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Nuriye METE[1]
Birgül IŞIK[1]
Levent ERDİNÇ[1]
Fuat GÜRKAN[2]

The Effect of Fish Oil on Liver and Plasma MDA and Antioxidant Status of Rats

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Abstract: The question how fish oil (FO) and soybean oil (SO) change the state of antioxidant and the lipid peroxidation in liver and plasma of rats and the protective role of vitamin E were investigated. Six-week-old forty male Sprague-Dawley rats were divided into five groups. One of the groups fed a control diet, another one fed SO, while the other three groups fed FO diet at the level of 10% Vitamin E supplementation was maintained to three different levels 3, 50, 150 IU/per kg diet in the FO diets and 3 IU vitamin E per kg of the SO diet. At the end of tenth week, blood was drawn under ether anesthesia and finally plasma and red blood cell samples were separated. Liver tissues were washed by 0.9% NaCl, then dried by a filter paper and stored at -60°C. The malondialdehyde (MDA) amount as an indicator of lipid peroxidation was not significantly affected in the SO group, while it was found increased both in serum and liver tissue in the FO group with vitamin E supplementation of 3 IU/kg diet ($P<0.001$). Erythrocyte GSH levels and liver GSH-Px activity were not significantly different between the SO and the control group, while there were significant differences in the FO group with the lowest dose of vitamin E ($p<0.001$). Catalase activity was not statistically different between groups. MDA production decreased and the antioxidant status was improved by higher doses of vitamin E fed together with FO. A statistically significant positive correlation was found between dietary vitamin E and plasma vitamin C, erythrocyte GSH and liver GSH-Px ($P<0.01$).

In conclusion decreased antioxidant defence and increased MDA amount in the FO fed rats changed to normal with vitamin E supplementation. As significant correlations were found between vitamin E and other antioxidants, vitamin E seems necessary to maintain appropriate levels of other physiological antioxidants.

Departments of [1]Biochemistry, [2]Pediatrics, Faculty of Medicine, Dicle University, Diyarbakır-Turkey

Introduction

Many studies showed that fish and fish oil (FO) preparations had many diverse actions that could alleviate vascular disease (1, 2). These included mild blood pressure reduction, definite hypotriglyceridemic effects, an antithrombotic action, inhibition of cellullar growth factors and monocyte migration, and enhancement of nitric oxide production by the endotelium.

Both the epidemiological evidence and the results of a subsequent clinical trial done in the United Kingdom suggest that two to three fish meals per week might provide considerable protection against coronary disease (1, 3). The clinical trial of n-3 fatty acids involved several thousand men who had survived from myocardial infarction. The men who ate fish at least twice a week for 2 years had a 29% reduction in total mortality, largely because they had fewer deaths from coronary disase (2). Besides these positive effects of fish oil, increased levels of long-chain n-3 fatty acids may render membranes more susceptible to oxidation and may increase the requirement for antioxidants.

Free radicals are well known reactive molecules mainly derived from univalent reduction of oxygen and giving rise to numerous by-products through reactions with almost all the unsaturated bonds found in natural living cells. The detrimental effects of such reactive molecules have been well described, since they can destabilize cell membranes by reacting with unsaturated fatty acids (4). Lipid oxidation products are well suited to induce arterial damage and based on their known cytotoxic effects, evidence also indicates the possibility of plaque formation and stimulation of thrombogenesis (5, 6).

Free radical production in cells is relatively low in normal conditions, due to the various and very active defence systems, including chemical scavengers or antioxidant molecules and the three enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase

(GSH-Px) (7, 8). However, due to the increasing tandance of peroxidation of the membrane lipids in the circumstances requirring fish oil usage, cellular immunity deficiency occurs and antioxidant usage is suggested (9, 10, 11, 12).

Antioxidants inhibit lipid peroxidation (LPO) by preventing peroxidation chain reaction or by accumulating the reactive oxygens. Antioxidants are divided into four subgroups according to their effects: scavenging, quencher, repairing and chain breaking. Vit E (DL-α-tocopherol) is the primary chain breaking antioxidant in membranes and reduces peroxyl, hydroxyl, and superoxide radicals and singlet oxygen. Adequate vitamin E dosage to preserve the normal antioxidant status during feeding by fish oil is not well determined in the literature (9, 10, 11, 12, 13, 14, 15, 16, 17).

The effect of fish oil administration on rat plasma and liver lipid peroxidation and the antioxidant role of three different doses of vitamin E were investigated in this study. The efffects of vitamin E were discussed by measuring the levels of liver and plasma malondialdehyde (MDA) content, liver glutathion peroxidase, catalase, eryhrocyte GSH, plasma uric acid and vitamin C. The probable effects of soybean oil (SO) which is also known rich for polyunsaturated fatty acids were investigated.

Materials and Methods

Menhaden fish oil (MO) was purchased from Sigma chemical Co (F8020) and soybean oil (SO) was obtained from a local market. DL-α-tocopheryl acetate was purchased from Pharmacia (Roche). Tertiary butylhdroxy- quinone was added to keep MO and SO from deteriorating (Sigma B 9161). β NADPH (Lot 95H7115), reduced glutathione (G 4251), glutathione reductase (G 4759), H_2O_2, EDTA, Sodium azid, Perchloric acid, Na_2HPO_4, 5.5' dithiobis (DTNB) and sodium citrate were purchased from Sigma Chemical Co.

Animals and Treatment

The study was carried out on adult male rats, mean body weight 180-200 g., provided by DUSAM (Research Center Health Sciences, Dicle Univercity). Six-week old male Sprague-Dawley rats were divided into five groups of eight animals each, and fed the five respective diets for ten weeks. The composition of the diets is shown in Table 1.

To prevent linoleic acid deficiency upon feeding MO, one part of SO was mixed with nine parts of MO for the FO diet at the level of (w/w) fat in the diet. The amounts of DL-α-tocopherol were adjusted to three different levels (Group I: 3 IU/kg diet, Group II: 50 IU/kg diet, Group III: 150 IU/per kg diet) in the FO diets, and to 3 IU per kg in the SO diet (Group 5).

Tissue Preparation

At the end of the feeding period, the rats were fasted for 16h before sacrifice. Under ether anesthesia, blood was drawn by cardiac puncture and plasma was prepared with EDTA. Liver tissues were washed by 0.9% NaCl, then dried by a filter paper and stored at -60°C. The red blood cells were washed twice with physiological saline, after which they were diluted in distilled water and frozen and thawn three times to break the cell membrans. The hemolysate was stored -60°C until analysis.

The livers were used to obtain 10% homogenates in 0.15 M KCl-10 mM potassium phosphate buffer, pH: 7.4, which were centrifuged at 600 g for 10 min and recentrifuged at 13000 g for 20 min for obtaining a postnuclear homogenate and postmitochondrial supernatant

Table 1. Composition of Experimental Diets.

Components	Control	Group I	Group II	Group III	Group IV
Digestible energy (Kj/g)*	16	16	16	16	16
Fat (g/kg)	50	50	50	50	50
Protein (g/kg)	120	120	120	120	120
Cellulose (g/kg)	40	40	40	40	40
Minerals (g/kg)	15	15	15	15	15
Trace elements (mg/kg)	104	104	104	104	104
Soybean oil (g/kg)	-	10	10	10	100
Menhaden fish oil (g/kg)	-	90	90	90	-
DL-α-Tocopheryl acetate	-	3	50	150	3

* Expressed per kg feed consisting of 90% dry matter. The study group have consumpted fish oil and soybean oil in addition with the control diet. Oil was the only dietary element that was changed and the effects on the oxidative and antioxidative status were investigated.

fractions.

Analytical Procedures

MDA content in liver homogenates and plasma were measured by the thiobarbituric acid method (18, 19). The MDA amount was calculated using a molar extinction coefficient of $1.56x10^5$ M^{-1} cm^{-1}. Plasma uric acid levels were measured enzymaticaly with an autoanalyzer (Beckman Synchron CX-5). Plasma ascorbic acid levels were measured spectrophotometrically, using dicholorophenol-indophenol reagent (20) The reduced glutathione (GSH) level of the hemolysate was measured spectrophotometrically using Ellman reagent (21).

Liver catalase activity was determined according to Aebi (22). The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240 nm, resulting from enzymatic decomposition of H_2O_2. The difference in absorbance (ΔA_{240}) per unit time is a measure of the catalase activity. Liver glutathione peroxidase (EC 1.11.1.9) activity was measured according to Paglie et. al. (23), with H_2O_2 as substrate, and was expressed as nmol NADPH oxidized/min per mg. protein. Liver protein level was measured by the method of Lowry et al (24).

Data was analyzed by analysis of variance and treatment differences were evaluated by Tukey's test. Correlations between dietary vitamin E levels and biochemical measurements were examined.

Results

MDA Amounts in Tissues

The plasma MDA content in the FO group was higher than in SO group when rats fed very low levels (3 IU) of vitamin E. MDA amounts decreased upon vitamin E supplementation. Supplementation with vitamin E up to 150 IU in the FO group decreased the levels of plasma MDA to those in the SO group. The FO group which fed very low levels (3 IU) of vitamin E had highest MDA production than other FO groups and control group (Table 2).

The accumulation of lipid peroxide is more pronounced in liver than in plasma. Liver MDA content (per g. tissue) was significantly increased, namely 1.6 fold, 1.2 fold and 1.1 fold in the FO groups with 3,50 and 150 IU, respectively, when compared to the SO group (Table 2). There was no significant difference between control group and FO group which fed high level (150 IU) vitamin E.

Tissue Levels of Physiological Antioxidants

Plasma uric acid levels was not significantly different between the groups. Ascorbic acid levels were significantly lower only in the FO group with 3 IU vitamin E as compared to the other groups. Erythrocyte GSH

Table 2. In vivo effects of FO and vitamin E on the plasma and liver LPO and antioxidants

Measurements (X±SD)	Control	Group I	Group II	Group III	Group IV
Plasma MDA production (nmol/ml)	2.98±0.14	3.86±0.6[a]	3.18±0.39[b]	2.81±0.21[b]	3.02±0.3[b]
Uric acid (mg/dl)	3.4±1.9	2.3±0.7	2.2±1.3	3.8±2.5	2.8±0.9
Vitamin C (mg/dl)	1.02±0.32	0.51±0.11[a]	0.72±0.15[b]	0.91±0.17[b]	0.98±0.13[b]
Erythrocyte GSH (mg/dl) hemolysate)	52.3±3.4	39.2±5.3[a]	43.5±3.8[c]	45.2±6.8[c]	60.8±2.5[d]
Liver MDA Production (nmol/g tissue)	161±28	268±34[a]	198±30[b]	179±20[b]	168±11
Glutathione peroxidase (U/g tissue)	169.8±11.4	128.7±10.1[a]	181.2±16.3[b]	190.2±10.3[b]	192.3±10.8[b]
Catalase (ΔE_{240}/min/mg protein)	5.9±1.5	7.3±0.9	6.1±1.3	5.02±1.1	6.02±0.9

Values represent the mean±SD of 8 experimental animals each group.

MDA: Malondialdehyde GSH: Reduced Glutathione

[a]p<0.001 (Compared to control)

[b]p<0.001, [c]p<0.01 (Compared to Group I)

[d]p<0.001 (Compared to Group I, II, III)

levels in the FO groups were lower than those in the SO group, but the levels were increased in the FO group at 150 IU vitamin E when compared with the FO group at 3 IU (Table 2).

In the liver homogenate, catalase activity was significantly different among the groups, but GSH-Px activity was significantly decreased in the FO group 3 IU vitamin E and the activity was increased in the FO group at 50 IU and 150 IU vitamin E (Table 2).

Correlation of Dietary Vitamin E with other Measured Parameters.

Using the data obtained for the FO groups correlations were calculated between the dietary vitamin E levels and the various variables measured. Results are presented in Table 3. Dietary vitamin E levels correlated negatively with plasma MDA and positively with plasma vitamin C and erythrocyte GSH. A significant positive correlation was also found between dietary vitamin E and liver GSH-Px activity. Liver MDA levels showed negative correlations with the status of erythrocyte GSH, liver GSH-Px and catalase activity (Table 3).

Discussion

Peroxidative damage has occurred in plasma and liver tissues after FO supplementation. Activities have changed in some components of antioxidative system. MDA accumulation was more prominent in liver tissues than in plasma. SO did not change the plasma and the liver MDA contents as much as fish oil diet. LPO increase in the liver tissues was reported to be correlated with the tissue fatty acids (14, 15, 25, 26, 27, 28, 29, 30). Reme et. al. has found fish oil to be more effective than soybean oil in increasing n-3, n-6 fatty acid levels in tissues (26). These fatty acids are known to be signficantly decreasing the plasma and liver vitamin E levels (31). n-3 fatty acids have also been shown to be decreasing plasma free fatty acids (32). Those features might have probably been the cause of the higher MDA content in liver than in plasma. Another reason for increased MDA amount in liver, even at higher levels of dietary vitamin E may be that fatty acid peroxides from dietary FO contain a considerable amount of lipid peroxides. The lipid peroxides accumulate and are not secreted in plasma lipoproteins. The peroxide levels of oils and the peroxidability indexes were compared and the levels in fish were found higher (33).

Sufficient antioxidant effect did not occur with the 3 IU/kg diet vitamin E doses, while significant MDA decreases were detected in plasma and liver after 50 IU/kg diet doses. These doseges were selected within the theropeutic ranges suggested. It has been reported that two times higher serum-α-tocopherol levels with the tenfold amounts of α-tocopherol added into the diet. Only fish oil was shown to increase plasma and liver MDA, but had an inverse effect when supplemented by vitamin E. It has been considered to have resulted from the fish oil's effect on decreasing membrane vitamin E levels. Microsomal and mitochondrial MDA content were shown to decrease by the enhancing vitamin E doses (31). In another study fish oil was reported to cause enhanced transfere of vitamin E from tho erythrocytes to plasma lipoprotiens that results in the decreased membrane α-tocopherol consitituent (34).

MDA amount in the plasma and in the liver of rats fed FO showed an inverse relationship to dietary vitamin E levels, although the negative correlation of plasma MDA amount with dietary vitamin E levels was stronger (r=-1.29) than that of liver MDA (r=-0.99) (Table 3). This might probably be due to the duration of α-tocopherol transfere from plasma lipoproteins through the liver cell membranes. In the present study we observed not only

Table 3. Correlation Coefficients Between the Levels of Dietary Vitamin E and Plasma and Liver Measurements in Rats Fed FO.

	PLASMA			Erythrocyte	Liver		
	MDA	Vitamin C	Uric acid	GSH	MDA	GSH-Px	Catalase
Dietary Vitamin E	-1.29	0.81		0.93		0.81	
Plasma MDA				-0.61		-0.72	-0.76
Vitamin C			0.69	0.79		0.91	0.88
Uric acid							
Erythrocyte GSH						1.09	1.11
Liver MDA				-0.89		-1.02	-0.99
GSH-Px							
Catalase						1.51	

Only statistically significant ($p<0.01$) values are shown in the table.

the effects of dietary vitamin E on the status of plasma and liver LPO but also the interrelationship with plasma and liver antioxidant status. Dietary vitamin E showed significantly positive correlations with the status of erythrocyte GSH, vitamin C and liver GSH-Px activity ($P<0.01$) (Table 3). Similar observations have been reported by other investigators (15,30, 33).

Fish oil had a decreasing effect on erythrocyte GSH concentration, while vitamin E was shown to improve this effect. Glutathion reductase (GR) is a NADPH dependent enzyme, that catalyzes GSSG reduction and takes role in regularizing GSH levels. Thus the production of GSH is also correlated with the glucose metebolism by the way of NADPH that is produced in the pentose monophosphate way. Glucose-6-phosphate dehydrogenase (G6PD) enzyme does also take place in the some way. Decrease in erythrocyte NADPH results in reduced GSH/GSSG ratios (7). FO has a decreasing effect on G6PD activity. However GR is known not to be affected by the dietary factors (1). SO was not as effective as fish oil on the erythrocyte GSH levels. Liver catalase activities were not significantly different between the groups, while GSH-Px activity was shown reduced by the fish oil. Vitamin E supplementation of 50 IU/kg diet doses was necesarry for the appropriate continuation of this physiologic antioxidant effect. GSH-Px and vitamin E show a complementary effect against free radicals. Vitamin E prevents the synthesis of peroxides and GSH-Px enzyme eliminates the pre-synthesized peroxides. The antioxidant effect of α-tocopherol is more effective at high oxygen concentrations and has an increased effect on erythrocyte membranes with the highest partial oxygen pressure. Actually, there was a positive significant correlation between the dietary vitamin E and the erythrocyte GSH levels (Table 3). This correlation was even higher than the relationship between vitamin E and the liver GSH-Px or catalase activity.

The reaction of catalase one of the two H_2O_2 detoxicating enzymes (i.e. CAT and GSH-Px) indicates that excessive amounts of peroxide are present since catalase protects cells against high H_2O_2 levels, whereas a GSH-dependent mechanism is sensitive to its low concentrations (35). GSH-Px has a high substrate affinity but its rate is limited by sluggish glutathione recycling (7, 8, 10). Biochemical reactions between vitamin E and selenium gathering the composition of GSH-Px are adopted to have protective endogenous antioxidant effect against peroxidative cell damage (36).

Plasma vitamin C levels were significantly decreased in the FO group fed 3 IU vitamin E, despite the fact that rats can synthesize vitamin C in vivo (12). Chen et al have observed the synergistic action of vitamin E and C in mutant rats (9). In humans who need relatively large amounts of vitamin C, the body status of vitamin C is more likely to be disturbed under conditions of oxidative stress brought about by the intake of FO and/or an insufficient amount of vitamin E. Ascorbic acid acts as seizer of free radicals by supplying vitamin E at the reductated active form. So vitamin C itself becomes a radical. The cycle is completed by the reduction of vitamin C radical with semiascorbate reductase (13, 36).

These results suggest that fish oil fed rats higher levels of plasma and liver LPO and lower levels of liver GSH-Px and catalase activity compared with the soybean oil fed rats and vitamin E should be supplemented in fish oil feeding to prevent the enhanced lipid peroxidation and protection antioxidant status. Vitamin E seems necessary to maintain appropriate levels of other physiological antioxidants. Soybean oil which is rich for polyunsaturated fatty acids was shown not reducing cellular defence mechanisms.

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