA levels decrease in old people upon omega-3 intake and LDL susceptibility to oxidation decreases in *in vitro* environment containing metallic ions (30). Carpentier et al. also depicted this decrease in oxidative stress by administering four grams of EPA or DHA in overweight subjects with hyperlipidemia (31) as did Tanaka in Japan by administering 2.7 gr of pure EPA in overweight 27-74 yr old subjects with non-alcoholic hepatic steatosis and showed decreases in thioreduction as an index for oxidative stress in hepatic cells. Regarding his remarkable results, Tanaka suggested addition of pure EPA to statins (13) and in his next Japan EPA Lipid Intervention Study (JELIS) he observed remarkable decreases in oxidative stress indices upon addition of 1.8 gr of pure EPA to statins in CHD patients with hyperlipidemia (32). However, Sumida *et al* observed the decreasing effects of three grams of EPA administration on serum ferritin and thioreduction, two hepatic oxidative stress markers, separately in 2000 and in 2003 (33, 34). They also observed a remarkable reduction in hepatic MDA concentration and 4-Hydroxynoneal content following an increase in the expression of SOD genes containing Cu, Mn and Zn (33, 34). Similarly, we demonstrated SOD enzyme activity in three groups of subjects taking EPA, EPA+E and E, respectively 7.3%, 5.7% and 4.7%, with statistically significant results in the group taking EPA ($P < 0.05$). Sumida reported

the reduction of triglyceride proportion to hepatic free fatty acid concentration as an effective factor in MDA reduction, contrary to their belief regarding the useful antioxidative effects of EPA (33, 34). Moreover, Tanaka et al. reported that EPA not only exerted its anti-inflammatory effects through decreased excavation of fatty acids by hepatocytes, it but also eliminated oxidative stress produced by arachidonic acid. They also showed that EPA reduced the nuclear factor [Kappa]-B and metamorphosis of its subunits. EPA also declined damage to hepatocytes by improving their membrane fluidity (13).

Although increases in the consumption of oily fish caused a source of concern, where increased LDL oxidation might aggravate atherosclerosis, but numerous studies have shown that the fatty acids in LDL, which originate from oil in food regimens leave LDL susceptible to oxidative changes. Therefore, it is expected that n3-PUFA consumption increase LDL oxidation (11) but in the present study the concentration of all serum enzymatic antioxidants were remarkably increased in the EPA group, especially SOD, G-PX and G-RD (Respectively, 7.3%, 5.1% and 8.4%, $P < 0.05$). Although Higgins et al showed that serum antioxidant enzymes undergo no changes by administering different doses of fish oil in healthy subjects aged 19-63 yr over a period of 16 wk but they had used EPA doses of 0.3, 0.6 and 0.9 grams which were smaller than the doses used in the present study (11).

In short-term supplementation with n3-PUFA, LDL and other membranous lipids undergo oxidation, which requires double-band fatty acids smaller than n3-PUFA. Brude et al. believed reduced oxidation rate following n3-PUFA consumption were due to tight packing of EPA and DHA in lipid membrane complex which provides fewer double bonds for interaction with free radicals (29).

Another hypothesis provided by Brude relies on the fact that n3-PUFAs internally regulate antioxidant systems (29). Upon introduction of this hypothesis, it was demonstrated that plasma and platelet concentrations of x-carotene increased after

n3-PUFA supplementation (11). Therefore, this adaptive effect was introduced to better justify the process when enhanced lipid oxidability is more often seen in short-term rather than long-term supplementation. Increased LDL oxidations have been reported in 3, 5 and 6 wk supplementations in contrast to 5, 6, and 8 up to 16 wk supplementations resulting in a decrease in LDL oxidation (11). In the present 12 wk study, MDA and protein carbonyl underwent significant decreases upon supplementation with two grams of EPA. Additionally, numerous studies on the propriety of applied doses of n3-PUFA have shown LDL oxidation will not occur in doses smaller than 2.5 gr/day but unwanted effects of n3-PUFA on lipid oxidation, especially LDL, will be seen in doses greater than or equal 2.5 gr/day (11).

In conclusion, it seems that EPA by itself has a statistically significant effect on serum total antioxidant capacity, enzymatic antioxidants and peroxidation indices in diabetic patients compared to EPA+E or vit E alone. Larger studies may be needed to confirm the results of the study.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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