The Effects of Fish Oil on Triglycerides, Cholesterol, Fibrinogen and Malondialdehyde in Humans Supplemented with Vitamin E

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ABSTRACT The effects of fish oils supplemented with 0.3 IU/g and 1.5 IU/g of vitamin E were compared in a double-blind, cross-over study. Twelve healthy volunteers were given 30 mL/day of either oil for 3 wk. Intake of the vitamin E-rich fish oil resulted in a marked decrease in serum triglycerides (48%) and in fibrinogen (11%). After administration of the low vitamin E-containing oil there was a considerably smaller reduction of serum triglycerides and no significant reduction of fibrinogen. Both oils caused an increase in high density lipoprotein cholesterol and a decrease in the atherogenic index, but neither oil altered the total cholesterol level. Serum vitamin E was decreased by 9% and plasma malondialdehyde was increased by 122% after intake of the low vitamin E-containing oil, but both remained normal after intake of the other oil. The effect of vitamin E may be due to inhibition of fatty acid peroxidation with less formation of malondialdehyde and a larger amount of active (*n*-3) fatty acids in their sites of action in the liver, resulting in a greater decrease in the synthesis of triglycerides and fibrinogen. J. Nutr. 121: 165–169, 1991.

INDEXING KEY WORDS:

- *(n-3) fatty acids*
- *vitamin E*
- *malondialdehyde*
- *triglycerides*
- *humans*

The low incidence of cardiovascular disease in some populations, for example Greenland Eskimos and coastland Japanese, is thought to be partially due to a high consumption of (*n*-3) fatty acids (1). There are numerous reports of potentially beneficial effects of fish oil on triglycerides, blood rheology, blood pressure and inflammation (2). There have been conflicting reports concerning fish oil's effects on cholesterol and its fractions and on plasma fibrinogen. Some of the discrepancies may be due to differences in the quality and dose of the fish oil (3). The effects of different supplementation of the fish oils with antioxidants, mainly vitamin E, have not been well explored in clinical trials. It is well known that administration of polyunsaturated fatty acids decreases the concentration of vitamin E in the body (4, 5). Vitamin E is an important chain-breaking antioxidant in the lipid phase of different cell membranes (6). It scavenges peroxy radicals by donating to them the phenolic hydrogen. In addition to its free radical scavenging activity, vitamin E is highly reactive toward singlet oxygen (7). It works in concert with vitamin C, glutathione peroxidase and β-carotene (8, 9). These agents protect different structures against oxidative damage and lipid peroxidation, often measured as the production of malondialdehyde (MDA)[1] in plasma or tissues (5).

In the present study we compared the effects of fish oils supplemented with different amounts of vitamin E on blood lipids, plasma fibrinogen and MDA in a double-blind cross-over study.

MATERIALS AND METHODS

Subjects. Twelve volunteers (10 men, 2 postmenopausal women) participated. Their mean age was 51 y (range 41–60). Fish oil supplemented with 0.3 or 1.5 IU/g of vitamin E was used. Subjects were given 30 mL/d of one type of fish oil for 3 wk, followed by a washout period of 2 wk, and then crossed over to the other oil for another 3 wk. Six of the volunteers started with one type of the oil and six with the other type. They were carefully instructed not to change their diets or lifestyle during the experimental period. The fish oil was thus added to their ordinary diet. Before the blood sampling, the volunteers fasted for 10 h. They were instructed to refrain from alcohol consumption for 2 d, vigorous physical

[1]Abbreviations used: HDL, high density lipoprotein; MDA, malondialdehyde; PV, peroxide value.

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TABLE 1

Fatty acid composition of the fish oil

Fatty acid	% (wt/wt)
14:0	6.8
16:0	15.6
16:1	8.5
16:2	1.1
18:0	1.2
18:1	3.7
18:2	10.8
18:3	1.8
18:4 (*n*-3)	4.1
20:1	1.2
20:4 (*n*-3)	1.0
20:5 (*n*-3)	19.1
22:1	1.2
22:5 (*n*-3)	2.8
22:6 (*n*-3)	13.0
Others	8.1

activity for 1 d, and the use of acetylsalicylic acid (aspirin) or similar medication at least 1 wk before the sampling. To minimize the effect of diurnal variation, blood was always sampled around 0800 h. Blood samples were taken and other tests performed before and after the various treatment periods. Informed consent was obtained and the study was approved by the local ethics committee.

Fish oil composition. The oil contained about 25% saturated fatty acids, 15% monosaturated fatty acids and 55% polyunsaturated fatty acids as triglycerides. Forty percent of the total fatty acids were of the (*n*-3) type, 19% eicosapentaenoic acid and 13% docosahexaenoic acid. The fatty acid composition of the fish oil is shown in Table 1. It had been stabilized against oxidation by natural antioxidants (Wallin, R. & Saldeen, T., unpublished results), and contained < 3 mg cholesterol/g. Dioxin was below detection level (< 0.74 pg/kg). One of the fish oils contained 0.3 IU/g of vitamin E (referred to as low vitamin E oil) and the other 1.5 IU/g (referred to as vitamin E–rich oil) (ESKIMO-3®, INUIT-3®, Cardinova, Sweden). Thirty milliliters of the oils correspond to ~1050 kJ (248 kcal). Compliance was determined by anamnesis, amount of fish oil left after the treatment periods and by the plasma vitamin E value.

Blood sampling. Venous blood samples were taken without stasis after 15 min of rest. The subject lay in the supine position.

Glucose was assayed in fresh plasma. For other analyses, plasma and serum were kept at –70°C until analyzed.

Analysis of blood samples. Triglyceride, total cholesterol and high density lipoprotein (HDL) cholesterol concentrations were determined in serum by enzymatic methods, using Boehringer-Mannheim kits 126012 and 124087 (Munich, Germany) modified for use in a Multistat III F/LS apparatus (Instrumentation Laboratories, Lexington, MA) (10). Serum HDL was obtained in the supernatant after selective precipitation with sodium phosphotungstate and magnesium chloride (11). The atherogenic index was calculated as (total cholesterol – HDL cholesterol)/HDL cholesterol. Lipoprotein (a) was measured by an enzyme immunoassay, TintElize™ Lp (a) (Biopool AB, Umeå, Sweden), according to the manufacturer's instructions. Apolipoprotein B was determined by means of the Pharmacia Apolipoprotein B RIA (Pharmacia Diagnostics AB, Uppsala, Sweden), which measures apolipoprotein B_{100} and B_{48}. According to the manufacturer, the mean value for healthy persons is 0.86 ± 0.23 g/L. Fibrinogen in plasma was determined as clottable fibrinogen by the method of Nilsson and Olow (12).

Malondialdehyde in plasma was measured by fast performance liquid chromatography with a PEP-RPC™ C-18 reversed phase column (Pharmacia LKB Biotechnology, Bromma, Sweden) after reaction with thiobarbituric acid (13). This new method has the advantage of being more specific than previously used methods, because MDA chromogens are separated from other reaction products, which can otherwise interfere with the MDA measurement. After blood sampling, 500 µL of the plasma was immediately mixed with 10 µL of 5% butylated hydroxytoluene in ethanol and then stored at –70°C until assayed.

Vitamin E (α-tocopherol), its isomers and retinol in serum were determined by HPLC with fluorescence detection (14). Glucose in plasma was assayed with a Reflotron®-Glucose (Boehringer Mannheim, Germany). The plasma value is 10–15% higher than the whole blood value. Insulin in serum was measured by the Pharmacia Insulin RIA (Pharmacia Diagnostics AB, Uppsala, Sweden) according to the manufacturer's instructions. Fructosamine in serum was determined by an automated colorimetric assay based upon the ability of fructosamines to act as reducing agents in alkaline solution (15).

For the determination of peroxide value (PV) in fish oil preparations, 5 g of fish oil was dissolved in 30 mL of acetic acid and chloroform (3:2, v/v). After 0.5 ml of saturated aqueous solution of potassium iodide was added, the mixture was shaken for 1 min and then titrated with sodium thiosulfate until colorless. The amount in milliliters (V) of sodium thiosulfate added expresses the PV as the peroxide quantity in milliequivalents active oxygen per kilogram according to the formula: $PV = V \cdot 2$.

Most analyses were performed in duplicate.

Statistics. Student's *t* test for paired observations was used to compare values in the same subjects before and after the intervention period. In the tables the percentage of change and statistical significance refer to the paired differences.

TABLE 2

Effects of 3 wk administration of fish oil containing two levels of vitamin E on triglycerides, cholesterol, high density lipoprotein (HDL) cholesterol and atherogenic index in serum[1]

	Triglycerides	Cholesterol	HDL-cholesterol	Atherogenic index
		mmol/L		
Before fish oil + vitamin E (0.3 IU/g)	2.6 ± 1.6	5.6 ± 0.9	1.0 ± 0.3	5.0 ± 1.7
After fish oil + vitamin E (0.3 IU/g)	2.0 ± 1.8 (–21%)	5.5 ± 0.9 (± 0%)	1.1 ± 0.3 (+11%**)	4.5 ± 1.9 (–11%**)
Before fish oil + vitamin E (1.5 IU/g)	3.4 ± 2.9	5.7 ± 1.1	1.0 ± 0.3	5.2 ± 2.2
After fish oil + vitamin E (1.5 IU/g)	1.8 ± 1.3 (–48%**)	5.6 ± 1.1 (–1%)	1.1 ± 0.3 (+9%*)	4.6 ± 1.9 (–11%**)

[1]Mean ± SD, n = 12. *P < 0.05, **P < 0.01. Student's t test for paired observations.

RESULTS

Both fish oil preparations were tolerated by all subjects without any major adverse effects. Both preparations lowered serum triglycerides, but the decrease was much larger (48%) with the vitamin E–rich oil (Table 2). Cholesterol concentration was unchanged. Levels of HDL cholesterol increased significantly with both oils. The atherogenic index was significantly decreased to the same extent by both oils (Table 2).

Plasma lipoprotein (a) and serum apolipoprotein B showed only insignificant changes with both types of oils (Table 3). Only the vitamin E–rich oil resulted in a significant (11%) reduction in plasma fibrinogen concentration (Table 3). After intake of the low vitamin E oil, MDA was significantly increased by 122%, but the vitamin E–rich oil had no effect (Table 3). The low vitamin E oil caused a decrease in the serum level of vitamin E, which was not observed after intake of the other oil (Table 3). Tocopherol isomers and retinol showed nonsignificant changes (data not shown).

Plasma glucose increased after treatment with both oils (Table 3). Insulin in serum showed only nonsignificant changes. Serum fructosamine showed a slight increase with both oils, but the change was statistically significant only after intake of the low vitamin E oil (Table 3).

The PV in the oils was ~1 mEq/kg and the MDA concentration was ~55 μmol/L, with no difference between the two oils.

DISCUSSION

This study showed that high levels of vitamin E markedly enhance the ability of fish oil to decrease serum triglyceride and plasma fibrinogen concentrations.

Triglycerides have now been generally accepted as an independent risk factor for ischemic heart disease (16). The triglyceride-lowering effect of fish oil is thought to be mediated by a decrease in the rate of synthesis of very low density lipoprotein triglyceride in the liver (17, 18). Why the low vitamin E oil caused a considerably smaller decrease in the triglycerides can only be conjectured. The lower vitamin E content in the fish oil may allow the production of fatty acid peroxides during storage. However, this possibility seems unlikely, as both the PV and the MDA concentrations were comparable in the two oils. Fatty acid peroxides may also be formed in the intestine or in the rest of the body in the presence of decreased amounts of vitamin E. Wherever the sites of production, fatty acid peroxidation will result in a smaller amount of active (n-3) fatty acids in their sites of action in the liver.

We found a significant reduction of vitamin E in the serum after administration of the low vitamin E oil. A low body content of vitamin E may result in an increase in MDA in the body, reflecting enhanced lipid peroxidation. In fact, we observed a very large increase in MDA after 3 wk of treatment with the low vitamin E oil. The corresponding changes after the period of treatment with the vitamin E–rich oil were not significant. Vitamin E in the body not only protects unsaturated fatty acids from oxidative changes (9) but is also thought to have a controlling influence upon linoleyl and arachidonyl residues within membrane phospholipids, which cannot be explained by the antioxidant function of the vitamin (6). Beside its antioxidant effect, vitamin E is thought to stabilize various membranes (19).

Cholesterol in serum was not significantly altered after the different treatments. However, it has been

TABLE 3

Effects of 3 wk administration of fish oil containing two levels of vitamin E on lipoprotein (a) (in plasma), apolipoprotein B (in serum), fibrinogen (plasma), malondialdehyde (plasma), vitamin E (serum), glucose (plasma), insulin (serum) and fructosamine (serum)[1]

	Lipoprotein (a)	Apolipoprotein B	Fibrinogen	MDA[2]	Vitamin E	Glucose	Insulin	Fructosamine
	mg/mL	*g/L*	*g/L*	*μmol/L*	*mg/L*	*mmol/L*	*μU/mL*	*mU/L*
Before fish oil + vitamin E (0.3 IU/g)	128 ± 149	1.27 ± 0.33	3.1 ± 0.4	0.6 ± 0.4	13.6 ± 3.6	7.0 ± 2.7	13.0 ± 11.9	2.1 ± 0.3
After fish oil + vitamin E (0.3 IU/g)	125 ± 136 (−2%)	1.29 ± 0.26 (+2%)	3.0 ± 0.4 (−3%)	1.3 ± 1.2 (+122%)*	12.4 ± 2.9 (−9%)**	7.6 ± 3.1 (+9%)**	13.0 ± 11.7 (± 0%)	2.2 ± 0.3 (+2%)**
Before fish oil + vitamin E (1.5 IU/g)	124 ± 135	1.28 ± 0.32	3.3 ± 0.7	0.7 ± 0.3	13.7 ± 3.6	6.9 ± 3.0	12.6 ± 12.2	2.1 ± 0.3
After fish oil + vitamin E (1.5 IU/g)	128 ± 133 (+3%)	1.29 ± 0.26 (± 0%)	2.9 ± 0.4 (−11%)**	0.7 ± 0.3 (+6%)	13.7 ± 2.9 (−1%)	7.6 ± 3.0 (+11%)***	11.7 ± 9.0 (−7%)	2.2 ± 0.2 (+2%)

[1]Values are means ± SD, $n = 12$. Student's t test for paired observations was used to compare values in the same subjects before and after the intervention period. The percentage of change and statistical significance refer to the paired differences. *$P < 0.01$, **$P < 0.05$, ***$P < 0.001$.

[2]MDA = malondialdehyde.

found to decrease after longer treatment with fish oil (3).

Our previous studies (3) showed that the levels of triglycerides and fibrinogen are more sensitive than is cholesterol or HDL cholesterol to the effects of fish oil and its composition. Triglyceride synthesis might also be more sensitive to an unchanged (not peroxidized) fatty acid than is the cholesterol synthesis. This could at least partly explain the differences between the effects of the two oils on the triglyceride levels.

With the vitamin E–rich oil plasma fibrinogen decreased 11%. This confirms our earlier results (3) and those of others (20). Interestingly, there was no significant decrease after the intake of the low vitamin E oil. The effect of fish oil is probably due to a decrease in the production of fibrinogen in the hepatocytes. The antioxidant effect of vitamin E ought to yield a larger amount of active (*n*-3) fatty acids at their sites of action in the liver. Increased fibrinogen has been stressed as a risk factor for stroke and myocardial infarction (21).

Oxidatively modified lipoproteins are now believed to play an important role in atherogenesis. Malondialdehyde and other lipoperoxidation products such as 4-hydroxy-nonenal modify these lipoproteins (5). Supplementation of the fish oils with adequate amounts of antioxidants, to prevent a reduction of the antioxidative capacity of the body and the formation of lipid peroxidation products, is therefore important.

The observed increase in plasma glucose caused by the fish oil confirms the findings in some studies (22, 23, 24). The significance of this potentially adverse effect of fish oil is unclear. Patients with type II diabetes may display many other abnormalities that might contribute to cardiovascular disease, including increased serum lipids, increased platelet aggregability and hypertension. Because fish oil has a favorable effect on these variables, the net effect of these oils in type II diabetes still needs to be elucidated in more detail.

The turnover of polyunsaturated fatty acids and of vitamin E differs in different cells (25, 26). This may influence the effect of the length of the washout period after administration of fish oils. We are aware that after a washout period of only 2 wk the levels of fatty acid and of vitamin E in some cells, e.g., platelets and leukocytes, have not returned to baseline. However, the short washout period in the present study does not seem to have had any major influence on our results.

In conclusion, some effects of the fish oil, especially on triglycerides and fibrinogen, were clearly more pronounced when the oil was adequately supplemented with vitamin E. This is probably due to the antioxidant effect of vitamin E, which leads to efficient inhibition of fatty acid peroxidation, less formation of MDA and larger amounts of active (*n*-3) fatty acids at their sites of action in the liver. The end result is pronounced decrease in the synthesis of triglycerides and fibrinogen.

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