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Effects of fish oil alone and combined with long chain (n-6) fatty acids on some coronary risk factors in male subjects

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The effects of fish oil alone were compared with those of fish oil combined 1:1 with evening primrose oil rich in long chain (n-6) fatty acids in a double-blind cross-over study. After administration of fish oil there was a highly significant increase in 20:5 (n-3) (eicosapentaenoic acid) and 22:6 (n-3) (docosahexaenoic acid) and a significant decrease in (n-6) fatty acids in plasma phospholipids. After consumption of the fish oil/evening primrose oil mixture, the increase in (n-3) and the decrease in (n-6) fatty acids were considerably smaller. Triglycerides in serum decreased by 36% ($P < 0.01$*) after the fish oil and by 29% (*$P < 0.05$*) after the fish oil/evening primrose oil mixture. Atherogenic index decreased by 12% (*$P < 0.05$*) after fish oil/evening primrose oil and by 6% (*$P = ns$*) after fish oil alone. This difference was statistically significant (*$P < 0.05$*). Plasma homocysteine was reduced by 10% (*$P < 0.05$*) after the fish oil/evening primrose oil mixture and decreased 4% (*$P = ns$*) after the fish oil alone. Plasma fibrinogen decreased after both oils. The combined oils did not raise plasminogen activator inhibitor-1 (PAI-1) antigen at all, whereas after fish oil there was a 49% (*$P < 0.05$*) increase. Fish oil increased the ratio C20:4 to C20:3, an index of delta-5-desaturase, by 96% (*$P < 0.001$*) and reduced the ratio of C20:3 to C18:2, an index of delta-6-desaturase, by 38% (*$P < 0.001$*), whereas the fish oil/evening primrose oil mixture left these indexes unchanged. A high index of delta-5-desaturase has been found to be correlated to increased insulin sensitivity. In conclusion, combination of fish oil and evening primrose oil had a more favorable effect on the atherogenic index and caused no increase in PAI-1 antigen. The effects on triglycerides and PAI-1 of the fish oil and the mixture appears to be a result of their (n-3) fatty acid content.* (J. Nutr. Biochem. 9:629–635, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Dietary fats have a profound impact on plasma lipids and lipoproteins, and this may explain many of the effects of lipids on risk factors for several major diseases in affluent societies.[1] To prevent ischemic heart disease (IHD), substitution of polyunsaturated and monounsaturated fatty acids for saturated fatty acids is generally recommended.[2] This also holds true to some extent for the prevention of some inflammatory diseases.[3] There are two series of essential polyunsaturated fatty acids (PUFAs): Alpha-linolenic acid (18:3 n-3) is the parent compound of (n-3) fatty acids, and linoleic acid (18:2 n-6) of (n-6) fatty acids. Long-chain (n-3) fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as (n-6) fatty acids [mainly linoleic and its derivative gamma-linolenic acid (18:3 n-6)], have been reported to possess lipid-lowering properties.[4] An increase in (n-3) fatty acids mainly decreases serum triglycerides and increases high density lipoprotein (HDL) cholesterol,[5] whereas an increase in linoleic acid and gamma-linolenic acid mainly decreases total cholesterol and low density lipoprotein (LDL) cholesterol.[6]

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In several previous studies we found a considerable decrease in triglycerides after administration of fish oil.[7–10] This was accompanied by a slight increase in HDL cholesterol and a decrease in fibrinogen. However, in many of our studies there was a slight increase in glucose and signs of a moderate decrease in fibrinolysis, as measured by an increase in plasminogen activator inhibitor-1 (PAI-1) activity and antigen.[8–10] The effects of fish oil on glucose homeostasis and on fibrinolysis have been used as reasons for not recommending this oil to persons with impaired glucose tolerance and decreased fibrinolysis.[11,12] Our previous studies showed that by increasing the vitamin E content of the fish oil[9] and supplementation with the B vitamins folic acid and pyridoxine,[10] these potentially adverse effects of fish oil could be partially diminished. Increased homocysteine in plasma has been reported to be an independent fisk factor for IHD.[13]

The goal of the present study was to determine whether the effects of fish oil could be improved by combining it with evening primrose oil, which is rich in long chain (n-6) fatty acids such as gamma-linolenic acid, as a 1:1 mixture. Therefore, we compared the effects of fish oil alone and a fish oil/evening primrose oil combination on fatty acids in plasma phospholipids, blood lipids, glucose, homocysteine, fibrinogen, and fibrinolysis in a double-blind cross-over manner.

Materials and methods

Participating in the study were 12 volunteers, 10 men and 2 postmenopausal women, who were healthy or had slightly to moderately increased blood lipid levels. Mean age of the group was 51 years (range 42–68 years) and mean body mass index was above average (27.8 ± 1.3 kg/m^2). No persons with known alcohol abuse or high gamma-glutamyl-transferase were accepted. Characteristics and the pretreatment laboratory values of the study group are shown in *Table 1*. Two types of treatment—30 mL of fish oil and 30 mL of a 1:1 mixture of fish oil and evening primrose oil—were used. In a double-blind cross-over manner patients received one type of treatment for 4 weeks, followed by a washout period of 5 weeks, after which the other treatment was given for 4 weeks. Six of the participants started with one type of treatment and six with the other.

The participants were carefully instructed not to change their diets and way of living during the trial, including the washout period, and also to record and report any significant changes in their diets or lifestyle. Thus, the fish oil added to their ordinary diet could result in an increased energy intake of 1,050 KJ (248 Kcal) per day. This may represent an increase of total dietary intake by 10% and a concomitant increase in the fat energy percent by approximately 6 units. Before the blood sampling, the subjects had fasted for 10 hours. They also were instructed to refrain from alcohol consumption for 2 days, from vigorous physical training for 1 day, and from the use of acetylsalicylic acid (aspirin) or similar medication for at least 1 week before the sampling. Blood was always drawn at approximately 8:00 AM to minimize the effect of diurnal variation. Blood samples were taken before and after the various treatment periods. Informed consent was obtained and the study was approved by the local ethics committee.

The fish oil that was used (ESKIMO-3®, Cardinova, Uppsala, Sweden) contained approximately 25% saturated fatty acids, 25% monounsaturated acids, and 45% polyunsaturated fatty acids in the form of triglycerides. Of the total fatty acids, 38 to 48% were of the (n-3) type, 19% EPA, and 13% DHA. The fatty acid composition of the fish oil is shown in *Table 2*. The oil had been stabilized against oxidation by natural antioxidants[14] and also contained 1.5 IU vitamin E (α-tocopherol) per gram of oil. The cholesterol content was less than 3 mg/g. Dioxin was below the detection level (<0.74 pg/kg). The fatty acid composition of the 1:1 mixture of fish oil and evening primrose oil is shown in *Table 2*. The mixture contained approximately 16% saturated fatty acids, 16% monounsaturated fatty acids, and 65% polyunsaturated fatty acids (ESKIMO-3® Cutan, Cardinova). Of the total fatty acids, 20% were of the (n-3) type and 44% of the (n-6) type. The mixture also contained 1.5 IU/g vitamin E (α-tocopherol). The volunteers also received a tablet containing 25 mg of pyridoxine and 0.4 mg of folic acid (Vitamin B, Cardinova) daily during both treatment periods. The oils were provided in bottles containing 105 mL, and the last portion of the oil of one bottle (along with some of the contents from a second bottle) was thus consumed on the fourth day. The bottles were stored in a refrigerator at 2 to 6°C. These measures precluded any oxidation of the fish oil. Compliance was determined by interviewing the subjects, by the amount of fish oil left after the treatment periods, and by the fatty acids in plasma phospholipids.

Table 1 Characteristics and pretreatment values of the study group

No. of subjects	12
Men	10
Women (postmenopausal)	2
Age (years)	51 ± 5.2
Age range (years)	42–68
Weight (kg)	79.8 ± 1.3
Body mass index (kg/m^2)	27.8 ± 1.3
Waist hip ratio (waist circumference/ hip circumference)	0.93 ± 0.02
Smokers[1]	2
Serum triglycerides (mmol/L)	1.8 ± 0.2
Total cholesterol in serum (mmol/L)	5.4 ± 0.3
Serum HDL cholesterol (mmol/L)	1.1 ± 0.1
Calculated LDL cholesterol in serum[2]	3.5 ± 0.3
Atherogenic index[3]	4.1 ± 0.4
Plasma glucose (mmol/L)	5.9 ± 0.2
Serum insulin (μU/mL)	7.5 ± 1.8
Serum C peptide (ng/mL)	2.5 ± 0.2
Plasma 4-hydroxynonal (nmol/L)	<D.L.[4]
Total homocysteine in plasma (nmol/L)	9.3 ± 1.0
Serum neopterin (nmol/L)	6.1 ± 0.2
Plasma fibrinogen (g/L)	3.0 ± 0.2
Plasma tPA antigen (ng/mL)	6.4 ± 0.7
Plasma PAI-1 activity (AU/mL)	26.8 ± 3.9
Plasma PAI-1 antigen (ng/L)	26.9 ± 5.0

[1]Current smokers.
[2]Total cholesterol − HDL cholesterol − 0.45 × triglycerides.
[3](Total cholesterol − HDL cholesterol)/HDL cholesterol.
[4]Below detection level.
HDL–high density lipoprotein. LDL–low density lipoprotein. tPA–tissue plasminogen activator. PAI-1–plasminogen activator inhibitor-1.

Blood sampling

Venous blood samples were taken without stasis after 15 minutes of rest, with the subject in the supine position. Glucose was assayed in fresh plasma. For other analyses, plasma and serum were kept for periods of up to 1 month at –70°C until analyzed.

Analysis of blood samples

For the determination of fatty acids in plasma phospholipids, plasma lipids were extracted with chloroform, to which 0.005% butylated hydroxytoluene had been added as an antioxidant. The

Table 2 Fatty acid composition (% wt/wt) of the fish oil and of a mixture of fish oil and evening primrose oil (FO/EPO)

Fatty acid	Fish oil	FO/EPO
14:0	6.8	3.4
16:0	15.6	10.7
18:0	2.7	2.0
Σ saturated	25.1	16.1
16:1 (n-7)	10.2	5.1
18:1 (n-9, n-7)	11.3	9.3
20:1 (n-9)	1.0	0.5
22:1 (n-11)	1.2	0.6
Σ monounsaturated	23.7	15.5
18:2 (n-6)	1.5	37.8
16:3 (n-4)	1.8	0.9
18:3 (n-6) GLA	0	5.3
18:3 (n-3) ALA	0.7	0.5
16.4 (n-3)	2.5	1.3
18:4 (n-3)	2.3	1.2
20:4 (n-6)	0.9	0.5
20:4 (n-3)	0.7	0.4
20:5 (n-3) EPA	19.1	9.6
22:5 (n-3)	2.8	1.4
22:6 (n-3) DHA	13.0	6.5
Σ polyunsaturated	45.3	65.4
Others	5.9	3.0
Σ (n-6)	2.4	43.6
Σ (n-3)	41.1	20.9

GLA–gamma-linolenic acid. ALA–alpha-linolenic acid. EPA–eicosapentaenoic acid. DHA–docosahexaenoic acid.

lipid esters were separated by thin layer chromatography. After transmethylation the fatty acid methyl esters were separated by gas liquid chromatography.[15] Triglycerides, total cholesterol, and HDL cholesterol concentrations were determined in serum by enzymatic methods, using Boehringer-Mannheim kits 126012 and 124084 (Munich, Germany) modified for use in a Multistat III F/LS apparatus (Instrumentation Laboratories, Lexington, MA USA).[15] Serum HDL cholesterol was obtained in the supernatant after selective precipitation with sodium phosphotungstate and magnesium chloride.[16] For calculation of LDL cholesterol, Friedewald's formula was used: total cholesterol – HDL cholesterol – 0.45 × triglycerides. The atherogenic index was calculated as: (total cholesterol – HDL cholesterol)/HDL cholesterol.

Glucose in plasma was measured with a Reflotron®-Glucose assay (Boehringer Mannheim). The plasma value is 10 to 15% higher than the value in whole blood. The accuracy of the test was checked with special reference probes. Insulin in serum was measured by the Pharmacia Insulin RIA 100 (Pharmacia Diagnostics AB, Uppsala, Sweden). C peptide in serum was assayed by RIA-gnost® hc-Peptide (Hoechst, Frankfurt/Main, Germany).

For measurement of total plasma homocysteine, blood samples were collected in evacuated tubes containing EDTA. The tubes were centrifuged within 10 minutes and the plasma was stored at –70°C until analyzed according to the method of Brattström et al.[17]

Fibrinogen in plasma was measured as clottable fibrinogen by the method of Nilsson and Olow.[18]

Tissue plasminogen activator (tPA) activity was measured amidolytically using a commercial kit (COA-SET® t-PA, Kabi Diagnostica, Mölndal, Sweden). The kit uses the chromogenic substrate S-2251 and the produced color is read spectrophotometrically at 405 nm. tPA antigen was measured by an enzyme-linked immunosorbent assay (Biopool, Umeå, Sweden), using a double antibody technique according to the instructions of the manufacturer. PAI-1 activity in plasma was assayed amidolytically by means of COATEST® PAI (Kabi Diagnostica), using the chromogenic substrate S-2403. The color is read spectrophotometrically at 405 nm. PAI-1 antigen was measured by an enzyme-linked immunosorbent assay (TintElize™ PAI-1, Biopool), with a double antibody technique.

Indexes of desaturase activity

The ratios of C20:4 to C20:3 and C20:3 to C18:2 are product-precursor ratios for the reactions controlled by delta-5-desaturase and delta-6-desaturase, respectively. They have been used as indexes for delta-5-desaturase and delta-6-desaturase.[19]

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 changes and statistical significance refer to the paired differences. A p-value less than 0.05 was considered significant. The data in the accompanying tables and figures are expressed as mean ±SEM. To test the difference in effect between the two treatments, possible interaction between the two treatments was first excluded. Then the change for each subject was compared using a *t*-test according to Hills and Armitage.[20]

Results

The relative proportions of various fatty acids in plasma phospolipids and the quotients between some fatty acids are shown in *Table 3* and *Figure 1*. After intake of fish oil there was a considerable increase in long-chain PUFAs of the (n-3) series (20:5 EPA and 22:6 DHA) and a concomitant decrease in (n-6) fatty acids (18:2, 20:3, and 20:4; $P < 0.001$ for all). The EPA/arachidonic acid ratio increased from 0.2 to 1.2 ($P < 0.001$) and the EPA/DHA ratio from 0.4 to 1.2 ($P < 0.001$). After intake of fish oil/evening primrose oil mixture, the decreases in (n-6) fatty acids and the changes in fatty acid quotients were considerably smaller (*Table 3* and *Figure 1*). Oleic acid (18:1 n-9) significantly decreased by 14% ($P < 0.001$) and 23% ($P < 0.001$) after treatment with fish oil and fish oil/evening primrose oil, respectively (*Table 3* and *Figure 1*). After fish oil, 20:3 (n-6) decreased by 57% ($P < 0.001$) whereas the decrease after the oil mixture was only 8%.

Delta-6-desaturase index decreased by 38% ($P < 0.001$) after fish oil alone, whereas after the fish oil/evening primrose oil mixture there was an increase of 10% (P = ns). Delta-5-desaturase index increased by 96% ($P < 0.001$) after the fish oil with no change after the oil mixture.

Serum triglycerides were decreased by 36% ($P < 0.01$) after the fish oil (*Figure 2*). The corresponding decrease after the fish oil/evening primrose oil mixture was 29% ($P < 0.05$). However, the difference between the two treatments was not statistically significant. The cholesterol and calculated LDL concentrations showed small changes: –2% and –5%, respectively, and 7% and –1%, respectively, after the fish oil and the oil mixture. HDL cholesterol increased by 6% and 5%, respectively, after the fish oil and fish oil/evening primrose oil mixture. Fish oil/evening primrose oil intake resulted in a 12% reduction ($P < 0.05$) in the atherogenic index, and the fish oil alone produced a somewhat smaller decrease of 6% (*Figure 2*). The differ-

Table 3 Fatty acid composition of plasma phospholipids before and after treatment with fish oil (FO) and a 1:1 mixture of fish oil and evening primrose oil (FO/EPO), 30 mL/day for 4 weeks

	Before FO	After FO	Before FO/EPO	After FO/EPO
16:0	22.5 ± 0.5	23.2 ± 0.5	23.2 ± 0.5	23.2 ± 0.5
16:1 (n-7)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
18:0	11.2 ± 0.4	12.1 ± 0.2	11.3 ± 0.2	11.9 ± 0.2
18:1 (n-9)	7.4 ± 0.3	6.3 ± 0.2[1]	7.6 ± 0.5	5.8 ± 0.2[1]
18:1 (n-7)	2.7 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.4 ± 0.1
18:2 (n-6)	17.2 ± 0.6	12.1 ± 0.7[1]	17.3 ± 0.6	14.8 ± 0.6[1]
18:3 (n-6)	<0.1	0.2 ± 0.0	<0.1	<0.1
18:3 (n-3)	<0.1	0.5 ± 0.1	<0.1	0.3 ± 0.1
20:1 (n-9)	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
20:3 (n-6)	2.3 ± 0.1	1.0 ± 0.1[1]	2.5 ± 0.1	2.3 ± 0.1
20:4 (n-6)	7.5 ± 0.3	6.3 ± 0.2[1]	7.3 ± 0.3	6.8 ± 0.2[2]
20:5 (n-3)	1.7 ± 0.2	7.5 ± 0.5[1]	1.7 ± 0.2	4.7 ± 0.3[1]
22:0	1.6 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1
22:5 (n-3)	2.6 ± 0.1	3.0 ± 0.1	2.7 ± 0.1	2.9 ± 0.1
22:6 (n-3)	4.6 ± 0.4	6.4 ± 0.3[1]	4.9 ± 0.3	6.1 ± 0.2[1]
24:1 (n-9)	2.5 ± 0.1	2.8 ± 0.1	2.5 ± 0.3	2.5 ± 0.1
Σ (n-6)	27.0 ± 0.6	19.6 ± 0.5[1]	27.1 ± 0.5	23.9 ± 0.5[1]
Σ (n-3)	8.9 ± 0.3	17.4 ± 0.5[1]	9.3 ± 0.3	14.3 ± 0.4[1]
C20:4 (n-6)/C20:3 (n-6)	3.3	6.5[1]	2.9	3.0
C20:3 (n-6)/C18:2 (n-6)	0.13	0.08[1]	0.14	0.16
C20:5 (n-3)/C20:4 (n-6)	0.23	1.19[1]	0.23	0.69[1]
C20:5 (n-3)/C22:6 (n-3)	0.37	1.17[1]	0.35	0.77[1]

Note: Relative proportions of various fatty acids in plasma. $n = 12$.
[1] $P < 0.001$.
[2] $P < 0.01$.

ence between the two treatments was statistically significant ($P < 0.05$).

After intake of fish oil/evening primrose oil mixture the plasma glucose concentration was unchanged, and after the fish oil alone there was a small nonsignificant increase by 4% (*Figure 2*). Insulin in serum (*Figure 2*) and C peptide in plasma (data not shown) showed only small nonsignificant changes.

Homocysteine in plasma was significantly decreased by 10% ($P < 0.05$) only after the period on the oil mixture (*Figure 2*). In 11 of 12 cases there was a decrease in homocysteine with this mixture. With the fish oil there was a small nonsignificant decrease of 4%. However, the difference between the two treatments did not reach statistical significance.

Fibrinogen in plasma decreased by 10% ($P < 0.05$) and 8% ($P < 0.05$) after intake of fish oil and fish oil/evening primrose oil, respectively (*Figure 3*). tPA antigen did not change with either oil. PAI-1 activity was increased by 50% ($P < 0.05$) after consumption of the fish oil and by 23% after fish oil/evening primrose oil consumption (*Figure 3*). PAI-1 antigen was significantly increased by 49% ($P < 0.05$) after intake of fish oil (*Figure 3*), but was unchanged after the period on the fish oil/evening primrose oil mixture. Of the fibrinolysis parameters, only the difference for PAI-1 antigen was statistically significant between the two treatments ($P < 0.05$).

Discussion

It is generally agreed that to prevent IHD and many inflammatory diseases, some saturated fatty acids should be replaced by polyunsaturated and monounsaturated fatty

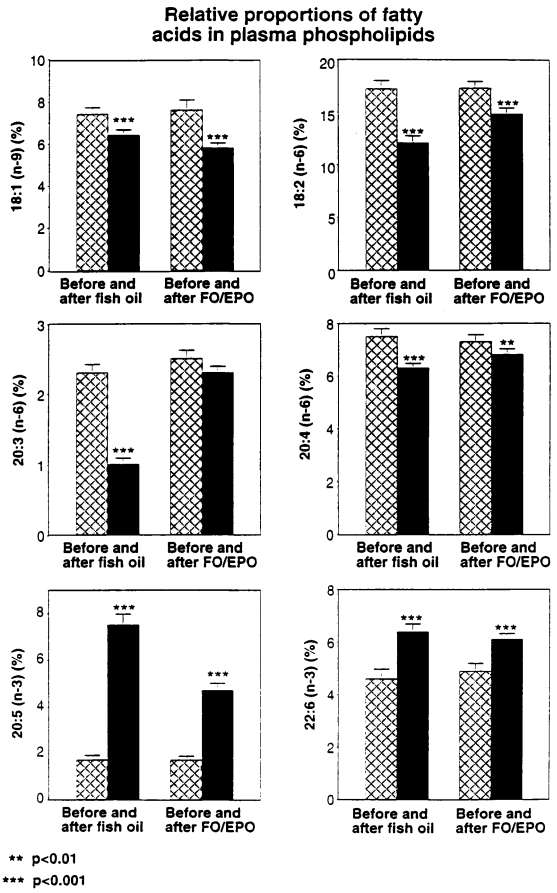

Figure 1 Relative proporations of fatty acids in plasma phospholipids. FO/EPO, fish oil/evening primrose oil mixture.

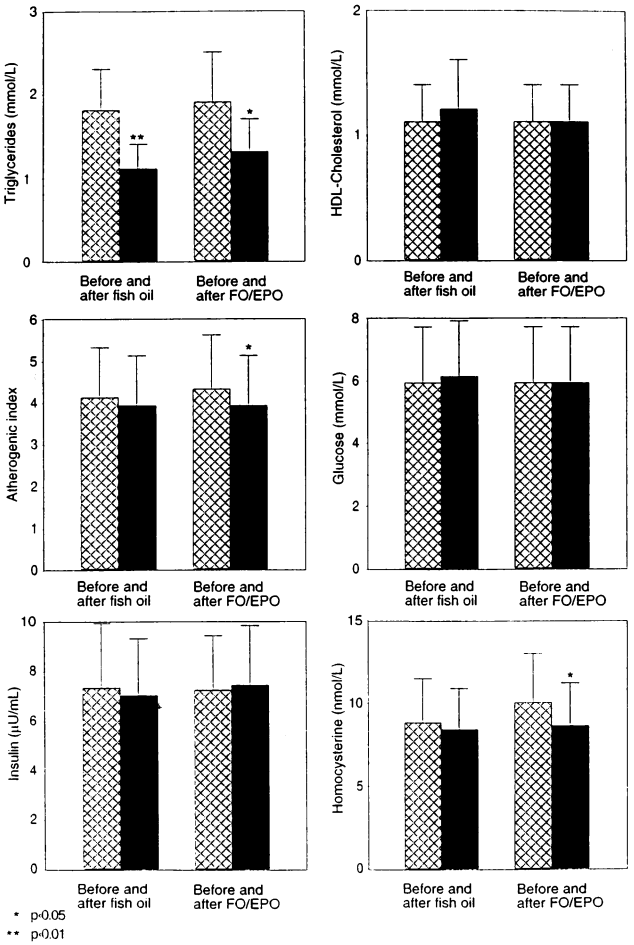

Figure 2 Effects of 4 weeks administration of 30 mL fish oil and a mixture of fish oil and evening primrose oil (FO/EPO) (1:1) on triglycerides, high density lipoprotein (HDL) cholesterol, atherogenic index, glucose, insulin, and homocysteine.

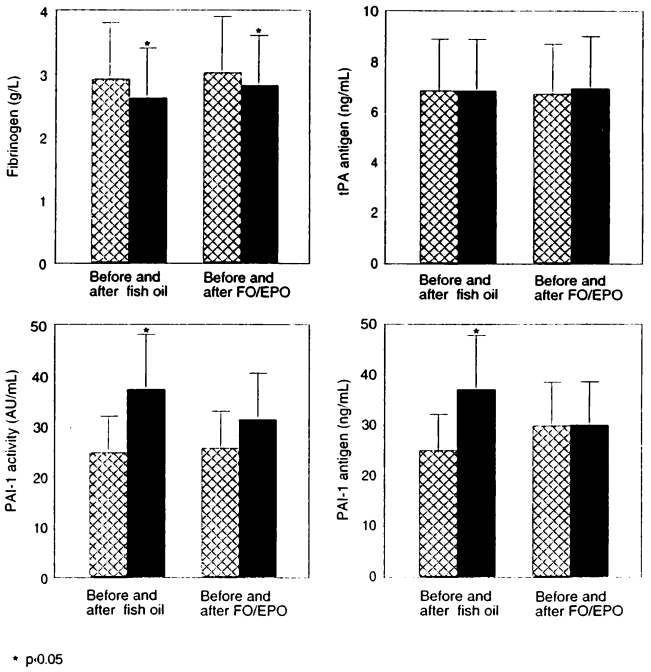

Figure 3 Effects of 4 weeks administration of 30 mL fish oil and a mixture of fish oil and evening primrose oil (FO/EPO) (1:1) on fibrinogen, tissue plasminogen activator (tPA) antigen, plasminogen activator inhibitor-1 (PAI-1) activity, and PAI-1 antigen.

acids.[2] There is considerable controversy about the relative importance of (n-3) and (n-6) PUFAs in the prevention of IHD and inflammatory diseases.[21–23] These views are evident in the different recommended dietary allowances that are published, with a recommended quotient between (n-6) and (n-3) fatty acids varying from 10 to 3.[2,22–24]

The goal of the present study was to determine whether the positive and the potentially negative effects of fish oil could be modulated by addition of evening primrose oil. After intake of fish oil there was a highly significant increase in EPA and DHA in plasma phospholipid fatty acids, with a concomitant decrease in (n-6) fatty acids. There were also considerable increases in the EPA/arachidonic acid and EPA/DHA ratios after intake of fish oil. After the period on fish oil/evening primrose oil mixture the corresponding changes were much smaller. These effects might be brought about by substitution of EPA and DHA for (n-6) fatty acids in the phospholipids, by competition for delta-6-desaturase and delta-5-desaturase, and by increased oxidation of fatty acids.[25] Both oils produced a decrease in (n-6) fatty acids, though to a varying extent. Fish oil/evening primrose oil also supplies gamma-linolenic acid, which is elongated to dihomo-gamma-linolenic acid, the precursor of anti-inflammatory prostaglandins of the series.[22,23]

Fish oil and fish oil/evening primrose oil significantly decreased triglycerides in serum by 36% and 29%, respectively. These changes correspond well to the decrease in triglycerides seen in a previous study using 30 and 15 mL, respectively, of fish oil,[7] and the effect of fish oil/evening primrose oil is thus probably due to its content of (n-3) fatty acids. The effects on total cholesterol and LDL cholesterol were rather small with both oils. The fish oil/evening primrose oil mixture produced a 12% ($P < 0.05$) decrease in the atherogenic index. The corresponding decrease with fish oil was 6%, which is smaller than has been seen in our previous studies after consumption of fish oil.[8–10] The significantly larger decrease in the atherogenic index after the oil mixture ($P < 0.05$) may reflect a somewhat better effect of linoleic acid and gamma-linolenic acid on total and LDL cholesterols. However, it cannot be excluded that the lower content of saturated fatty acids in the oil mixture may have contributed to this effect.

There has been much concern about the potential deterioriation of glucose homeostasis on consumption of fish oil. In a previous study blood glucose increased less when 80 mg pyridoxine and 10 mg folic acid were added together with the fish oil compared with unsupplemented fish oil.[10] In the present study 25 mg pyridoxine and 0.4 mg folic acid were given together with both oils, resulting in only a small (4%) increase in plasma glucose after fish oil alone, whereas intake of the fish oil/evening primrose oil mixture produced no change at all. Intake of fish oil led to a nonsignificant 4% decrease in serum insulin, and intake of fish oil/evening primrose oil mixture produced a corresponding 2% increase

in insulin. Elevated insulin and increased insulin resistance have been demonstrated to be a risk factor for IHD.[26,27] The question as to whether fish oil should be given to patients with type II diabetes has not yet been settled. There is some evidence that fish oil increases the gluconeogenesis in the liver and increases insulin sensitivity.[28] Variations in insulin sensitivity have been found to be related to differences in the membrane content of long-chain polyunsaturated fatty acids.[19] Thus, the ratio of 20:4 to 20:3, an index for delta-5-desaturase activity, directly correlated to insulin sensitivity.[19] Interestingly, this index increased by 96% after fish oil alone, but was unchanged after the oil mixture, which might indicate an advantage for (n-3) fatty acids over (n-6) fatty acids on insulin sensitivity. Fish oil has positive effects on several other risk factors present in diabetic patients. Supplementation with pyridoxine and folic acid and a higher concentration of vitamin E in the fish oil may reduce the risk for deterioriation of glucose homeostasis. There is a need for well-controlled long-term studies with small doses of (n-3) fatty acids to clarify these questions.

Interestingly, the fish oil/evening primrose oil mixture produced a 10% ($P < 0.05$) decrease in the plasma homocysteine concentration. In 11 of 12 subjects homocysteine decreased with this treatment. In the twelfth volunteer plasma homocysteine was greatly increased before treatment, probably because of some defect in homocysteine metabolism. Recently, fish oil was found to reduce homocysteine in hyperlipemic men.[29] Hypercholesterolemic men have been found to have increased levels of homocysteine in the LDL and other lipoprotein fractions of plasma.

A decreased fibrinolytic capacity has been suggested to be a risk factor for IHD and peripheral vascular disease.[30] In this study and in most other studies fish oil produced a moderate increase in PAI-1 activity and antigen.[10,31] In the current study fish oil and fish oil/evening primrose oil increased PAI-1 activity in plasma by 50% ($P < 0.05$) and 23%, respectively. Fish oil increased PAI-1 antigen by 49% ($P < 0.05$). Interestingly, the fish oil/evening primrose oil mixture did not change PAI-1 antigen at all. The effects on fibrinolytic parameters of the fish oil and the mixture thus appears to be a result of their (n-3) fatty acid content. The significance of an elevated level of plasma PAI-1 in general and after consumption of fish or fish oil is not well known. It has been suggested that the increase in PAI-1 on intake of (n-3) fatty acids might be a compensatory reaction without which there would be an increased risk of bleeding.[31] In support of this, Eskimos, who have a low incidence of IHD, also show increased levels of PAI-1 activity.[32]

In a previous study,[10] 30 mL of fish oil supplemented with 80 mg pyridoxine and 10 mg folic acid attenuated the rise in PAI-1 antigen after pure fish oil. In the present study, fish oil supplemented with only 25 mg pyridoxine and 0.4 mg folic acid resulted in almost the same attenuated level of PAI-1 antigen, suggesting that the lower doses used in the present investigation may be sufficient.

The members of the two different series of PUFAs exhibit many complex interactions at several levels. They have different affinities for enzymes regulating the production of prostanoids and leukotrienes.[25] The products often show considerably different actions.[33] The different PUFAs also compete for the desaturase enzymes.[34] Generally (n-3) fatty acids have a greater affinity for these enzymes, inhibiting, for example, the formation of arachidonic acid from linoleic acid. Recently, the interaction of free fatty acids with cytoplasmic and nuclear steroid hormone receptors has received great attention.[35,36] For cell functioning, both (n-6) and (n-3) PUFAs are needed and they require each other for optimal function. At the level of desaturases, in the competition for eicosanoid-producing enzymes and in their reaction with, for example, the intracellular steroid receptor superfamily, there are complex, partially unknown interactions between the two series of PUFAs.

There is much support for the view that overactivity of some arachidonic acid pathways greatly contributes to many chronic diseases in affluent countries.[21,24,37] With the introduction of agriculture and with the industrial revolution, there was an increase in saturated fat and (n-6) fatty acids and a decrease in (n-3) fatty acids in the diets of more affluent populations. These dietary changes—together with smoking, hypertension, obesity, some forms of stress, and decreased physical activity—are believed to have contributed to the increase in many chronic diseases.[21] It has also been proposed that the best preparation of fatty acids might be one containing a combination of EPA/DHA and gamma-linolenic acid.[23] This bypasses the sensitive delta-6-desaturase, supplies important (n-3) fatty acids, and inhibits the production of arachidonic acid. Gamma-linolenic acid is elongated to dihomo-gamma-linolenic acid, which is the precursor of anti-inflammatory prostaglandins of the series.[22,23]

In conclusion, although it produced a relatively large decrease in serum triglycerides, the fish oil/evening primrose oil mixture had a somewhat more favorable effect on the atherogenic index and caused a smaller increase in PAI-1 antigen than fish oil alone. In addition, there was a smaller decrease in PAI-1 activity after the fish oil/evening primrose oil mixture. The effects on triglycerides and fibrinolytic parameters of the fish oil and the mixture mainly appears to be a result of their (n-3) fatty acid content. The larger decrease in atherogenic index after fish oil/evening primrose oil may reflect a better effect of (n-6) than (n-3) fatty acids on total and LDL cholesterol. The increase in delta-5-desaturase index by fish oil possibly reflects increased insulin sensitivity. The interactions between the two series of PUFAs warrant further studies. This might aid in the optimal formulation of recommended dietary allowances of fatty acids and in the design of fatty acid mixtures for various therapeutic aims.

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