

Effects of Eicosapentaenoic Acid and Vitamin E on the Plasma Levels of Antioxidant Vitamins and Inflammatory Markers, and on Erythrocyte Antioxidant Enzyme Activities, in Male Basketball Players

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Abstract- Strenuous aerobic exercise is associated with oxidative stress and tissue damage. Therefore, we have investigated the effects of exercise and eicosapentaenoic acid supplementation, with or without vitamin E, on the plasma levels of vitamin C, E and A, IL-6, and glutathione peroxidase activity in basketball players. Thirty four male basketball players, enrolled in the study. Subjects received 2g EPA and/or 400 IU vitamin E or placebo depending on their groups. For 6 weeks, eight subjects took a daily EPA supplement together with vitamin E (group 1), nine received an EPA supplement together with placebo (group 2), nine were administered placebo along with vitamin E (group 3), and finally, eight subjects received placebo alone (group 4). As compared with group 4 (placebo), there were significant increases in vitamins C, E, and A in groups 1 and 3, but significant decreases in these parameters in group 2 ($P < 0.01$). In addition, there were significant decreases in IL-6 in groups 1 and 3 ($P < 0.01$), whereas there were significant increases in glutathione peroxidase in groups 1 and 3 ($P < 0.01$). There were significant differences in vitamin C between groups 3 and 4 ($P < 0.05$), and in vitamin E between groups 1 and 2, and groups 2 and 3 ($P < 0.01$), and in vitamin A between groups 1 and 2 ($P < 0.05$), and groups 1 and 4, 2 and 3, and 3 and 4 ($P < 0.01$), and in IL-6 between groups 1 and 2 ($P < 0.05$), groups 1 and 3 ($P < 0.01$), 2 and 3 ($P < 0.01$), 2 and 4 ($P < 0.01$), and 3 and 4 ($P < 0.05$).

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Introduction

Evidence has accumulated in the past decade indicating that strenuous aerobic exercise is associated with oxidative stress and tissue damage. It is, therefore, conceivable that dietary supplementation with specific antioxidants would be beneficial (1). During severe oxidative stress, the enzymatic and nonenzymatic antioxidant systems of skeletal muscle are not able to cope with the massive free radical formation, resulting in an increase in lipid peroxidation. Exercise and training, however, appear to augment the body's antioxidant defense (2). Whether this augmented defense can keep up with the increase in lipid peroxidation due to exercise is un-

known. Vitamin E is reported to decrease exercise-induced lipid peroxidation. Exercise may increase free radical generation in the heart, and the increase in the activity of glutathione peroxidase (GPX) in skeletal muscle may be an indirect evidence for exercise-induced free radical formation (3). However, incorporation of the highly unsaturated fatty acids in membranes may increase the membrane susceptibility to lipid peroxidation, especially in combination with exercise (4).

Exercise can alter the release of numerous cytokines and modulate their receptor systems. Such changes may trigger inflammatory and acute phase responses. Inflammation in athletes may be caused by mechanical stress, local ischemia, and/or free radical generation in

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the active skeletal muscle. After high-intensity exercise, the immune system becomes involved in tissue repair processes. Suppression of IL-2 and increases in IL-1 and TNF- α production are reported after exercise (5-7). Physical exercise, including eccentric muscle contractions, induces the production of chemokines (8).

Strenuous exercise is accompanied by an increase in circulating pro-inflammatory and inflammation-responsive cytokines, having some similarities with the response to sepsis and trauma. The sequential release of TNF- α , IL-1 β , IL-2, and IL-6 in the blood is comparable to that observed in relation to bacterial diseases (9). Specific fatty acids can lower the levels of certain pro-inflammatory cytokines. These fatty acids may have a protective role to defend against the inflammatory responses caused by exercise (9).

Dietary ω -6 fatty acids generally increase the levels of pro-inflammatory cytokines and inflammatory prostaglandins (PGs), whereas ω -3 fatty acids may decrease the levels of these mediators (10).

Therefore, we have investigated the effects of exercise and EPA supplementation, with or without vitamin E, on the plasma levels of vitamin C, E, A, IL-6 and glutathione peroxidase activity in male basketball players.

Materials and Methods

The present study was a randomized double blind placebo-controlled clinical trial. Thirty-four apparently healthy, well-trained male basketball players, aged 17-35 yrs, enrolled in 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 subjects. Participants were instructed not to take any antioxidant supplements during and 2 weeks preceding the study. Exclusion criteria included the existence of pathologies interfering with immune functions, such as inflammatory diseases, and hemophilia.

Venous blood samples were obtained from all subjects between 5:00 and 6:00 p.m., after exercising for 2

hours, at the baseline and after intervention. Subjects received 2g EPA and/or 400 IU vitamin E or placebo depending on their groups.

For 6 weeks, eight subjects took a daily EPA supplement together with vitamin E (group 1), nine received an EPA supplement with placebo (group 2), nine were administered placebo along with vitamin E (group 3), and finally, eight subjects received placebo alone (group 4). EPA and corresponding placebo soft gels were supplied by Minami Nutrition (Belgium). Vitamin E and corresponding placebo soft gels were obtained from Zahravi Pharmaceutical Inc. (Iran). N7505 NADPH, G3664 GSSG-R, and GSH were purchased from Sigma (USA), and NaN₃, NaCl, NaHCO₃, KCN, K₃Fe(CN)₆, HCl, Na₂HPO₄.2H₂O, NaH₂PO₄, H₂O₂, trichloro acetic acid (TCA), H₂SO₄, CuSO₄, DNPH, T5500 thiobarbituric acid (TBA), and ascorbic acid were obtained from Merck (Germany). Plasma levels of vitamins A and E were determined by HPLC. Plasma levels of vitamin C were measured by Lowry method (11). determination of glutathione peroxidase (GPx) activity is based on the method of Paglia and Valentine (12). IL-6 ELISA kits were obtained from Bender Medsystems GmbH (Vienna, Austria).

Student paired t-test was used for comparison between two groups and Tukey post test for multiple comparisons. SPSS (version 10; SPSS Inc., Chicago, IL, USA) and Stata (version 7; Stata Corp., College Station, TX, USA) were used for statistical analysis. Values are expressed as means \pm standard errors.

Results

Thirty-seven subjects were collected with a median age of 24 ranging between 17 and 35 years. Thirty four of them completed the 6 week intervention. The groups were well matched for gender and age. Some of characteristics of subjects are seen in table 1. Withdrawal from the study was due to personal reasons, unrelated to the protocol. Body weights were similar in all groups (group 1: 86.7 \pm 8.5; group 2: 91.5 \pm 7.3; group 3: 83.5 \pm 6.5; group 4: 88.2 \pm 7.6 kg) throughout the study.

Table 1. Characteristics of subjects ($\bar{x} \pm SD$)

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)
Age (year)	27.5 \pm 5.3	23.7 \pm 3.3	23.5 \pm 2.4	21.2 \pm 2.2
Weight (Kg)	88.4 \pm 5.6	89.8 \pm 5.4	88.1 \pm 5.5	87.9 \pm 4.8
Height (m)	1.91 \pm 0.07	1.94 \pm 0.03	1.93 \pm 0.07	1.92 \pm 0.05
BMI (Kg/m ²)	24.1 \pm 1.1	23.7 \pm 1.3	23.5 \pm 0.8	23.8 \pm 0.7

Mean plasma levels of vitamins C, E, and A, IL-6, and glutathione peroxidase activity in erythrocytes are shown in table 2. There were significant increases (paired *t*-test) in vitamins C, E, and A in groups 1 and 3, but significant decreases (paired *t*-test) in these parameters in group2 ($P < 0.01$). Moreover, there were significant decreases in IL-6 in groups 1 and 3 ($P < 0.01$), whereas there were significant increases in glutathione peroxidase activity in groups1 and 3 ($P < 0.01$) (Table 2).

There were significant differences (Turkey) in vitamin C between groups 3 and 4 ($P < 0.05$), and in vitamin E between groups 1 and 2 and groups 2 and 3 ($P < 0.01$), and in vitamin A between groups 1 and 2 ($P < 0.05$), and groups 1 and 4, 2 and 3, and 3 and 4 ($P < 0.01$), and in IL-6 between groups1 and 2 ($P < 0.05$), groups 1 and 3 ($P < 0.01$), 2 and 3 ($P < 0.01$), 2 and 4 (P

< 0.01), and 3 and 4 ($P < 0.05$), but not in glutathione peroxidase activity among groups after 6 weeks of intervention (Table 3).

Discussion

The present study examined the effects of EPA and vitamin E supplementation on plasma levels of vitamins C, E, and A, IL-6, and glutathione peroxidase activity in erythrocytes, in male basketball players.

EPA is an omega-3 (n-3) polyunsaturated fatty acid (PUFA) derived from fish oil that competitively inhibits n-6 PUFA arachidonic acid (AA) metabolism and thus reduces the generation of inflammatory 4-series leukotriene and 2-series PG mediators (13), and the production of cytokines from inflammatory cells (14).

Table 2. Comparisons of parameters before and after 6 wk supplementation

Parameters	Group 1 (EPA+vitamin E)			Group 2 (EPA+placebo)			Group 3 (Vitamin E +placebo)			Group 4 (placebo)		
	Baseline values	Final values	<i>P</i> value (paired <i>t</i> -test)	Baseline values	Final values	<i>P</i> value (paired <i>t</i> -test)	Baseline values	Final values	<i>P</i> value (paired <i>t</i> -test)	Baseline values	Final values	<i>P</i> value (paired <i>t</i> -test)
Vitamin C (mg/dl)	0.29 ± 0.16	0.59 ± 0.29	0.001	0.9 ± 0.29	0.58 ± 0.34	0.008	0.54 ± 0.45	1.06 ± 0.58	0.004	0.36 ± 0.21	0.43 ± 0.26	0.6
Vitamin E (mg/dl)	11.74 ± 5.82	13.99 ± 7	0.016	9.73 ± 6.81	5.81 ± 4.14	0.005	8.5 ± 5.14	13.46 ± 6.55	0.004	8.72 ± 3.32	8.29 ± 2.48	0.4
Vitamin A (mg/dl)	0.58 ± 0.22	0.81 ± 0.26	0.004	0.72 ± 0.14	0.51 ± 0.13	0.014	0.48 ± 0.17	0.86 ± 0.15	0.001	0.53 ± 0.21	0.46 ± 0.24	0.2
IL-6 (pg/ml)	7.5 ± 2.42	4.67 ± 1.6	0.005	5.44 ± 1.43	1.63 ± 0.75	0.000	7.65 ± 2.11	7.55 ± 1.85	0.8	4.77 ± 2.34	4.93 ± 2.16	0.4
Glutathione peroxidase (u/gHg)	43.02 ± 18.83	55.26 ± 18.72	0.005	61.42 ± 18.75	60.31 ± 13.59	0.6	44.66 ± 17.47	63.87 ± 16.63	0.002	63.89 ± 16.68	63.16 ± 14.01	0.6

Table 3. Multiple comparisons of parameters among groups after 6 wk supplementation

Parameters	Group 1 (EPA+vitamin E)	Group 2 (EPA+placebo)	Group 3 (Vitamin E +placebo)	Group 4 (placebo)
Vitamin C*(mg/dl)	0.59 ± 0.29	0.58 ± 0.34	1.06 ± 0.58	0.43 ± 0.26
Vitamin E**(g/dl)	13.99 ± 7	5.81 ± 4.14	13.46 ± 6.55	8.29 ± 2.48
Vitamin A***(mg/l)	0.81 ± 0.26	0.51 ± 0.13	0.86 ± 0.15	0.46 ± 0.24
IL-6****(pg/ml)	4.67 ± 1.6	1.63 ± 0.75	7.55 ± 1.85	4.93 ± 2.16
Glutathione peroxidase(u/gHg)	55.26 ± 18.72	60.31 ± 13.59	63.87 ± 16.63	63.16 ± 14.01

Results were expressed as the Mean ± SD

* Significant differences between groups 3 and 4 ($P < 0.05$) (Tukey)

** Significant differences between groups1 and 2 and groups 2 and 3 ($P < 0.05$) (Tukey)

*** Significant differences between groups1 and 2 ($P < 0.05$), groups 1 and 4 ($P < 0.01$), groups 2 and 3($P < 0.01$) and groups 3 and 4 ($P < 0.01$).

****Significant differences between groups1 and 2 ($P < 0.05$), groups 1 and 3 ($P < 0.01$), groups 2 and3 ($P < 0.01$), groups 2 and 4 ($P < 0.01$), and groups 3 and 4 ($P < 0.05$).

Fish oil consumption results in partial replacement of AA in inflammatory cell membranes by EPA (13,14). This response alone is a potentially beneficial anti-inflammatory effect of n-3 PUFA.

In this randomized double blind placebo-controlled clinical trial, there were significant differences in vitamins C, E, and A-antioxidant vitamins- between groups, and these vitamin levels were higher after intervention in groups 1 and 3, but significant decreases in these vitamins in group 2 were observed, whereas there were no significant differences in glutathione peroxidase among the groups after 6 weeks of intervention.

A decrease in plasma antioxidant concentrations after fish oil supplementation has been reported by Nair et al. (15), a result confirmed by the present study but not by other groups (16).

The results of several studies suggest that increased consumption of n-3 fatty acids may result in an increased potential for oxidative stress in vivo. These studies were based primarily on the results of the TBA assay (17). Many studies have shown that exercise may result in oxidative damage (18,19) which can be intervened by vitamin E.

Data from this study indicate that EPA in well-trained basketball players have variable effects on the antioxidant levels, some of which being suppressive, such as those on the plasma levels of vitamins C, E, and A and some of which being inert, such as those on glutathione peroxidase activity in erythrocytes. Nevertheless, vitamin E supplementation enhanced the antioxidant vitamins and enzymes.

One of the major functions of the immune system is the production of soluble and cellular components that provide immunity and protection against foreign materials (20). Cytokines are a group of low molecular weight regulatory proteins secreted by white blood cells and a variety of other cells in response to a number of inducing stimuli. Cytokines generally function as intercellular messenger molecules that evoke particular biological activities after binding to a receptor on a responsive target cell. Exercise is accompanied by an increase in pro-inflammatory and inflammation responsive cytokines, having some similarities with sepsis and trauma (21,22).

The results of the present study, also showed significant differences in IL-6 between groups 1 and 2 ($P < 0.05$). On the other hand there were significant decreases in IL-6 in groups 1 and 3 ($P < 0.01$). These findings confirm those of Endres et al. (21). However, Venkatraman et al. (23) found no effect of ω -3 fatty acids on the induction and reduction of anti- and pro-inflammatory cytokines production.

Some investigators have shown that dietary supplementation with n-3 PUFA results in decreased monocyte synthesis of TNF- α and IL-1 β in healthy subjects (24). However, Hodge and coworkers (25) demonstrated reductions in TNF- α production after fish oil supplementation. The mechanism involved is probably that exercise causes release of pro-inflammatory cytokines that in turn will trigger the production of anti-inflammatory cytokines such as IL-2 and IL-10 (26-30). The sources of these cytokines are the skeletal muscle and peripheral blood mononuclear cells (26,31). The sequence of production of these pro- and anti-inflammatory cytokines appears to include initial production of TNF- α and IL-1 β by the peripheral blood leukocytes and muscle. Meanwhile, EPA has anti-inflammatory actions; however, the exact mechanism is unknown.

This is the first study to assess the effect of EPA and vitamin E supplementation on inflammatory markers, antioxidant enzymes and lipid peroxidation in basketball players. In conclusion, this study shows that 6 weeks of EPA plus vitamin E supplementation enhances the plasma levels of vitamins C, E, and A, and activity of glutathione peroxidase in erythrocytes, whereas it reduces IL-6, and that 6 week of EPA supplementation alone reduces the plasma levels of vitamins C, E, and A, IL-6, and finally that 6 week of vitamin E supplementation alone increases the plasma levels of vitamins C, E, and A and enhances the glutathione peroxidase activity of erythrocytes.

The differences between reports on the effect of EPA and vitamin E supplementation on inflammatory markers and antioxidant status are probably methodologic. The small number of studies and the different methods used for the assessment of inflammation and antioxidant status call for further trials (32). In addition, due to the discrepancy seen between the findings of this study and those of some others, further investigation is warranted.

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EPA, vitamin E, inflammation and antioxidant status

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