EFFECTS OF EICOSAPENTAENOIC ACID AND VITAMIN E SUPPLEMENTATION ON SERUM LIPID PROFILE, BLOOD PRESSURE, ANTIOXIDANT STATUS AND INFLAMMATORY RESPONSES IN MALE ATHLETES

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Abstract

INTRODUCTION: The relationship between ω -3 fatty acids and surrogate circulating markers of cardiovascular disease (CVD) risk, especially in healthy individuals remains to be determined. We investigated the effects of Eicosapentaenoic acid (EPA) supplementation, with or without vitamin E, on serum lipid profile, C-reactive protein (CRP), blood pressure (BP) and total antioxidant capacity in a sample of male athletes.

METHODS: This randomized double blind placebo-controlled clinical trial was conducted in 2006 on 34 apparently healthy, well-trained male basketball players, aged 17-35 years, . Venous blood samples were obtained between 5:00 and 6:00 p.m., after exercising for 2 hours, at the baseline and after intervention. Participants received 2 g EPA and/or 400 IU vitamin E and/or placebo depending on their groups. For 6 weeks, eight subjects received an EPA supplement with vitamin E (group 1), nine subjects received an EPA supplement with vitamin E placebo (group 2), nine subjects received an EPA supplement placebo and vitamin E (group 3), and eight subjects received an EPA supplement placebo and vitamin E placebo (group 4).

RESULTS: Significant decreases were documented in the serum levels of total cholesterol (TC), triglycerides (TG), LDL-C and CRP in group1 ($p<0.01$), in TC, TG, LDL-C, CRP, and BP in group 2 ($p<0.01$), and significant increase in total antioxidant capacity in group 3 ($P<0.05$). No significant difference was found in LDL between groups 1 and 4 ($P<0.05$), and in total antioxidant capacity between groups 2 and 3 ($p<0.001$) and groups 3 and 4 ($p<0.001$), and in CRP level between groups 2 and 3 ($P<0.05$). There were no significant differences in TC, TG, HDL-C and BP between the groups after 6 weeks of intervention.

CONCLUSIONS: Six weeks of EPA+ vitamin E supplementation improved the lipid profile and reduced the CRP level, whereas six weeks of EPA supplementation without vitamin E improved the lipid profile, but increased CRP and BP. Six weeks of vitamin E supplementation alone increased total plasma antioxidant capacity.

Keywords: Eicosapentaenoic acid, vitamin E, lipid, antioxidant, inflammation, healthy men, exercise.

ARYA Atherosclerosis Journal 2007, 3(2): 87-93

Date of submission: 28 Oct 2006, *Date of acceptance:* 10 Mar 2007

Introduction

The association of dietary ω-3 fatty acids and the risk of developing cardiovascular disease (CVD) began to emerge in the late 1970s. Thereafter, a limited number of intervention trials reported lower rates of CVD mortality, after supplementation with the long-chain ω-3 fatty acids eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA). Potential mechanisms for the cardioprotective effects of ω-3 fatty acids include anti-arrhythmic, anti-thrombotic and anti-inflammatory effects,as well as its effects on lowering blood pressure (BP), improving endothelial function, and reducing serum triglycerides (TG) levels.[1]

Although the relationship of dietary fish and fish oil with CVD risk factors is well-documented, the relationship between ω-3 fatty acids and surrogate circulating markers of CVD risk remains to become clear.

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The long-chain fatty acids are integral constituents of cell membrane phospholipids that can affect properties of ion transport, receptor binding, enzyme activities, electrical potentials, and gene expression. They are also precursors for local mediators called eicosanoids (prostaglandins, thromboxanes, leukotrienes), which modulate vascular tone, platelet aggregation, and inflammatory responses.[2]

EPA and DHA lower triglyceride levels in the fasting and post-prandial states in a dose-dependent manner, and do so reliably and effectively.[3-5] Low-density lipoprotein cholesterol (LDL-C) levels are slightly increased probably because the more buoyant, fast-floating LDL subclasses increase, while the denser, slow-floating LDL subclasses decrease. The effects of …..on other serum lipids, i.e. cholesterol or high-density lipoprotein (HDL) are less clear.[5]

Some investigators have shown that supplementation with ω-3 fatty acids may lower serum TG concentrations. A 25-30% reduction in TG concentration is reported with approximately 4 g/d of ω-3 fatty acids.[6] An increase in LDL-C concentrations has been observed both in healthy subjects[7] and hyperlipidemic individuals receiving ω-3 fatty acid supplements.[6] In addition, HDL-C levels increase with fish oil supplementation (3.5-4 g/d).[8] It is reported that DHA supplementation (1.2 g/d for 6 weeks) in hyperlipidemic children improves the lipoprotein subclass profile without significant changes in total cholesterol (TC), LDL-C, HDL-C and/or TG concentrations.[9]

Predominance of small dense LDL-C particles (subclass pattern B) is associated with increased atherogenicity compared to larger, more buoyant LDL-C particles (subclass pattern A).[9] The results of these studies suggest that ω-3 fatty acids may affect the quality of lipoproteins. A shift in the distribution of lipoprotein particles to large, buoyant LDL-C and HDL-C particles may partially contribute to the anti-atherogenic effects of ω-3 fatty acids.

Some human and animal studies revealed that fish oil rich in EPA and DHA lowers BP. Meta-analyses of controlled trials on fish oil supplementation suggest that the BP-lowering effect in hypertension is both dose- (>3 g/d) and time-dependent.[10,11] Increasing evidence also indicates that DHA alone can reduce BP.[12] Among athletes, inflammation may be caused by mechanical stress, local ischemia and/or free radical generation in the active skeletal muscle. After high-intensity exercise, the immune system becomes involved in tissue repair processes.[13]

Strenuous exercise is accompanied by an increase in circulating pro-inflammatory markers, bearing some similarities with the response to sepsis and trauma.[14] Specific fatty acids can lower the levels of certain pro-inflammatory cytokines; these fatty acids may have a protective role against the inflammatory responses caused by exercise.[14]

Dietary ω-6 fatty acids generally increase the levels of inflammatory markers, whereas ω-3 fatty acids may decrease the levels of these markers.[15]

ω-3 fatty acids have anti-inflammatory effects.[16] Observational data suggest that dietary fish oil and α-linolenic acid are inversely associated with CRP level, whereas, the intervention data are less consistent.[17,18] Intervention studies have also reported an anti-inflammatory effect of fish and fish oil supplements on other inflammatory markers.[19,20]

The anti-inflammatory properties of ω-3 fatty acids may contribute to their cardioprotective effects. Inflammation is now believed to be a central process in the development of atherosclerosis and coronary heart disease (CHD).[20]

However, incorporation of the highly unsaturated fatty acids in membranes may increase the membranes' susceptibility to lipid peroxidation, especially in combination with exercise.[20]

We investigated the effects of exercise and EPA supplementation, with or without vitamin E, on the plasma levels of TC, TG, LDL-C, HDL-C, CRP and BP in male athletes.

Materials and methods

In this randomized double blind placebo-controlled clinical trial, 34 apparently healthy, well-trained male basketball players, aged 17-35 years, were enrolled into the study between May 4 and 19, 2006. Ethical approval was obtained from the Medical Ethics Committee of Tehran University of Medical Sciences and informed consent was obtained from all individuals. The participants were instructed not to take any antioxidant supplements during, and 2 weeks preceding the study. Exclusion criteria included pathologies interfering with immune functions i.e. inflammatory diseases and hemophilia.

Venous blood samples were obtained from all participants at 5:00 - 6:00 p.m., after 2 hours of exercise, at the baseline and after intervention. Subjects received 2 g EPA and/or 400 IU vitamin E or placebo depending on their groups.

For a period of 6 weeks, 8 participants received daily EPA supplements with vitamin E (group 1), 9 took

EPA supplements and vitamin E placebo (group 2), 9 took EPA supplement placebo and vitamin E (group 3), and 8 received EPA supplement placebo and vitamin E placebo (group 4).

EPA and EPA placebo soft gels were supplied from Minami Nutrition (Belgium). The vitamin E and their placebo soft gels were obtained from Zahravi Pharmaceutical Inc. (Iran).

Serum TG and TC levels were determined using the enzymatic method. LDL-C and HDL-C levels were measured by the turbidimetric method. CRP levels were determined by the immunoturbidimetric method(Reference). Microsoft Excel® was used for data handling and graph generation. SPSS (version 10; SPSS Inc., Chicago, IL, USA) and Stata (version 7; Stata Corp., College Station, TX, USA) were used for statistical analysis**.** Value**s** are expressed as means and standard errors for each group.The significant level was set at P<0.05.

Results

Thirty-seven athletes with a median age of 24 years (range: 17-35 years) were recruited. Thirty-four participants completed the 6-week intervention. Withdrawals from the study were due to personal reasons, unrelated to the study protocol. The groups were matched for gender and age. Some of the participants' characteristics are shown in Table 1. Body weights were statistically similar in all groups (group 1: 86.7±8.5; group 2: 91.5±7.3; group 3: 83.5±6.5; group 4: 88.2±7.6 kg, respectively) throughout the study.

Mean (±SD) plasma levels of TC, LDL-C, HDL-C, TG, CRP, total antioxidant capacity and BP are presented in Table 2. There were significant decreases in TC, TG, LDL-C and CRP in group 1 (p<0.01), and in Tc, TG, LDL-C, CRP, and BP in group 2 (p<0.01). A significant increase in total antioxidant capacity was seen in group 3 (p=0.002) (Table 2).

There was a significant difference in LDL-C between groups 1 and 4 (p<0.05). Total antioxidant capacity was significantly different between groups 2 and 3 (p<0.001) and between groups 3 and 4 (p<0.001). There was a significant difference in CRP level between groups 2 and 3 (p<0.05), but no difference in TC, TG, HDL-C and/or BP was seen between the groups after 6 weeks of intervention (Table 3).

Discussion

This randomized double-blind placebo-controlled clinical trial in male basketball players demonstrated a decrease in total cholesterol, TG, LDL, and CRP levels (P<0.01) in group 1, a decrease in these parameters and BP in group 2, and an increase in total

TABLE 1. Characteristics of participants (Mean±SD)

Characteristics	group 1(n=8) (EPA+vitamin E)	Group 2 (n=9) (EPA+placebo)	group 3 (n=9) (Vitamin E +placebo)	group 4 (n=8) (placebo+placebo)
Age (year)	27.5±5.3	23.7±3.3	23.5±2.4	21.2±2.2
Weight (Kg)	88.4± 5.6	89.8 ± 5.4	88.1 ± 5.5	87.9 ± 4.8
Height (m)	1.91±0.07	1.94±0.03	1.93±0.07	1.92±0.05
BMI (Kg/m^2)	24.1±1.1	23.7±1.3	23.5±0.8	23.8±0.7

TABLE 2. Comparisons of variables assessed in different groups before and after 6 weeks of intervention

Parameters	Group1			Group2		
	Baseline values	Final values	P value (paired t-test)	Baseline values	Final values	P value (paired t-test)
Total cholesterol (mg/dl)	184.25±21.54	161.5±20.36	0.005	177.11±24.48	158.88±23.3	0.000
TG (mg/dl)	193.37±70.72	170.5±57.86	0.003	167.55±31.82	148.33±30.09	0.001
LDL (mg/dl)	88±15.03	72.87±12.74	0.000	89.33±16.62	75.66±12.56	0.000
HDL (mg/dl)	34.62±4.75	35.75±5.36	0.26	35.33±3.57	35.33±4.58	1.0
Total antioxidant (nmol/dl)	3.14±0.23	3.15±0.25	0.85	3.01±0.29	3±0.22	0.8
CRP (mg/dl)	4.84±3.86	1.67±1.8	0.006	2.85±0.96	1.22±0.42	0.000
BP (mmHg)	11.75±1.58	11.25±0.7	0.38	12.66±1.0	11.22±0.66	0.01
	Group3			Group4		
Total cholesterol (mg/dl)	171.44±22.4	168.33±16.68	0.25	162.37±22.55	164.62±20.11	0.33
TG (mg/dl)	177.22±72.75	175.22±66.75	0.46	133±31.42	135.5±38.66	0.61
LDL (mg/dl)	76.74±16.13	76.77±14.36	0.82	92.75±19.48	94±18.83	0.62
HDL (mg/dl)	35.66±3.96	35±4.7	0.47	36±8.55	34.5±8.46	0.21
Total antioxidant (nmol/dl)	2.9±0.27	3.4±0.15	0.002	2.99±0.07	2.96±0.15	0.45
CRP (mg/dl)	2.86±1.23	2.99±1.39	0.45	2.29±1.07	2.43±1.27	0.75
BP (mmHg)	12.22±1.64	11.88±0.6	0.58	10.12±1.12	11±1.3	0.08

TABLE 3. Comparisons of variables assessed between different groups after 6 weeks of intervention

Parameter	Group1	Group2	Group3	Group4
Total cholesterol (mg/dl)	161.5±20.36	2.88±9.8	168.33±16.68	164.62±20.11
TG (mg/dl)	170.5±57.86	0.88±4.45	175.22±66.75	135.5±38.66
LDL (mg/dl)*	72.87±12.74	1.41±1.27	76.77±14.36	94±18.83
HDL (mg/dl)	35.75±5.36	21.01±111.23	35±4.7	34.5±8.46
Total antioxidant (nmol/ml)**	3.15±0.25	0.65±1.47	3.4±0.15	2.96±0.15
CRP (mg/dl)***	1.67±1.8	1.22±0.42	2.99±1.39	2.43±1.27
BP(mmHg)	11.25±0.7	11.22±0.66	11.88±0.6	11±1.3

Results were expressed as the Mean±SD
* Significant differences between groups 1 and 4 (P<0.05) (Tukey)
** Significant differences between groups 2 and 3 and between groups 3 and 4 (P<0.001) (Tukey)
*** Significant differences between groups 2 and 3 (P<0.05) (Tukey)

antioxidant capacity in group 3 following 6 weeks of supplementation, suggesting that EPA with or without vitamin E can decrease the cardiovascular markers in plasma, but only along with vitamin E can it reduce the blood pressure.

We examined the effects of EPA and vitamin E supplementation on serum lipid profile, total antioxidant capacity and BP in male basketball players. Six weeks of EPA+ vitamin E supplementation improved the lipid profile and reduced the CRP level, whereas six weeks of EPA supplementation without vitamin E improved the lipid profile, but increased CRP and BP. Six weeks of vitamin E supplementation alone increased total plasma antioxidant capacity. These findings are consistent with the results of some previous studies.[20,21] Others, however, report either no effect,[26-28] or a harmful effect[29] of increased ω-3 fatty acid intake. Many epidemiologic and interventional studies have evaluated the associations and effects of the intake of ω-3 fatty acids[22-25] on CVD endpoints in a variety of populations. Healthy subjects and those at high risk for CHD, including patients who had suffered a myocardial infarction have been evaluated. Many different study designs for both epidemiologic and interventional studies have been used.

Reductions in CVD events have been demonstrated in endpoint studies with two servings of fatty fish per week, 1 g/day of a 85% EPA + DHA concentrate in the form of an ethyl ester, and 1.8 g/day of EPA as an ethyl ester.[30-32]

Individual fatty acids have a wide range of effects on biochemical and physiologic functions that are determined by a combination of chain length, number and placement of double bonds, and isomerism around these bonds. The nutritionally important ω-6 fatty acids, linoleic acid (LA) (18 carbons with 2 double bonds) and arachidonic acid (AA) (20 carbons with 4 double bonds) differ substantially from their ω-3 counterparts, α-linolenic acid (ALA) (18 carbons with 3 double bonds) and EPA (20 carbons with 5 double bonds), in their impact on plasma lipids and lipoproteins, BP, and other prominent biomarkers of CVD risk such as fasting plasma glucose and insulin, lipoprotein (a), inflammatory markers, and clotting factors. In addition, DHA, an ω-3 fatty acid with 22 carbons and 6 double bonds, contributes substantially to total bioactivity of ω-3 fatty acids, but does not appear to have an ω-6 fatty acid counterpart with comparable biologic activity.[33]

In many publications, mechanisms potentially responsible for these effects have been examined. This attests to the fact that, like other fatty acids, EPA and DHA form part of the cell membrane, replacing other mostly unsaturated fatty acids upon incorporation, and thereby modulating cellular function. Therefore, a number of changes of cell function can be observed upon incorporation of EPA and DHA into the cell membrane. Among them are the modulation of the eicosanoid system towards vasodilatation and less proinflammation, a lowering of blood TG and anti-arrhythmic effects, as well as reductions in pro-atherogenic cytokines and growth factors. In animal models, in dogs, swine and primates, but not in rodents, beneficial effects have been observed in models of vasoocclusion and atherosclerosis.[3,4,34]

The results of the present study also showed significant differences in LDL-C between groups 1 and 4 (P<0.05). There were significant differences in total antioxidant capacity between groups 2 and 3 and between groups 3 and 4 (P<0.001). A significant difference in CRP was seen between groups 2 and 3 (P<0.05) (Tukey).

Clinical trial data provide compelling evidence on the favorable effects of ω-3 fatty acids in reducing the risk of CHD. The Diet and Reinfarction Trial (DART) included 2033 Welsh men after a recent myocardial infarction randomized to 2 servings of fish/week or equivalent in ω-3 supplements, if fish intolerant. Over 2 years, a significant 29% reduction in cardiovascular- and total mortality rate was reported in patients who consumed fish regularly compared to those without fish in their diet. [21] A smaller study included 360 patients admitted to hospital for suspected myocardial infarction who were randomized to either fish oil supplements rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), mustard seed oil rich in α-linolenic acid, or placebo.[35] After 1 year, total cardiac events decreased significantly by 25% in the fish oil group and 28% in the mustard seed oil group. The GISSI-Prevention Trial is by far the largest clinical trial to test the efficacy of ω-3 fatty acid supplements for secondary prevention of CHD.[31] The trial included 11,324 patients with a recent myocardial infarction who were randomized to either ω-3 fatty acid supplements (850 mg/d), vitamin E (300 mg/d), both ω-3 fatty acids and vitamin E, or none for 3.5 years in conjunction with a Mediterranean diet. The Mediterranean diet emphasizes less meat consumption with more bread, vegetables, fruit, and fish intake. ω-3 fatty acid supplementation significantly reduced all-cause death by 20% and nonfatal myocardial infarction and stroke by 15%. Subsequent reanalysis of the data demonstrated that total mortality was lowered at 4 months of treatment suggesting an anti-dysrhythmic effect of ω-3 fatty acids and a decreased risk of sudden cardiac death.[36]

At 25-year follow-up of the SCS, a significant inverse relationship was found between ω-3 fatty acid intakes and CHD mortality, but the relationship was not independent of saturated fat intake.[37] The CWES, a study specifically examining fish intake, determined that fish intake ≥35 g/day compared with no fish consumption, significantly reduced the risk for CAD in healthy men with a relative risk of 0.62;.[22] In a nested case-control study of the CVHS, the relationship between CHD and polyunsaturated fatty acid (PUFA) concentration of plasma phospholipids in 179 cases and equal number of controls was determined. Among cases, plasma EPA and DHA, expressed as a percentage of total fatty acids, were 3.3% (±0.8%) compared with 3.8% (±1.3%) for cases of fatal ischemic heart disease, and plasma ALA was 0.16% (±0.06) compared with 0.17% (±0.06%) for controls. Linoleic acid was 20.1% (±2.3%) for cases and 19.2% (±2.4%) for controls.[38] Evidence from three epidemiologic studies, the NHANES I Follow-up Study, a study conducted by Osler and associates,[28] and the JPHC Study Cohort I,[39] did not reveal any significant reduction in CHD incidence with increasing intakes of fish. In addition, the prospective cohort study in Danish men and women by Osler and associates did not reveal any significant relationship between fish intake and CHD mortality. In a subgroup analysis of Danish adults at high risk for CVD based on gender-adjusted age (men aged >50 years and women aged >60 years), serum TC levels, and smoking, the relative risk for CAD incidence and mortality was not significant. Evidence from the Finnish cohort of the EUROASPIRE study, an interventional study, confirmed these findings and demonstrated that fish intake was not associated with reductions in 5-year mortality due to CHD .[27]

In conclusion, this study showed that 6 weeks of EPA+ vitamin E supplementation reduced the plasma levels of TC, TG, LDL-C, and CRP levels, 6 weeks of EPA supplementation alone reduced the plasma levels of total cholesterol, TG, LDL, and increased CRP and blood pressure, and that 6 weeks of vitamin E supplementation alone increased total plasma antioxidant capacity. The differences between reports on the effect of EPA and vitamin E supplementation on inflammatory markers and antioxidant status are probably because of differences in study methodology. Further studies are warranted in this regard.

Acknowledgments

This study was supported by the Vice-Chancellery for Research, Tehran University of Medical Sciences and the Sport Office of the Iranian Ministry of Health and Medical Education. We thank Mrs. Chamari from Tehran University of Medical Sciences for her assistance in the analysis of total antioxidant capacities.

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