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550 __ |**a** Pharmacy

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450 __ |**a** Subject dictionaries

550 __ |**w** g |**a** Reference books

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consisting of comprehensive alphabetical lists of the words of a specific language, usually with definitions, are entered under the heading for the language with subdivision Dictionaries, e.g. |**a** English language--Dictionaries. |**i** Works about dictionaries of a specific language are entered under the heading for the language with subdivision Lexicography, e.g. |**a** English language--Lexicography.

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ATTACHMENT 4

(Mori)

The American Journal of
CLINICAL NUTRITION
Official Journal of The American Society for Clinical Nutrition, Inc

FULL TEXT ONLINE AT WWW.AJCN.ORG

MAY 2000 • VOLUME 71 • NUMBER 5 ISSN 0002-9165

Continued on Table of Contents inside

The American Journal of
CLINICAL NUTRITION
VOLUME 71 NUMBER 5 May 2000

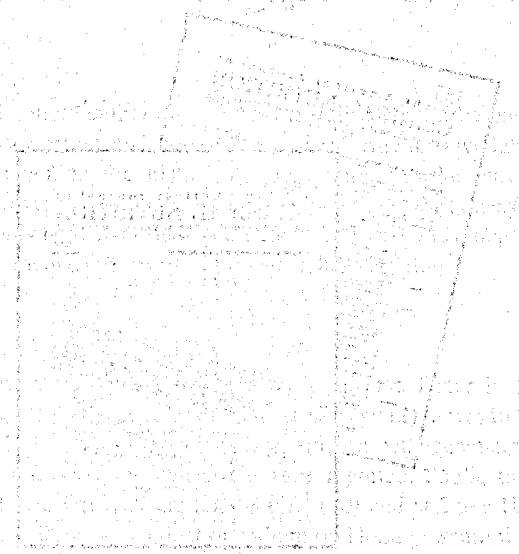
(continued)

(continued)

Book Review

Supplement: Maternal Nutrition: New Developments and Implications

(bound separately)



Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men[1–3]

Trevor A Mori, Valerie Burke, Ian B Puddey, Gerald F Watts, David N O'Neal, James D Best, and Lawrence J Beilin

ABSTRACT

Background: Regular consumption of n−3 fatty acids of marine origin can improve serum lipids and reduce cardiovascular risk.

Objective: This study aimed to determine whether eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids have differential effects on serum lipids and lipoproteins, glucose, and insulin in humans.

Design: In a double-blind, placebo-controlled trial of parallel design, 59 overweight, nonsmoking, mildly hyperlipidemic men were randomly assigned to receive 4 g purified EPA, DHA, or olive oil (placebo) daily while continuing their usual diets for 6 wk.

Results: Fifty-six men aged 48.8 ± 1.1 y completed the study. Relative to those in the olive oil group, triacylglycerols fell by 0.45 ± 0.15 mmol/L (≈20%; $P = 0.003$) in the DHA group and by 0.37 ± 0.14 mmol/L (≈18%; $P = 0.012$) in the EPA group. Neither EPA nor DHA had any effect on total cholesterol. LDL, HDL, and HDL_2 cholesterol were not affected significantly by EPA, but HDL_3 cholesterol decreased significantly (6.7%; $P = 0.032$). Although HDL cholesterol was not significantly increased by DHA (3.1%), HDL_2 cholesterol increased by ≈29% ($P = 0.004$). DHA increased LDL cholesterol by 8% ($P = 0.019$). Adjusted LDL particle size increased by 0.25 ± 0.08 nm ($P = 0.002$) with DHA but not with EPA. EPA supplementation increased plasma and platelet phospholipid EPA but reduced DHA. DHA supplementation increased DHA and EPA in plasma and platelet phospholipids. Both EPA and DHA increased fasting insulin significantly. EPA, but not DHA, tended to increase fasting glucose, but not significantly so.

Conclusions: EPA and DHA had differential effects on lipids, fatty acids, and glucose metabolism in overweight men with mild hyperlipidemia. *Am J Clin Nutr* 2000;71:1085–94.

KEY WORDS Eicosapentaenoic acid, docosahexaenoic acid, EPA, DHA, hyperlipidemia, fish oil, n−3 fatty acids, lipids, LDL particle size, glucose metabolism, insulin metabolism, men

INTRODUCTION

There is considerable evidence to support a protective effect of dietary n−3 polyunsaturated fatty acids against atherosclerotic heart disease (1). The 2 principal n−3 fatty acids in marine oils, eicosapentaenoic acid (EPA; 20:5n−3) and docosahexaenoic acid (DHA; 22:6n−3), have a wide range of biological effects (1–3). Those relevant to heart disease include influences on lipoprotein metabolism (4, 5), platelet and endothelial function, vascular reactivity, neutrophil and monocyte cytokine production, coagulation, fibrinolysis, and blood pressure (1–3, 6, 7). In addition, the effect of n−3 fatty acids may be dependent, to some extent, on the presence of underlying disorders such as dyslipidemia, hypertension, diabetes mellitus, and vascular disease.

n−3 Fatty acid supplementation in animals and humans results in substantial increases in plasma and tissue EPA and DHA as well as variable incorporation in different phospholipid classes in different tissues (8–10). These differences may be important to the subsequent utilization and metabolism of EPA and DHA. Although both fatty acids are considered to be biologically active, most studies have focused on the relative importance and effects of EPA, primarily because of its predominance in marine oils and fish species. The recent availability of purified EPA and DHA, however, has enabled studies of the independent biological effects of these fatty acids.

Evidence from in vitro studies suggests differential effects of EPA and DHA (11, 12). In vitro (13) and animal (10, 14, 15) studies have also suggested that EPA may be primarily responsible for the hypotriglyceridemic effect of n−3 fatty acids. Rambjor et al (16) concluded that EPA is responsible for the triacylglycerol-lowering effect of fish oils in humans, but their study had small numbers of subjects and was of short duration. In contrast, a hypotriglyceridemic effect of DHA was shown in healthy subjects (17) and in patients with combined hyperlipidemia (18).

[1] From the Department of Medicine, The University of Western Australia and The West Australian Heart Research Institute, Perth, and the Department of Medicine, University of Melbourne and St Vincent's Hospital, Melbourne.

[2] Supported by a grant (Studies in Hypertension and Cardiovascular Disease) from the National Health and Medical Research Council of Australia. Purified eicosapentaenoic and docosahexaenoic acids and olive oil capsules were kindly provided by the Fish Oil Test Materials Program and the US National Institutes of Health.

[3] Address reprint requests to TA Mori, University Department of Medicine, Medical Research Foundation Building, Box X 2213 GPO, Perth, Western Australia 6847. E-mail: tmori@cyllene.uwa.edu.au.

Received August 10, 1999.

Accepted for publication October 20, 1999.

Grimsgaard et al (19) reported that EPA and DHA have similar triacylglycerol-lowering effects compared with corn oil placebo. However, DHA significantly increased HDL cholesterol, whereas EPA significantly lowered both total cholesterol and apolipoprotein (apo) A-I concentrations (19). Neither fatty acid altered LDL-cholesterol concentrations significantly.

Possible benefits of n−3 fatty acids have to be weighed against the potential for impairment of glycemic control, particularly in patients with type 2 diabetes (20–23). However, studies in healthy subjects, in patients with dyslipidemia (24), and in patients with untreated hypertension (25) showed no adverse effects of n−3 fats on plasma glucose concentrations. To our knowledge, there have been no studies in which the effects of pure EPA were compared with those of DHA on indexes of glucose and insulin metabolism in humans.

In view of the increasing use of n−3 fatty acids in the diet as food additives or as therapeutic substances, it is important to determine the extent of any differential effects of EPA and DHA. This study examined the independent effects of EPA and DHA on fatty acid and lipid metabolism, as well as on fasting glucose and insulin concentrations. The study also aimed to determine whether EPA and DHA differ in their effects on HDL-cholesterol subfractions and LDL particle size.

SUBJECTS AND METHODS

Study population

Mildly hypercholesterolemic but otherwise healthy, nonsmoking men aged 20–65 y were recruited from the general community by media advertising. Entry criteria included a serum cholesterol concentration >6 mmol/L, a triacylglycerol concentration >1.8 mmol/L, or both; a body mass index (BMI; in kg/m^2) between 25 and 30; and no recent (previous 3 mo) symptomatic heart disease, diabetes, or liver or renal disease (plasma creatinine >130 μmol/L). None of the subjects were regularly taking nonsteroidal antiinflammatory, antihypertensive, or lipid-lowering drugs or other drugs known to affect lipid metabolism. All of the men had a usual weekly consumption of not more than one fish meal and drank <210 mL ethanol/wk. Fifty-nine of the 136 subjects screened satisfied the entry criteria. The study was approved by the ethics committee of the Royal Perth Hospital and all subjects gave written consent.

Dietary education and intervention

All subjects maintained their usual diets and alcohol intakes during a 3-wk familiarization period. Baseline measurements were collected and the men were stratified for age and BMI before being randomly assigned to 1 of 3 groups: 4 g daily of EPA, DHA, or olive oil (placebo) capsules for 6 wk. Capsules contained either purified preparations of EPA ethyl ester (≈96%), DHA ethyl ester (≈92%), or olive oil (≈75% oleic acid ethyl ester). All participants were instructed to maintain their usual diets, alcohol intakes, and physical activities, and not to make any changes to their lifestyle throughout the intervention period.

At an initial interview, subjects were given written and verbal instructions by a dietitian on how to keep diet records, with food weighed or measured. The same dietitian monitored the dietary intake of all the volunteers at 2-wk intervals and ensured that usual eating habits were maintained. A 3-d diet record (2 weekdays and 1 weekend day) was completed by the volunteers at baseline and postintervention.

Lifestyle assessment and anthropometry

Alcohol intakes, physical activities, and any medications taken were monitored every second week during the intervention by using 7-d retrospective diaries. Weight was measured every second week with an electronic scale.

Serum lipids, glucose, and insulin

Fasting serum lipids, lipoproteins, glucose, and insulin were measured twice at baseline and twice at the end of the intervention. Serum glucose was measured with an automated Technicon Axon Analyzer (Bayer Diagnostics, Sydney, Australia) by using a hexokinase method within 12 h of collection. The assay precision for serum glucose at 4.9 mmol/L was 3.1%. Serum insulin was measured by radioimmunoassay with an automated immunoassay analyzer (Tosoh Corporation, Tokyo). The CV for serum insulin at 21 and 102 pmol/L was 14.0% and 8.0%, respectively. The precision in the range of 234–720 pmol/L was 7.0%.

Serum total cholesterol and triacylglycerols were determined enzymatically on the Cobas MIRA analyzer (Roche Diagnostics, Basel, Switzerland) with reagents from Trace Scientific (Melbourne). The assay CVs were 2.2% at 4.2 mmol/L and 1.4% at 10.5 mmol/L for total cholesterol, and 1.6% at 4.0 mmol/L and 2.5% at 1.2 mmol/L for triacylglycerol. HDL cholesterol was determined on a heparin-manganese supernate (26); the CV at 1.1 mmol/L was 1.9%. HDL_2 and HDL_3 cholesterol were determined by using a single precipitation procedure (27). LDL cholesterol was calculated by using the Friedewald formula (28). Serum for the analyses of lipids, lipoproteins, and insulin was snap-frozen in liquid nitrogen and stored at − 80°C. Samples obtained at baseline and at the end of the intervention were measured in a single assay to minimize interassay variation.

LDL particle size

LDL particle size was determined from LDL isolated by vertical density-gradient ultracentrifugation of 4 mL plasma collected into EDTA (29). LDL particle diameter was determined by using a previously published method (30, 31) with use of commercially available 3–13% nondenaturing native gels (Gradipore, Sydney, Australia). Markers used were 28-nm latex beads (Duke, Palo Alto, CA) and high-molecular-weight standards (Pharmacia, Peapack, NJ). Gels were scanned by Tracktel video densitometry (Vision System Ltd, Adelaide, Australia) to provide a quantitative estimate of the dominant peak size. Particle diameter was obtained from a standard curve of the logarithm of the diameter of the standards (latex beads, 28 nm; thyroglobulin, 17.nm; and ferritin, 12.2 nm) against their positions on the scanned gel. A statistical package was used to derive a regression equation that allowed test samples to be sized. The CV of a 26.1-nm quality-control sample run on every gel was 0.8%.

Plasma and platelet phospholipid fatty acids

Plasma (1 mL) and washed platelets prepared from blood collected into EDTA were extracted with chloroform:methanol (2:1 by vol, 5 mL). The phospholipid fraction was obtained from total lipid extracts by thin-layer chromatography by using a solvent system of hexane:diethyl ether:acetic acid:methanol (170:40:4:4, by vol) on silica gel 60 F_{254}-precoated aluminum sheets (Merck, Darmstadt, Germany). Fatty acid methyl esters were prepared by

TABLE 1
Characteristics of participants in the 3 groups at baseline[1]

	Olive oil (control) (n = 20)	EPA (n = 19)	DHA (n = 17)
Age (y)	48.4 ± 2.0	48.9 ± 1.7	49.1 ± 2.2
Body weight (kg)	88.7 ± 2.0	89.1 ± 2.3	90.8 ± 2.8
BMI (kg/m^2)	28.4 ± 0.5	29.0 ± 0.7	28.9 ± 0.7
Waist-to-hip ratio	0.94 ± 0.01	0.93 ± 0.01	0.94 ± 0.01

[1] $\bar{x}$ ± SEM. There were no significant differences by one-way ANOVA. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

treating phospholipid extracts with 4% H_2SO_4 in methanol at 90°C for 20 min and analyzed by gas-liquid chromatography with a model 5980A gas chromatograph equipped with a 3393A computing integrator (Hewlett-Packard, Rockville, MD). The column was a BPX70 (25 m × 0.32 mm, 0.25-μm film thickness; SGE, Ringwood, Australia) with a temperature programmed from 150 to 210°C at 4°C/min and with nitrogen as the carrier gas at a split ratio of 30:1. Peaks were identified by comparing them with a known standard mixture. Individual fatty acids were calculated as a relative percentage with the evaluated fatty acids set at 100%.

Statistical analysis

Diet records were analyzed by using DIET/1 (version 4; Xyris, Brisbane, Australia), which is based on the Australian Food Composition Database NUTTAB 1995A (32). Data were analyzed by using SPSS (SPSS Inc, Chicago) with general linear models to assess the effects of EPA or DHA relative to the olive oil group. Significance levels were adjusted for multiple comparisons by using the Bonferroni method. Values are reported as means ± SEMs.

RESULTS

Study population

Fifty-six of the 59 subjects completed the study. Two subjects withdrew because they were unable to maintain the schedule of laboratory visits and one subject withdrew because of gastrointestinal symptoms. Baseline characteristics of the 3 groups confirmed that they were well matched for the entry criteria (**Table 1** and **Table 2**).

Energy and macronutrient intakes

Evidence of adherence to the diets was from analysis of diet records and capsule counts. There was no significant difference in body weight between the groups at baseline (Table 1) and no significant change during the intervention. Weight changes in the 3 groups were as follows: 0.2, 0.2, and 0.3 kg in the control, EPA, and DHA groups, respectively. Analysis of diet records indicated that total energy and major macronutrient intakes were not significantly different between groups at baseline (**Table 3**) and did not change significantly in any of the groups during the intervention. Alcohol drinking and physical activity were unchanged during the intervention in all groups.

Plasma and platelet phospholipid fatty acids

At baseline, there were no significant differences between groups in plasma and platelet phospholipid fatty acid composition. The changes in plasma (**Figure 1**) and platelet (**Figure 2**) phospholipid fatty acids in each group indicated compliance with capsule intake. There were no significant changes in fatty acid composition in the control group.

Plasma fatty acids

In plasma phospholipids, EPA supplementation increased EPA by 494% ($P < 0.01$) and docosapentaenoic acid (DPA; 22:5n−3) by 87% ($P < 0.01$), without significantly changing DHA (9% change; NS). In the DHA group, DHA and EPA increased by 167% ($P < 0.01$) and 52% (NS) respectively, whereas DPA was not affected significantly. Oleic acid (18:1n−9) concentrations were significantly decreased by both EPA (by 11%; $P < 0.01$) and DHA (by 11%; $P < 0.01$) supplementation. There was a significantly larger ($P < 0.01$) decrease in linoleic acid (18:2n−6) in the EPA group (by 21%; $P < 0.01$) than in the DHA group (by 12%; $P < 0.01$). EPA and DHA decreased arachidonic acid (20:4n−6) by 25% ($P < 0.01$) and 22% ($P < 0.01$), respectively, and decreased 20:3n−6 by approximately the same extent, 36% ($P < 0.01$) and 28% ($P < 0.01$), respectively.

Platelet fatty acids

EPA supplementation significantly increased platelet phospholipid EPA by 370% ($P < 0.01$) and DPA by 56% ($P < 0.01$), but also significantly decreased DHA by 28% ($P < 0.01$). DHA supplementation significantly increased DHA by 155% ($P < 0.01$) and EPA by 54% (NS). DPA, however, unlike in plasma phospholipids, decreased significantly by 34% ($P < 0.01$). Both EPA and DHA significantly decreased stearic acid (18:0) ($P < 0.01$), whereas only EPA decreased 20:3n−6 (by 25%; $P < 0.01$). Similar to plasma phospholipids, 20:4n−6 decreased significantly more ($P < 0.01$) after EPA (by 15%; $P < 0.01$) than after DHA (by 7%; $P < 0.01$).

Serum lipids

There were no significant differences in fasting serum lipids at baseline between groups (Table 2). Changes in fasting lipids and lipoproteins are shown in **Figures 3** and **4**. There were no significant changes in lipids with olive oil supplementation. Neither EPA nor DHA supplementation had an effect on serum total cholesterol concentrations. After adjustment for baseline values, fasting triacylglycerols decreased significantly by 18.4% with EPA ($P = 0.012$) and by 20% with DHA ($P = 0.003$), relative to the placebo group. Serum LDL cholesterol increased significantly with DHA (by 8%; $P = 0.019$), but not with EPA (by 3.5%; NS). In the EPA group, the nonsignificant 3% decrease in HDL cholesterol was attributable to a significant 6.7% reduction in HDL_3 cholesterol ($P = 0.032$) and no change in HDL_2 cholesterol. A small, albeit nonsignificant increase (3.1%) in HDL cholesterol after DHA supplementation was due to a significant increase (29%) in the HDL_2-cholesterol subfraction ($P = 0.004$) with no significant change in the HDL_3-cholesterol subfraction.

LDL particle size

LDL particle size was not significantly different between groups at baseline (Table 2). Neither olive oil nor EPA had a significant effect on LDL particle size, whereas DHA supplementation significantly increased LDL particle size ($P = 0.002$) after adjustment for baseline values (Table 2 and **Figure 5**). At baseline, LDL particle size was inversely correlated with triacylglycerol ($r = -0.58$, $P < 0.0001$) and positively correlated with

TABLE 2
Fasting serum lipids, glucose, and insulin at baseline and postintervention in the 3 groups[1]

	Olive oil (control) ($n = 20$)	EPA ($n = 19$)	DHA ($n = 17$)	Treatment effect (P)[2]	
				EPA	DHA
Cholesterol (mmol/L)					
Baseline	6.47 ± 0.21	6.20 ± 0.20	6.18 ± 0.18		
Postintervention	6.22 ± 0.10	6.16 ± 0.11	6.34 ± 0.11	−0.06 ± 0.15 (NS)	0.11 ± 0.15 (NS)
Triacylglycerols (mmol/L)					
Baseline	2.04 ± 0.19	2.01 ± 0.19	2.25 ± 0.40		
Postintervention	1.95 ± 0.10	1.58 ± 0.10	1.50 ± 0.11	−0.37 ± 0.14 (0.012)	−0.45 ± 0.15 (0.003)
LDL cholesterol (mmol/L)					
Baseline	4.41 ± 0.19	4.28 ± 0.19	4.27 ± 0.17		
Postintervention	4.31 ± 0.09	4.46 ± 0.10	4.64 ± 0.10	0.15 ± 0.13 (NS)	0.34 ± 0.14 (0.019)
LDL particle size (nm)					
Baseline	25.68 ± 0.14	25.64 ± 0.09	25.69 ± 0.11		
Postintervention	25.72 ± 0.05	25.69 ± 0.05	25.96 ± 0.06	0.03 ± 0.07 (NS)	0.25 ± 0.08 (0.002)
HDL cholesterol (mmol/L)					
Baseline	1.12 ± 0.07	1.00 ± 0.04	0.96 ± 0.04		
Postintervention	1.02 ± 0.02	0.99 ± 0.02	1.05 ± 0.02	−0.03 ± 0.03 (NS)	0.03 ± 0.03 (NS)
HDL_2 cholesterol (mmol/L)					
Baseline	0.33 ± 0.05	0.25 ± 0.02	0.24 ± 0.03		
Postintervention	0.26 ± 0.02	0.27 ± 0.02	0.33 ± 0.02	0.01 ± 0.02 (NS)	0.07 ± 0.02 (0.004)
HDL_3 cholesterol (mmol/L)					
Baseline	0.80 ± 0.03	0.74 ± 0.03	0.72 ± 0.03		
Postintervention	0.76 ± 0.01	0.72 ± 0.02	0.72 ± 0.02	−0.05 ± 0.02 (0.032)	−0.04 ± 0.02 (NS)
Glucose (mmol/L)					
Baseline	4.95 ± 0.12	5.03 ± 0.09	5.15 ± 0.13		
Postintervention	5.03 ± 0.08	5.24 ± 0.08	5.08 ± 0.09	0.21 ± 0.11 (0.062)	0.05 ± 0.12 (NS)
Insulin (pmol/L)					
Baseline	9.79 ± 1.24	8.78 ± 0.83	9.59 ± 0.99		
Postintervention	8.76 ± 0.51	10.34 ± 0.52	11.38 ± 0.55	1.58 ± 0.73 (0.035)	2.62 ± 0.74 (0.001)

[1] $\bar{x}$ ± SEM. There were no significant differences between the 3 groups at baseline (by one-way ANOVA). Postintervention values were not adjusted. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[2] A general linear model was used to assess treatment effects on postintervention values adjusted for baseline values. Data represent the change and P values for each of the 2 treatment groups relative to the olive oil group.

HDL-cholesterol ($r = 0.62$, $P < 0.0001$) concentrations. The change in LDL particle size from baseline to postintervention was inversely correlated with the change in triacylglycerols ($r = -0.35$, $P = 0.009$) and positively correlated with the change in HDL cholesterol ($r = 0.28$, $P = 0.035$). In regression analysis, DHA supplementation remained the strongest independent predictor of postintervention LDL particle size (adjusted $R^2 = 0.776$, $P = 0.002$) after adjustment for baseline values and for the change in LDL particle size (adjusted $R^2 = 0.216$, $P = 0.023$). DHA supplementation remained significant in regression models, which included changes in serum triacylglycerols and other lipids.

Glucose and insulin

At baseline there were no significant differences between groups in fasting serum glucose or insulin concentration (Table 2). Postintervention, however, there were significantly different responses between the EPA and DHA groups (**Figure 6**). Olive oil did not change either fasting glucose or insulin. After adjustment for baseline values, there was a trend toward increased fasting glucose concentrations with EPA ($P = 0.062$), but not with DHA (NS), relative to the control group. Both EPA and DHA significantly increased fasting insulin, by 18% ($P = 0.035$) and 27% ($P = 0.001$), respectively. DHA supplementation also significantly decreased the glucose-insulin ratio by 0.13 ± 0.05 ($P = 0.018$).

DISCUSSION

This study addressed whether purified EPA and DHA have different effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. We found that DHA, but not EPA, improved serum lipid status, in particular a small increase in HDL cholesterol and a significant increase in the HDL_2-cholesterol subfraction, without adverse effects on fasting glucose concentrations. Neither EPA nor DHA affected total cholesterol and both fatty acids reduced

TABLE 3
Total energy and macronutrient intakes at baseline and changes during the intervention in the 3 groups[1]

	Olive oil (control) (n = 20)	EPA (n = 19)	DHA (n = 17)
Total energy intake (kJ/d)			
Baseline	10441 ± 588	9516 ± 677	10550 ± 588
Change	−471 ± 497	82 ± 844	−188 ± 421
Total fat (% of energy)			
Baseline	34.2 ± 1.2	30.9 ± 1.6	32.6 ± 1.6
Change	−0.4 ± 1.3	2.8 ± 1.6	2.3 ± 1.2
Fatty acids (% of energy)			
Saturated fat			
Baseline	13.6 ± 0.8	12.1 ± 0.9	13.6 ± 0.9
Change	0.0 ± 0.6	1.2 ± 0.9	0.8 ± 1.0
Monounsaturated fat			
Baseline	12.2 ± 0.6	11.1 ± 0.8	11.5 ± 0.7
Change	−0.2 ± 0.6	1.4 ± 0.8	0.3 ± 0.6
Polyunsaturated fat			
Baseline	5.0 ± 0.3	4.2 ± 0.3	4.6 ± 0.2
Change	0.0 ± 0.4	0.2 ± 0.4	0.9 ± 0.5
Protein (% of energy)			
Baseline	18.0 ± 0.5	19.8 ± 0.9	18.1 ± 0.6
Change	−0.5 ± 0.6	−0.9 ± 0.8	0.1 ± 0.7
Carbohydrate (% of energy)			
Baseline	42.1 ± 1.7	44.6 ± 1.7	41.8 ± 1.8
Change	1.9 ± 1.6	−1.0 ± 1.5	−1.2 ± 1.4
Fiber (g/d)			
Baseline	30.8 ± 2.9	27.4 ± 1.8	26.1 ± 1.1
Change	−3.3 ± 2.3	−2.8 ± 2.1	−0.1 ± 1.7

[1] $\bar{x}$ ± SEM. Baseline measures were compared by one-way ANOVA. A general linear model was used to test for treatment effects on postintervention values adjusted for baseline value. There were no significant differences between the groups in any of the dietary nutrients at baseline and no significant changes during the intervention. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

triacylglycerols and increased fasting insulin concentrations to a similar extent. DHA supplementation significantly increased LDL cholesterol; however, this was associated with an increase in LDL particle size, which may represent a shift to a less atherogenic LDL particle.

Although numerous studies have examined the effect of n−3 fatty acids on serum lipids, glucose, and insulin (1–5, 20–22), few have assessed the independent effects of EPA and DHA. In vitro, both EPA (13, 33–35) and DHA (13, 35, 36) inhibit triacylglycerol synthesis and secretion. In rats, EPA lowered triacylglycerols, whereas DHA lowered cholesterol (10, 14, 15). These studies, however, used very high doses (1–2 $g \cdot kg^{-1} \cdot d^{-1}$) of fatty acids, equalling 12–24 g/d in humans.

In humans, n−3 fatty acids reduce triacylglycerols (4, 5, 37), with more variable effects on total cholesterol, LDL cholesterol, and HDL cholesterol (4, 5). These contradictory findings may be explained, in part, by variations in the amount of n−3 fatty acids consumed, the manner in which they are presented (fish, fish oils, or purified oils), and the lipoprotein phenotype of the patients. Our own studies have shown that the background dietary fat intake influences serum lipid responses to n−3 fatty acids (37).

Trials in humans using mixtures enriched in EPA and DHA have suggested different effects of the 2 fatty acids on serum lipids (38, 39). In a placebo-controlled study, 4 g EPA/d reduced triacylglycerols by 35% (40). It was also shown in a single-blind crossover study that EPA reduced triacylglycerols and VLDL cholesterol, increased LDL cholesterol and HDL cholesterol, but had no effect on total cholesterol (16). DHA did not affect cholesterol, triacylglycerols, VLDL cholesterol, LDL cholesterol, or HDL cholesterol, but increased the HDL_2-cholesterol subfraction and reduced the HDL_3-cholesterol subfraction (16). That study, however, had only a small number of subjects in the DHA group, was short in duration, and included only a 2-wk washout period between treatments (16).

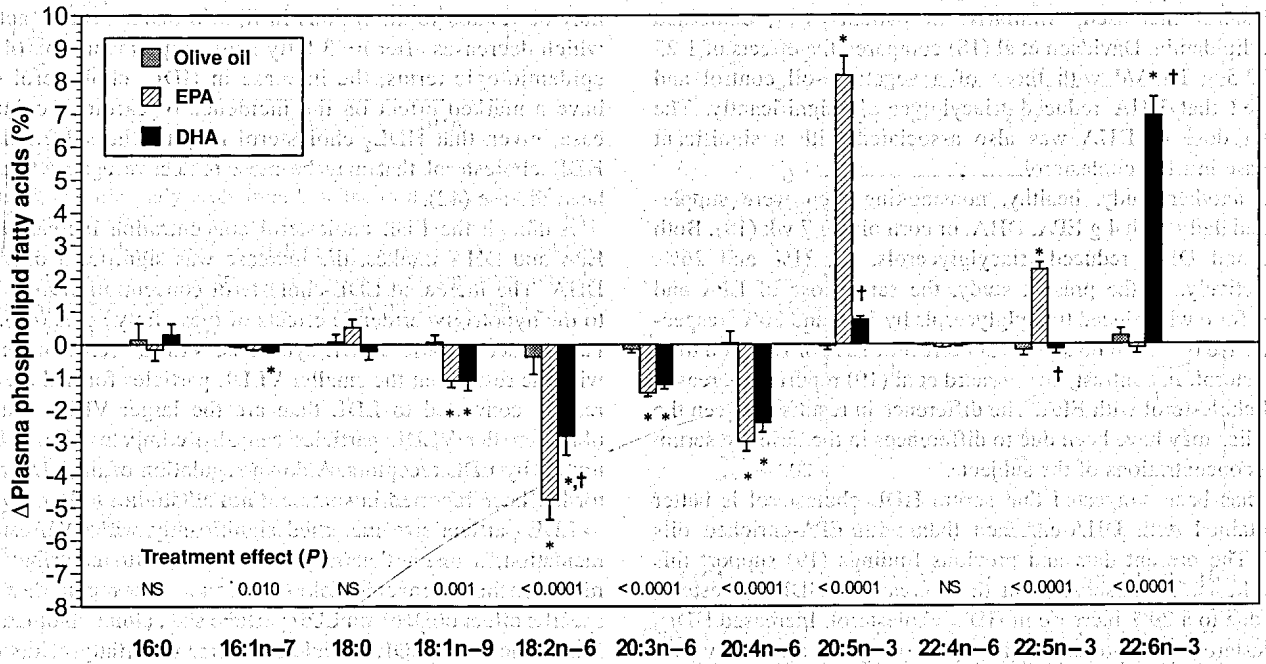


FIGURE 1. Mean (±SEM) changes in plasma phospholipid fatty acids from baseline to the end of the intervention in the olive oil (control; n = 20), eicosapentaenoic acid (EPA; n = 19), and docosahexaenoic acid (DHA; n = 17) groups. ANOVA was used to assess treatment effects. *Significantly different from the olive oil group, $P < 0.01$. †Significantly different from the EPA group, $P < 0.01$.

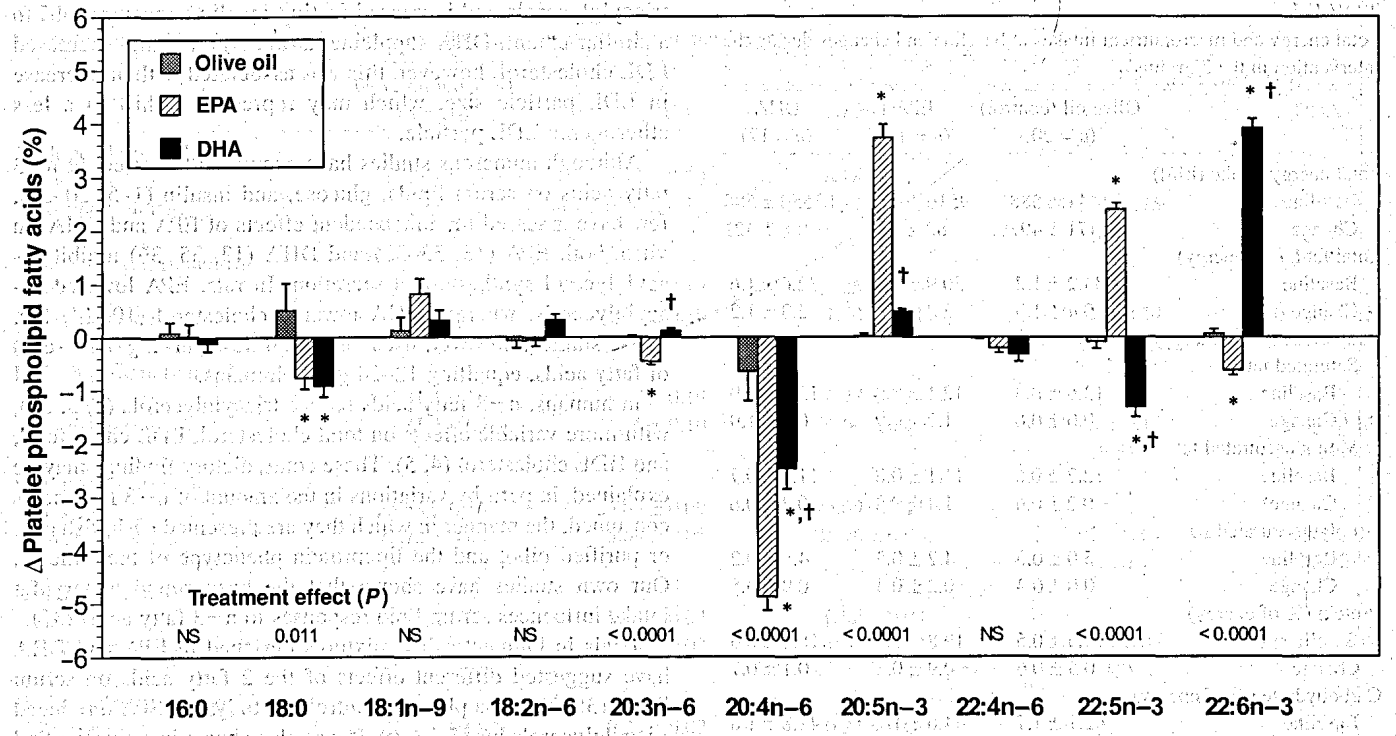


FIGURE 2. Mean (±SEM) changes in platelet phospholipid fatty acids from baseline to the end of the intervention in the olive oil (control; $n = 20$), eicosapentaenoic acid (EPA; $n = 19$), and docosahexaenoic acid (DHA; $n = 17$) groups. ANOVA was used to assess treatment effects. *Significantly different from the olive oil group, $P < 0.01$. †Significantly different from the EPA group, $P < 0.01$.

Several reports have described the effects of DHA supplements on serum lipids in humans. Nelson et al (17), in a single-blind study of healthy men, compared the effects of 6 g DHA/d with those of a control diet. They reported that after 90 d total cholesterol, LDL cholesterol, apo A-I, apo B, and lipoprotein(a) were unchanged, whereas triacylglycerols decreased and HDL cholesterol increased. Similarly, in patients with combined hyperlipidemia, Davidson et al (18) compared the effects of 1.25 and 2.5 g DHA/d with those of a vegetable-oil control and showed that DHA reduced triacylglycerols significantly. The higher dose of DHA was also associated with a significant increase in LDL cholesterol.

In another study, healthy, nonsmoking men were supplemented daily with 4 g EPA, DHA, or corn oil for 7 wk (19). Both EPA and DHA reduced triacylglycerols, by 21% and 26%, respectively. In the present study, the same dose of EPA and DHA for 6 wk reduced triacylglycerols by 18% and 20%, respectively. We observed no significant effect of EPA or DHA on total cholesterol. In contrast, Grimsgaard et al (19) reported increased total cholesterol with EPA. The difference in results between the 2 studies may have been due to differences in the baseline serum lipid concentrations of the subjects.

It has been suggested that serum HDL cholesterol is better maintained with DHA-enriched than with EPA-enriched oils (38). The present data and previous findings (19) support this hypothesis. We observed that the increase in HDL cholesterol was due to a 29% increase in HDL_2 cholesterol. Increased HDL_2 cholesterol was reported previously by our group after daily consumption of fish or fish oils by subjects with type 2 diabetes or at risk of heart disease (23, 37). In contrast, Grimsgaard et al (19) surmised that both EPA and DHA increase HDL_2 cholesterol because both fatty acids increased the ratio of HDL cholesterol to apo A-I. DHA increased HDL cholesterol and EPA decreased apo-AI, suggesting an increased surface-to-core ratio of the HDL particle and a redistribution of the HDL subclasses toward the larger HDL_2 particles (41). The mechanisms by which DHA increases HDL cholesterol are not known, but may be related to alterations in lipid transfer protein activity, which decreases after n−3 fatty acid supplementation (41). In epidemiologic terms, the increase in HDL_2 cholesterol could have a marked effect on the incidence of cardiovascular disease, given that HDL_2 cholesterol may be the subfraction of HDL cholesterol that may be most protective against coronary heart disease (42).

Although the LDL-cholesterol concentration increased after EPA and DHA intakes, the increase was significant only after DHA. The increased LDL-cholesterol concentration may relate to the hypotriglyceridemic effects of these fatty acids (43). n−3 Fats reduce hepatic VLDL synthesis, VLDL secretion, or both with the result that the smaller VLDL particles formed are more readily converted to LDL than are the larger VLDL particles (44). Smaller VLDL particles can also compete with LDL for uptake by LDL receptors. A down-regulation of the LDL receptor has been reported in some but not all studies (43).

LDL particle size increased significantly with DHA supplementation, a result that might be expected to contribute to a reduction in atherogenic risk. Ours is the first report showing a specific effect of DHA on LDL particle size, although others have shown increased LDL particle size after n−3 fatty acid supplementation (45, 46). Small, dense LDL particles are associated with an increased risk of coronary artery disease (47) and an increase in plasma triacylglycerol concentrations (48). Both

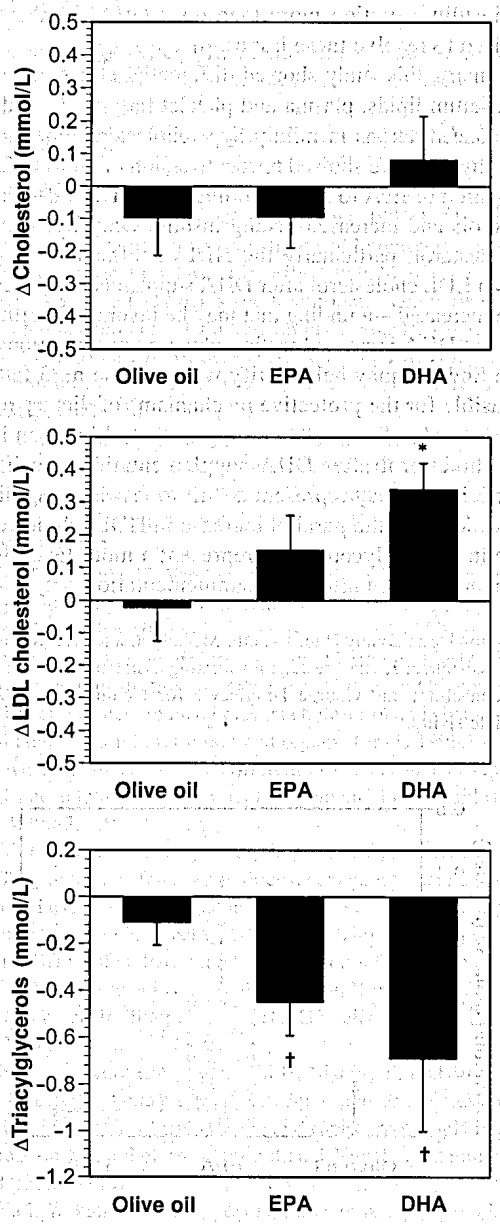


FIGURE 3. Mean (±SEM) changes in serum total cholesterol, LDL cholesterol, and triacylglycerols from baseline to the end of the intervention in the olive oil (control; n = 20), eicosapentaenoic acid (EPA; n = 19), and docosahexaenoic acid (DHA; n = 17) groups. A general linear model was used to assess treatment effects on postintervention values adjusted for baseline values. *,†Significantly different from the olive oil group: $^{*}P < 0.05$, $^{\dagger}P < 0.01$.

triacylglycerols and HDL cholesterol are major determinants of LDL particle size (49), partly because the exchange of triacylglycerols from VLDL for cholesterol ester in LDL, which is mediated by cholesteryl ester transfer protein (CETP). It is possible that as serum triacylglycerols decrease after n−3 fatty acid supplementation, fewer triacylglycerols are transferred to LDL by CETP, reducing the formation of triacyglycerol-enriched LDL, which minimizes the opportunity for lipoprotein lipase to convert large LDL particles to small LDL particles. This hypothesis is supported by reports of reduced CETP activity after n−3 fatty acid supplementation (41). Given the similarity in triacylglycerol lowering by EPA and DHA, our results may be related to a more pronounced effect of DHA on CETP activity.

Olive oil supplementation did not alter plasma or platelet phospholipid oleic acid and other fatty acid concentrations; therefore, its use as a placebo was justified (5). Supplementation with EPA increased plasma and platelet phospholipid EPA and DPA concentrations, whereas the concentration of DHA was decreased. These findings can be explained by the inhibitory effect of EPA on Δ^6-desaturase, which converts DPA to DHA (50), and confirm results of previous studies that humans do not synthesize DHA from EPA unless, perhaps, the EPA concentration is high (19).

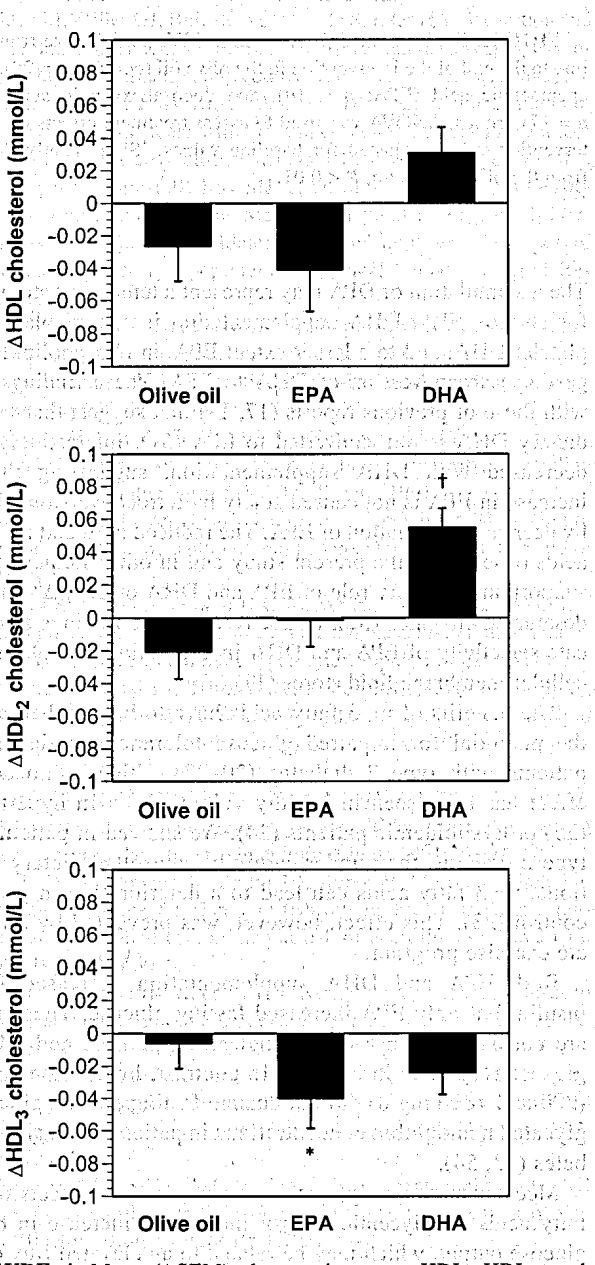


FIGURE 4. Mean (±SEM) changes in serum HDL, HDL_2, and HDL_3 cholesterol from baseline to the end of the intervention in the olive oil (control; n = 20), eicosapentaenoic acid (EPA; n = 19), and docosahexaenoic acid (DHA; n = 17) groups. A general linear model was used to assess treatment effects on postintervention values adjusted for baseline values. *,†Significantly different from the olive oil group: $^{*}P < 0.05$, $^{\dagger}P < 0.01$.

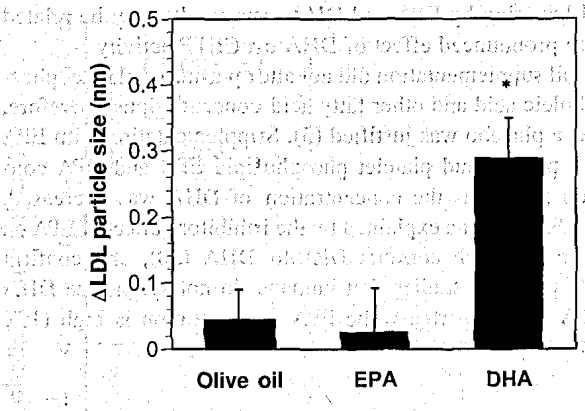


FIGURE 5. Mean (±SEM) changes in LDL particle size from baseline to the end of the intervention in the olive oil (control; $n = 20$), eicosapentaenoic acid (EPA; $n = 19$), and docosahexaenoic acid (DHA; $n = 17$) groups. ANOVA was used to assess treatment effects on postintervention values adjusted for baseline values. *Significantly different from the olive oil group, $P < 0.01$.

The accumulation of DPA may represent a temporary storage site for surplus EPA. DHA supplementation increased plasma and platelet DHA, and to a lesser extent EPA, in phospholipids, suggesting retroconversion of DHA to EPA. These findings agree with those of previous reports (17, 19) that suggest that ≈9% of dietary DHA is retroconverted to EPA (51). Interestingly, DPA decreased with DHA supplementation, suggesting that the increase in EPA is not caused solely by retroconversion, but also by decreased elongation of EPA. The reduced n−6 and n−9 fatty acids observed in the present study and in other studies (17–19) support an inhibitory role of EPA and DHA on Δ^5-, Δ^6-, and Δ^9-desaturase enzymes (52), respectively. These findings also indicate selectivity of EPA and DHA incorporation into plasma and cellular membrane lipid stores (17).

The benefits of n−3 fatty acids have to be weighed against the potential for impaired glucose tolerance, particularly in patients with type 2 diabetes (20–22), although no adverse effect has been seen in healthy volunteers or in hypertensive (25) or dyslipidemic patients (24). We showed in patients with type 2 diabetes that, under carefully controlled dietary conditions, n−3 fatty acids can lead to a deterioration in glycemic control (23). This effect, however, was prevented by a moderate exercise program.

Both EPA and DHA supplementation increased fasting insulin, but only EPA increased fasting glucose. These results are consistent with a differential effect of EPA and DHA on glucose responses in humans. In contrast, lower doses of EPA (900 and 1800 mg/d) did not change fasting plasma glucose or glycated hemoglobin concentrations in patients with type 2 diabetes (53, 54).

Mechanisms underlying the putative adverse effects of n−3 fatty acids on glycemic control include an increase in hepatic glucose output, which may be related to an elevated flux of gluconeogenic precursors to the liver, increased plasma glucagon concentrations, changes in hepatic insulin or glucagon sensitivity, or decreased insulin secretion rates (20–22). The mechanisms responsible for the increase in fasting glucose after EPA but not after DHA supplementation are not known, but may be because EPA increases hepatic glucose production or decreases hepatic insulin secretion more than does DHA. Further studies are required to resolve these issues.

In summary, this study showed differential effects of EPA and DHA on serum lipids, plasma and platelet fatty acids, and fasting glucose concentrations in mildly hypercholesterolemic but otherwise healthy men. We showed retroconversion of DHA to EPA, but not elongation of EPA to DHA. Both EPA and DHA decreased triacylglycerols and increased fasting insulin. Only DHA increased HDL cholesterol, particularly the HDL_2 subfraction. Despite an increase in LDL cholesterol after DHA supplementation, LDL particle size increased—a finding that may be favorable. Furthermore, EPA but not DHA increased fasting glucose concentrations.

These findings may help clarify which of the n−3 fatty acids is responsible for the protective mechanisms of dietary n−3 fats on cardiovascular disease. They suggest that, despite an increase in LDL cholesterol after DHA supplementation, the increased LDL particle size may represent a shift to less atherogenic particles, in which case the parallel increase in HDL_2 cholesterol and decrease in triacylglycerol may represent a more favorable lipid profile than that seen after EPA supplementation.

We acknowledge Danny Bao, Lynette McCahon, and Ken Robertson for technical assistance; Jessie Prestage for nursing assistance; Nardia Ward for dietary counseling; and George Dragicevic for technical assistance with LDL particle sizing.

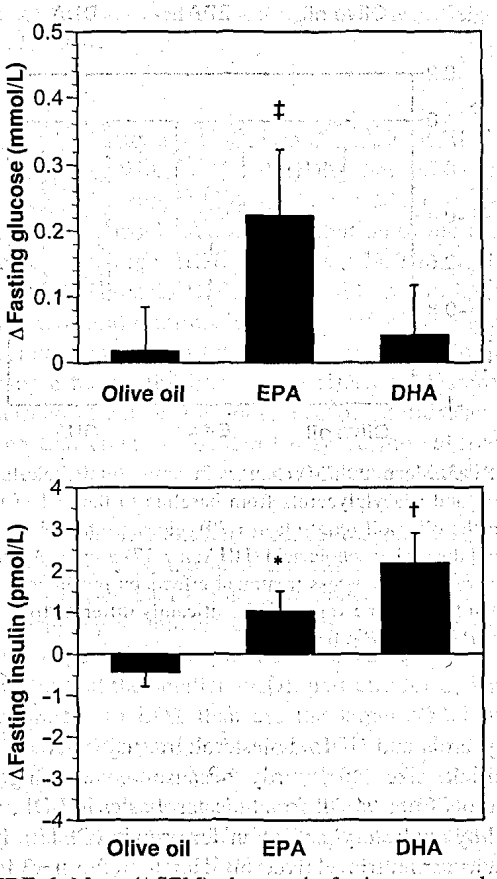


FIGURE 6. Mean (±SEM) changes in fasting serum glucose and insulin from baseline to the end of the intervention in the olive oil (control; $n = 20$), eicosapentaenoic acid (EPA; $n = 19$), and docosahexaenoic acid (DHA; $n = 17$) groups. A general linear model was used to assess treatment effects on postintervention values adjusted for baseline values. *,†,‡Significantly different from the olive oil group: *$P < 0.05$, †$P < 0.01$, ‡$P = 0.062$.

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Now in its sixtieth year of publication, this journal serves as a forum for the scientific discussion of vitamin and nutrition research from around the world. The primary emphasis lies in the high scientific quality of the articles submitted for publication. Manuscripts dealing with experimental as well as epidemiological vitamin and nutrition research are welcome. Also papers related to deficiency syndromes, interactions of food stuffs, pharmacology of nutrition, nutrition dependent health risks or dealing with clinical nutriton and dietetics are gladly accepted. New analytical methods inthe general area of nutrition can also be considered. However, articles in the area of animal husbandry, poultry science as well as papers dealing with molecular mechanisms of biological reactions do not have first priority.
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Contents (continued from outside cover)

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Eicosapentaenoic acid
Hypercholesterolemia
Cholesteryl ester transfer protein
Low density lipoprotein
High density lipoprotein

Effects of Purified Eicosapentaenoic Acid Ethyl Ester on Plasma Lipoproteins in Primary Hypercholesterolemia

Shuichi NOZAKI, Yuji MATSUZAWA, Ken-ichi HIRANO, Naohiko SAKAI, Masaharu KUBO, Seiichiro TARUI

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Summary: We investigated the effects of purified eicosapentaenoic acid (EPA) ethyl ester capsules (90% purity), which are free from cholesterol, saturated fatty acids and docosahexaenoic acid (DHA), on plasma lipoproteins and cholesteryl ester transfer protein (CETP) activity. We administered 2.7 g of EPA per day as capsules for 6 months to 14 primary hypercholesterolemic subjects. Total cholesterol, triglyceride and low density lipoprotein (LDL)-cholesterol levels in plasma were significantly reduced. The LDL cholesterol/apoB ratio and LDL particle size did not change. The ratio of high density lipoprotein (HDL)2/HDL3 cholesterol increased from 1.04 to 1.35 ($p<0.05$), while the HDL cholesterol level did not change. CETP activity was significantly reduced. The reduction of CETP activity may explain the increase in the HDL2/HDL3 cholesterol ratio. These results suggest that purified EPA not only reduces LDL cholesterol levels but also acts on HDL metabolism in patients with hypercholesterolemia and therefore will be useful for the treatment of hypercholesterolemia.

Abbreviations used: Cholesteryl ester transfer protein, CETP; docosahexaenoic acid, DHA; eicosapentaenoic acid, EPA; low density lipoprotein, LDL; high density lipoprotein, HDL; hepatic triglyceride lipase, HTGL; very low density lipoprotein, VLDL.

Introduction

Since it has been suggested [1] that high content of eicosapentaenoic acid (EPA) in diet is linked to low incidence of coronary heart disease in Greenland Eskimos, many studies reported that EPA has a variety of beneficial effects protecting against atherosclerotic disease [2–8]. The effect of EPA on plasma lipoproteins has been studied [9] since lipoprotein abnormalities are an important factor in the development of atherosclerosis.

In previous studies, fish oil has been used since purified EPA has not been available. These studies have confirmed the reduction of serum triglyceride and very low density lipoprotein (VLDL) concentrations by fish oil [9, 10]. There is still controversy concerning the effects of fish oil on low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol levels [9]. Fish oil conventionally used contains at most 25% EPA in addition to various amount of other fatty acids including docosahexaenoic acid (DHA) and cholesterol [2].

The insufficient purity of fish oil may be one of the reasons for inconsistent data about the effects of fish oil on LDL and HDL cholesterol levels. Furthermore, it is suggested that EPA and DHA have different properties against lipoprotein metabolism [11]. In our previous study, we reported the effect of a capsule containing 80% pure EPA on platelet and plasma

lipids, showing a substantial reduction in total plasma cholesterol level [12]. However even this capsule still contained much DHA and we could not differentiate the effects of EPA and DHA.

This report describes the effect of purified EPA ethylester, recently developed in Japan, on concentrations and composition of plasma lipoproteins in primary hypercholesterolemia.

Materials and Methods

Subjects: Fourteen patients (11 females and 3 males) with primary hypercholesterolemia were studied as outpatients. The patients were selected from the Lipid Clinic of Osaka University Medical School. Familial hypercholesterolemia was excluded. We further excluded patients with diabetes mellitus, renal disease, and liver disease. Their ages ranged from 36 to 65 years old (mean±SD, 55±8.8 years). Their body mass indexes averaged 22.9±2.2 kg/m^2. Patients did not take any medicine affecting lipid metabolism for at least 2 months before the start of this study. Eight of the patients had type IIa hyperlipidemia and 6 of them had type IIb. Their mean total cholesterol levels and triglyceride levels were 277±30 and 165±25 mg/dl respectively. The mean HDL cholesterol level was 52±13 mg/dl.

Study protocol: The subjects were instructed to take 20% fat diet on the entry of this study. It was confirmed that their serum lipids levels were plateau at least for 2 months before the onset of treatment with EPA ethyl ester. They were also instructed to maintain this diet throughout the study. The subjects took 2.7 g of EPA ethyl ester daily for 6 months. The encapsulated EPA ethyl ester was provided by Mochida Pharmaceutical Company. The EPA used in this study was purified from sardine oil. Each capsule contained over 90% EPA ethyl ester. The content of DHA (C22:6) was less than 1% and no cholesterol was present. This EPA capsule contains 0.2% vitamin E to inhibit peroxidization in capsule and in vivo. Venous blood samples were obtained before and during the study period. All patients gave informed consent to participate in the investigation.

Lipoprotein analyses: Venous blood was drawn in a glass tube with EDTA 3.5 mM (final concentration) from patients after overnight fasting. Total cholesterol and triglycerides were measured by an enzymatic method (Kyowa Medex Co., Tokyo, Japan). HDL cholesterol was measured by the heparin manganese precipitation method [13]. Total protein was measured in the LDL fraction [14]; it was assumed to consist entirely of apoB100. Very low density lipoprotein (VLDL, d<1.006 g/ml), intermediate density lipoprotein (IDL, 1.006<d<1.019 g/ml), low density lipoprotein (LDL, 1.019<d<1.063 g/ml), high density lipoprotein 2 (HDL2, 1.063,d<1.125 g/ml) and high density lipoprotein 3 (HDL3, 1.125<d g/ml) were separated by sequential preparative ultracentrifugation according to the method of Havel [15] as we previously reported [16]. LDL size was estimated using 2–16% polyacrylamide gradient gel electrophoresis according to the method of KRAUSS [17]. Size was determined by scanning each lane with a densitometer and comparing this value to a standardized curve of compounds of known size as we previously reported [18]. Cholesteryl ester transfer protein (CETP) activity was measured by ALBERS' method [19] as we previously reported [20]. Briefly, the activity in the patient's plasma is assayed by examining the transfer of carbon-14 cholesteryl ester from HDL3 to VLDL and LDL. The CETP activity was expressed as the percentage of donor cholesteryl ester transferred to LDL.

Fatty acids analyses: Total plasma fatty acids were extracted with chloroform methanol solution (2/1,v/v). The fatty acids were analyzed as their methyl esters by gas chromatography using Yokogawa Hewlett Packard HP-5731 before and 6 months after EPA administration. Plasma fatty acids composition was calculated as percentage of total mass.

Plasma peroxide analysis: Plasma peroxide levels were measured before and after 6 months of EPA treatment by using Determiner LPO kit (Kyowa Medex Co., Tokyo, Japan), which depends on the method of OHISHI and YAGI *et al* [21].

Statistical analysis: The significance of differences between mean values was determined by paired t-test.

Results

EPA concentrations of serum fatty acids are increased in all subjects (data non shown) and the mean plasma EPA concentration of 14 subjects was significantly increased from 2.8% to 9.7% after EPA administration as shown in Table I.

The change of plasma levels of total cholesterol, triglyceride and HDL cholesterol before and after EPA administration are shown in Table II. Total cholesterol levels were significantly reduced by 10%. The triglyceride levels were also significantly decreased by 16%. The HDL cholesterol levels did not significantly change.

Lipoprotein fractionation was done before and after EPA administration (Tab. III). Mean VLDL triglyceride levels decreased by 18%. The levels of IDL cholesterol and triglyceride were not changed. A significant reduction in LDL cholesterol was noted ($p<0.05$). The LDL apolipoprotein (apo) B100 levels were significantly reduced ($p<0.05$). The LDL cholesterol

Table I: Changes of serum fatty acids compositions after EPA administration

	Before	After EPA
C 14:0	1.15±0.39	1.01±0.36
C 16:0	24.9 ±2.42	23.7 ±2.74
C 16:1 n-7	4.17±1.06	3.60±1.35*
C 18:0	5.35±0.46	5.90±0.64**
C 18:1 n-9	20.3 ±2.05	18.2 ±2.82**
C 18:2 n-6	30.0 ±4.09	25.3 ±4.30**
C 18:3 n-3	0.88±0.36	0.84±0.36
C 20:0	0.11±0.06	0.13±0.04
C 20:1 n-9	0.16±0.06	0.16±0.05
C 20:2 n-6	0.18±0.04	0.13±0.02**
C 20:3 n-9	0.79±0.26	0.51±0.16**
C 20:4 n-6	4.68±1.10	4.96±1.01
C 22:0	0.08±0.06	0.12±0.07
C 20:5 n-3	2.88±1.47	9.75±3.13**
C 22:5 n-3	0.50±0.14	1.36±0.37**
C 22:6 n-3	3.86±0.90	4.26±1.06

Each value shows percent of total fatty acids and presented as mean±SD.
*: $p<0.05$, **: $p<0.01$, Significantly different from before EPA administration

Table II: Effects of EPA administration on plasma lipids

		Before	After EPA administration
Total cholesterol	mg/dl	277±30	249±28* (−10%)
Triglyceride	mg/dl	165±25	139±27* (−16%)
HDL-cholesterol	mg/dl	52±13	50±12

Values are presented as mean±SD
* Significantly different from before EPA administration at $p<0.05$ (student's t-test; paired)

Table III: Changes of lipid levels, ratio of high density lipoprotein subfractions and CETP activities before and after EPA administration

		Before	After	p value
VLDL	CH (mg/dl)	29.8 ±14.2	25.4 ±18.1	ns
	TG (mg/dl)	106.5 ±42.6	87.0 ±45.0	<0.05
IDL	CH (mg/dl)	10.8 ± 7.9	11.8 ± 8.3	ns
	TG (mg/dl)	9.3 ± 5.7	9.8 ± 6.3	ns
LDL	CH (mg/dl)	185.4 ±49.5	156.2 ±43.2	<0.01
	TG (mg/dl)	37.6 ±10.5	34.0 ±13.0	ns
	apoB (mg/dl)	142.2 ±27.9	122.5 ±27.9	<0.05
HDL2	CH (mg/dl)	25.2 ±11.2	28.8 ±11.5	ns
HDL3	CH (mg/dl)	26.1 ± 6.9	21.9 ± 4.4	ns
HDL2-CH/ HDL3-CH		1.04± 0.6	1.35± 0.53	<0.05
CETP activity (%)		29.0 ± 6.3	24.2 ± 5.5	<0.05

Values are presented as mean±SD.
Abbreviations: CH, cholesterol; TG, triglyceride; CETP, cholesteryl ester transfer protein activity; ns, not significant

to apo B ratio did not change (1.2±0.11 vs. 1.19±0.18, before and after treatment of EPA, respectively. There was no statistically significant relationship between the increase of EPA concentration and the decrease of LDL cholesterol level.

There was an increase in HDL2 cholesterol (from 25.2±11.2 mg/dl to 28.8±11.5) and decrease in HDL3 cholesterol (from 26.1±6.9 mg/dl to 21.9±4.4), although the changes did not reach statistical significance. There was a significant increase in the HDL2/HDL3 cholesterol ratio (from 1.04±0.6 to 1.35±0.53, $p<0.05$).

LDL fractions were analyzed by 2-16% PAG gradient electrophoresis. EPA treatment did not cause any change in the electrophoretic mobility of LDL before (267Å±8.1) and during EPA (268Å±8.8), suggesting the size of LDL particles had not changed.

CETP activities were significantly decreased from 29.0±6.3% to 24.2±5.5%, $p<0.05$, (Tab. III).

The weight percent chemical compositions of LDL before and after EPA administration is shown in Figure 1. No statistically significant changes could be observed.

There was no significant increase of plasma peroxide level before and after 6 months of EPA treatment (mean±SD: 1.2±0.24 to 1.1±0.31 nmol/ml, before and after).

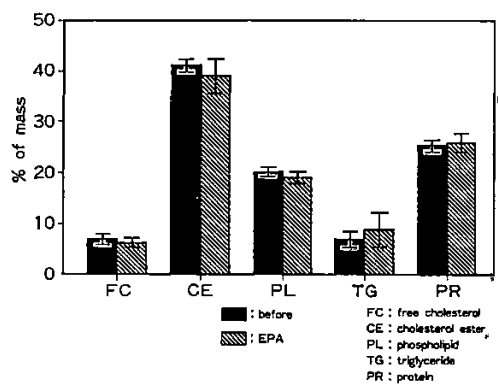

Figure 1: Chemical compositions of LDL before and after EPA administration.

Discussion

We demonstrated that purified EPA reduced plasma LDL cholesterol levels in primary hypercholesterolemic patients.

It has been confirmed that n-3 fatty acids consistently reduce serum triglyceride levels [9, 10]. However, the results from the various studies regarding the effect of n-3 fatty acids on serum cholesterol levels have been inconsistent [9]. The inconsistency of the results among the reports concerning the effect of fish oil on LDL levels might be, in part, relative to the fact that the subjects with different LDL composition show different responses to fish oil [22]. Fish oil had been used as a preparation containing much n-3 fatty acids since purified EPA had not been available. While, fish oil contains much cholesterol and fatty acids other than EPA. It also contains much DHA, another major n-3 fatty acid. Furthermore, the quality of fish oil is different in each study. This heterogeneity of fish oil used in those studies may be another cause of the inconsistency of the results regarding the effect of fish oil on LDL levels. We previously reported the reduction of LDL cholesterol by administration of the capsules which contain 80% EPA, but as this capsule also contained much DHA, we could not ascribe this effect to EPA alone [12].

In the present study, EPA itself reduced VLDL and LDL levels and the reduction in LDL cholesterol levels was associated with the reduction in apo B100 levels. The cholesterol/apo B100 ratio of LDL did not change. These results suggest that the number of LDL particles is reduced by EPA treatment.

The present study also demonstrated that EPA treatment resulted in the increase of HDL2/HDL3 cholesterol ratio. We also measured CETP activity as an important factor in HDL and LDL metabolism and found a reduction in CETP activity. This finding is well in accordance with the recent observations made in a study where fish oil was used as a supplement [23]. Patients with absent or markedly reduced CETP activities have increased HDL cholesterol, mainly associated with HDL2 cholesterol [24]. Patients with heterozygous CETP deficiency have CETP levels between those of normal persons and those with homozygous deficiency and also have an increased HDL2/HDL3-cholesterol ratio [20], indicating that even a partial reduction in CETP activity is associated with changes in distribution of HDL subfractions. Lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) are well known to play an important role in HDL metabolism. We and other researchers [25, 26] reported no change of LPL and HTGL activity in either hypertriglyceridemia or normolipidemia using fish oil, suggesting LPL and HTGL might not be responsible for the increase in the HDL2/HDL3 cholesterol ratio. As these studies were done using fish oil, further study of the effect of pure EPA on LPL and HTGL will be needed. Increased catabolism of VLDL particles due to a change in VLDL composition may also be related to the increase of the HDL2/HDL3 cholesterol ratio [27, 28]. In the patients studied, there was an increase in TG content and a decrease in the CE content of LDL although these changes did not reach statistical significance. The reduction of CETP activity may explain, in part, the change of LDL composition since LDL from CETP deficient patients is TG rich and CE poor as we reported [29]. There is, recently, much concern about feeding large amounts of highly unsaturated fatty acids such as EPA because this may increase the risk of lipid peroxidation. In order to reduce the problem of excess peroxidation in vivo, the capsule used in this study contains 0.2% vitamin E. This amount of vitamin E did not produce any increase of peroxide at least for 1 year in the capsule. Further, as studied in this study, the plasma peroxide levels were not increased. From these observations, we believe this EPA capsule is safe for use even for relatively long period, 6 months.

Regarding to the study design, although this study was not placebo-controlled study, their serum lipids levels were confirmed to be plateau before the entry (data not shown) and their good compliance to the EPA capsule administration was ascertained by the increase of plasma EPA levels (Tab. III). These observations suggest that the results obtained in this study was due to the effect of EPA administration.

In conclusion, the reduction in LDL cholesterol level and the change in HDL subfractions by EPA will provide clinical benefits and pure EPA capsules will be useful in the treatment of primary non-familial hypercholesterolemia.

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Purified Eicosapentaenoic Acid Reduces Small Dense LDL, Remnant Lipoprotein Particles, and C-Reactive Protein in Metabolic Syndrome

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Eicosapentaenoic acid (EPA), one representative of n-3 unsaturated fatty acids (n-3 PUFAs), is clinically used for its lipid-lowering effects (1). n-3 PUFAs were shown to exert various physiological functions such as antiplatelet actions (by antagonizing effects of arachidonic acid) and plaque stabilization (2,3). Several epidemiological studies have explored antiatherogenic and cardioprotective effects of n-3 PUFA that are abundantly contained in fish oil (4). Dyslipidemia accompanying the metabolic syndrome is often associated with elevated levels of remnant lipoprotein particles and small dense LDL (sdLDL), which are newly recognized risk factors for cardiovascular disease (CVD) (5). It was reported that fish oil improved lipoprotein subclass profiles in subjects with an atherogenic lipoprotein phenotype (6). Besides EPA, docosahexaenoic acid and cholesterol are present in fish oil (7), but it is not clear whether purified EPA independently affects lipoprotein subclass profiles. Therefore, we used purified EPA ethyl ester and examined effects of EPA on atherogenic sdLDL particles and remnant lipoprotein particles in the metabolic syndrome, a precursor of CVD. Furthermore, sdLDL has been reported to synergistically interact with inflammation in pathophysiologic processes leading to CVD (8). Therefore, we simultaneously measured effects of EPA on C-reactive protein (CRP), a marker of inflammation, and examined how alteration of lipoprotein profiles by EPA affects systemic inflammation.

RESEARCH DESIGN AND METHODS

— A total of 44 Japanese obese type 2 diabetic patients were recruited in our clinics (Table 1). All patients satisfied the definition and diagnostic criteria of the metabolic syndrome proposed by the National Metabolic Syndrome Criteria Study Group of Japan in 2005 (9). Accordingly, an individual is diagnosed with metabolic syndrome if he or she has central adiposity plus two or more of the following three factors: *1*) raised concentration of triglycerides (≥150 mg/dl) or reduced concentration of HDL cholesterol (<40 mg/dl); 2) raised blood pressure: systolic blood pressure (≥130 mmHg) or diastolic blood pressure (≥85 mmHg) or treatment of previously diagnosed hypertension; and 3) raised fasting plasma glucose concentration (≥110 mg/dl). The thresholds for waist circumference to define central adiposity were ≥85 cm for men and ≥90 cm for women. The study protocol was approved by the ethical committee on human research of the Kyoto Medical Center, and all participants gave written informed consent. Patients were assigned to one of the following treatment groups (a single-blind and a run-in period randomization, which patients received): *1*) treated for 3 months with either diet alone (the control group) (8 men and 14 women; mean ± SE age 51.6 ± 3.2 years) or 2) diet plus EPA (1.8 g daily) (the EPA group) (8 men and 14 women; mean age 51.6 ± 2.8 years). The subjects in the EPA group received an EPA capsule containing highly purified (>98%) EPA ethyl ester. Patient's diets are based on the Japan Atherosclerosis Society Guidelines for Diagnosis and Treatment of Atherosclerotic Cardiovascular Diseases, consisting of 25 kcal/kg of ideal body weight per day (60% of total energy as carbohydrates, 15–20% as protein, and 20–25% as fat with the ratio of polyunsaturated, monounsaturated, and saturated fatty acids being 3:4:3). Lipid-lowering medications such as statins and fibrates were excluded.

At the beginning and at the end of the study, we measured BMI, serum levels of EPA and arachidonic acid, and glycolipid parameters according to standard procedures. Remnant lipoprotein particle (RLP) cholesterol and RLP triglycerides were measured using an assay kit (Japan Immunoresearch Laboratories, Takasaki, Japan) (10). Plasma cholesteryl ester transfer protein (CETP) activity was measured using an assay kit (BioVision, Mountain View, CA) (11). LDL cholesterol subfractions were separated using the Quantimetrix Lipoprotein LDL system (12). According to the specific subfractions of LDL cholesterol obtained by this system (LDL3–7:sdLDL), the sdLDL proportion was defined as the percentage of sdLDL over the whole amount of LDL (13). Plasma level of CRP was measured

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Abbreviations: CETP, cholesteryl ester transfer protein; CRP, C-reactive protein; CVD, cardiovascular disease; EPA, eicosapentaenoic acid; n-3 PUFA, n-3 unsaturated fatty acid; RLP, remnant lipoprotein particle; sdLDL, small dense LDL.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—*Baseline characteristics and effects of EPA on metabolic parameters, lipoprotein profiles, and CRP*

	Control		EPA	
	Before	3 months	Before	3 months
BMI (kg/m^2)	29.2 ± 0.91	29.1 ± 0.92	31.0 ± 1.22	30.8 ± 1.26
EPA (μg/ml)	79.2 ± 17.8	64.6 ± 11.6*	77.2 ± 11.5	125 ± 12.3†‡
Arachidonic acid (μg/ml)	145 ± 12.1	148 ± 8.08	165 ± 7.34	152 ± 9.72†
EPA/arachidonic acid	0.52 ± 0.10	0.44 ± 0.08*	0.48 ± 0.07	0.88 ± 0.11†‡
FPG (mmol/l)	6.88 ± 0.47	7.16 ± 0.62	6.16 ± 0.57	6.00 ± 0.52
A1C (%)	6.57 ± 0.25	6.47 ± 0.26	6.11 ± 0.20	6.02 ± 0.24
Insulin concentration (pmol/l)	101 ± 14.5	100 ± 12.0	104 ± 23.1	95.4 ± 15.1
Total cholesterol (mmol/l)	5.38 ± 0.21	5.30 ± 0.24	5.46 ± 0.19	5.15 ± 0.16
LDL cholesterol (mmol/l)	3.18 ± 0.16	2.97 ± 0.19	3.44 ± 0.15	3.18 ± 0.15
HDL cholesterol (mmol/l)	1.46 ± 0.07	1.38 ± 0.11	1.42 ± 0.08	1.39 ± 0.07
Triglyceride (mmol/l)	1.89 ± 0.19	1.62 ± 0.21	1.93 ± 0.20	1.57 ± 0.11
RLP cholesterol (mmol/l)	0.19 ± 0.02	0.16 ± 0.03	0.19 ± 0.02	0.15 ± 0.01
RLP triglyceride (mmol/l)	0.44 ± 0.06	0.58 ± 0.16	0.43 ± 0.09	0.26 ± 0.02*§
CETP activity (nmol · ml^{-1} · h^{-1})	203 ± 1.47	202 ± 2.26	205 ± 3.05	200 ± 1.75*
sdLDL (mmol/l)	0.26 ± 0.08	0.23 ± 0.08	0.23 ± 0.04	0.18 ± 0.04†
sdLDL proportion (%)	7.33 ± 2.18	6.82 ± 2.36	6.78 ± 1.28	5.51 ± 1.09†
CRP (mg/dl)	0.11 ± 0.03	0.10 ± 0.03	0.22 ± 0.08	0.08 ± 0.02*

Data are means ± SE. *$P < 0.05$, †$P < 0.01$ vs. before determined by two-tailed, paired *t* test. ‡$P < 0.01$, §$P < 0.05$ vs. control determined by Student's *t* test. FPG, fasting plasma glucose.

by the latex-enhanced assay using the particle-enhanced technology performed on the Behring BN nephelometer (Dade Behring, Marburg, Germany) (14). Data are presented as mean ± SE, and $P < 0.05$ was considered statistically significant. Repeated-measures ANOVA (control and EPA groups × before and after treatment) was used to access the comparative effects of EPA treatment on the measured variables. A two-tailed, paired *t* test was applied for the evaluation of changes from baseline conditions to those at 3 months. Comparisons of the means between the two groups at baseline or posttreatment were performed by Student's *t* test. All statistical analyses were performed using the Stat View program version 5.0 for Windows (SAS Institute, Cary, NC).

RESULTS — There were no significant differences between the control and EPA groups for all measured variables at baseline (Table 1). Treatment with EPA significantly increased EPA and EPA/arachidonic acid levels, while it decreased arachidonic acid levels compared with baseline levels ($P < 0.01$). Differences of EPA and EPA/arachidonic acid levels at 3 month were observed between the control and EPA groups ($P < 0.01$). EPA also caused significant overall effects on RLP triglyceride, CETP activity, sdLDL, and the proportion of sdLDL and CRP by repeated-measures ANOVA. There were also significant reductions in values compared with baseline by paired *t* test, despite no changes in BMI, fasting plasma glucose, A1C, insulin concentration, and HDL cholesterol in both groups. Significant reductions of total cholesterol, LDL cholesterol, triglycerides, and RLP cholesterol in the EPA group was observed ($P = 0.035$, 0.004, 0.047, and 0.035, respectively) by two-tailed, paired *t* test, although there were no significant overall effects on those parameters by repeated-measures ANOVA. Increases in EPA/arachidonic acid for 3 months inversely correlated with decreases in RLP cholesterol, sdLDL, and CRP for 3 months ($P = 0.0379$, 0.0479, and 0.0467, respectively). Furthermore, reduction in CRP with EPA treatment for 3 months showed a significant positive correlation with reductions in RLP cholesterol and sdLDL for 3 months ($P = 0.0075$ and 0.0142, respectively).

CONCLUSIONS — This study is the first to demonstrate that EPA significantly reduces serum sdLDL and CRP in the metabolic syndrome. Reduction of sdLDL by EPA treatment in this study is believed to be due to a suppression of triglycerides production in the liver by EPA. In addition, since CETP is an important enzyme in cholesterol metabolism—responsible for the transfer of cholesteryl esters from HDL to LDLs (11)—degradation of CETP activity by EPA treatment may also have contributed to the decrease in the generation of remnants and sdLDL. Furthermore, we detected that reductions in RLP cholesterol and sdLDL also correlated with a decrease in CRP by EPA, which was consistent with a previous report (8) showing that LDL particle size had a strong inverse association with CRP. Atherogenic sdLDL particles are susceptible to oxidative modifications; then, oxidized LDL is easily taken into macrophages through damaged endothelial cells, thereby inducing inflammation and early atherosclerotic lesions (5,15). On the other hand, CRP has also been shown to accelerate LDL modifications during inflammatory processes (8). These findings suggest that EPA may be capable of preventing the progression of atherosclerosis in the metabolic syndrome by suppressing reciprocal interactions of atherogenic lipoproteins and inflammation. There are several reports demonstrating that n-3 PUFA does not decrease CETP protein mass and CRP (16,17). This may be caused by the differences outlined in the research designs and methods of each report.

Recently, the Japan EPA Lipid Intervention Study reported that EPA provided further benefits in preventing major coronary events without changing reductions in LDL cholesterol levels (18). Considering the improvements in lipoprotein profiles by EPA in this study, EPA may exert cardioprotective effects not by changing the quantity but by improving the quality of LDL cholesterol.

Collectively, the present study is the first to demonstrate that purified EPA re-

duces sdLDL, remnants, and CRP, thereby potentially leading to the reduction in development of atherosclerosis and CVD in the metabolic syndrome.

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ATTACHMENT 6a

Title
Diabetes care.

Creation Date
1978

Publisher
New York : American Diabetes Association.
v. 1- Jan./Feb. 1978-

Format
volumes : illustrations ; 28 cm

Language
English

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Diabetes -- Rehabilitation -- Periodicals

MESH subjects
Diabetes Mellitus

Contributor
American Diabetes Association.

Additional Info
Issued also in electronic format.
The American Diabetes Association clinical practice recommendations are published yearly 1990- as suppl. 1 or 2.

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680 __ |**i** Here are entered works on diabetes in general as well as works specifically on insulin-dependent diabetes.
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360 __ |**i** subdivision |**a** Rehabilitation |**i** under classes of persons and ethnic groups; and subdivision |**a** Patients--Rehabilitation |**i** under individual diseases, e.g. |**a** Cancer--Patients--Rehabilitation

680 __ |**i** Here are entered general works on the means and methods used to improve or restore an individual's impaired ability to lead a normal life. Works on the physical, medical, psychiatric, educational or vocational methods of rehabilitating specific groups of persons are entered under specific headings, with subdivision |**a** Rehabilitation, |**i** e.g. |**a** People with disabilities--Rehabilitation; Criminals--Rehabilitation.

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ATTACHMENT 7

(Shinozaki)

Journal of Atherosclerosis and Thrombosis Vol. 2, No. 2

The Long-Term Effect of Eicosapentaenoic Acid on Serum Levels of Lipoprotein (a) and Lipids in Patients with Vascular Disease

Koji Shinozaki, Jun-ichi Kambayashi, Tomio Kawasaki, Yoshio Uemura, Masato Sakon, Eiichi Shiba, Takashi Shibuya, Takashi Nakamura, and Takesada Mori

Department of Surgery II, Osaka University Medical School, Osaka, Japan.

The effects of eicosapentaenoic acid (EPA) on serum lipoprotein (a) (Lp(a)) and other lipid levels in patients with vascular disease were examined. The serum levels of Lp(a), total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were measured in 24 patients with vascular disease. An elevated serum Lp(a) level (39±22 mg/dl) was noted in 9 patients, elevated total cholesterol level (263±31 mg/dl) in 12 patients, elevated triglyceride level (240±98 mg/dl) in 10 patients and elevated LDL level (651±88 mg/dl) in 6 patients before administration of EPA. EPA (1,800 mg/day) was given to these patients for long periods ranging from 6 to 24 months. The serum levels of Lp(a), TC, TG and LDL were lowered significantly ($p<0.05$) after EPA administration for 12 and 18 months, for 6, 12, 18 and 24 months, for 18 months and for 12 and 18 months, respectively. These findings indicated that long-term administration of EPA may lower Lp(a) and serum lipids, which is beneficial for patients with various arterial diseases in terms of preventing progression of the disease. ***J Atheroscler Thromb, 1996; 2: 107-109.***

Key words: Atherosclerosis, Oral Administration, Total cholesterol, Triglyceride

Eicosapentaenoic acid (EPA) has been suggested to prevent arterial thrombosis and development of atherosclerosis by altering lipid metabolism in addition to its known antiplatelet effect (1). Lipoprotein (a) (Lp(a)), described by Berg in 1963, is a variant of low-density lipoprotein (LDL), which has been reported to be correlated with an increased risk of atherosclerotic vascular disease (2). A high concentration of Lp(a) in plasma has been reported to be an independent risk factor for acute myocardial infarction and to be increased in patients with peripheral vascular diseases (3). Some studies have suggested that the Lp(a) level is under tight genetic control and is not influenced by diet (4). However, nicotinic acid and stanozolol have been reported to lower the Lp (a) level (5, 6).

We examined whether the long-term administration of EPA influenced the serum levels of Lp (a) and other lipids in patients with vascular disease. In this study, the serum levels of Lp (a), total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were measured in patients with arteriosclerosis obliterans (ASO), Buerger's disease (TAO) and abdominal aortic aneurysm (AAA) before and after administration of EPA.

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Methods

The subjects were 24 patients with vascular disease who visited our clinic from February 1991 to March 1992. The 24 patients were comprised of 21 men and 3 women aged 38-75 years. Sixteen patients with ASO, 6 patients with TAO and 2 patients with AAA received oral administration of 1,800 mg/day of EPA (Epadel, Mochida Pharmaceutical Co., Tokyo, Japan). Informed consent was obtained from all patients before administration of EPA. The serum levels of Lp(a) and various lipid parameters including TC, TG, LDL and VLDL were measured before and 6, 12, 18 and 24 months after the administration of EPA. Blood samples were obtained under strict fasting and serum was obtained by centrifugation of the blood at 1,200×g for 5 min. The determination of Lp(a) was performed in the Biochemical

Laboratory (Sumitomo Metal Bio-Science, Inc., Tokyo, Japan) by enzyme immunoassay (7). A Tint Elisa Lp(a) determination kit (Biopool AB, Umea, Sweden) was used. The principle of the ELISA is based on the sandwich technique in which two monoclonal antibodies react with different antigenic determinations on the apo(a) molecule. Blood Lp(a) level was diagnosed as elevated when determined to be above 20 mg/dl. Measurement of LDL and VLDL was performed by turbidometric assay using sodium heparin. The values of blood lipid levels were diagnosed as elevated when TC, TG, LDL and VLDL were above 221, 151, 571 and 410, respectively. Statistical analysis was performed using the paired Wilcoxon test to compare pre- and post-treatment values.

Results

Of the 24 patients studied, an elevated serum Lp(a) level of more than 20 mg/dl (39±22 mg/dl) was noted in 9 patients (7 in ASO, 1 in TAO and 1 in AAA), elevated TC level of more than 220 mg/dl (263±31 mg/dl) in 12 patients (9 in ASO and 3 in TAO), elevated TG level of more than 150 mg/dl (240±98 mg/dl) in 10 patients (7 in ASO, 2 in TAO and 1 in AAA) and elevated LDL level of more than 570 mg/dl (651±88 mg/dl) in 6 patients (5 in ASO, 1 in TAO) before administration of EPA. The levels of VLDL were within the normal limit in all patients. EPA (1,800 mg/day) was given to these patients for 24 months and the data from these patients were analyzed. The serum level of Lp(a) in the patients with elevated Lp(a) levels before EPA administration were lowered significantly ($p<0.05$) after administration for 12 and 18 months. The levels of Lp(a) in patients in which it was initially normal did not change significantly (Fig. 1). The serum level of TC in patients with elevated TC level was lowered significantly after administration of EPA for 6, 12, 18 and 24 months (Fig. 2). The TG level in patients in which it was elevated before administration was lowered significantly after EPA administration for 18 months (Fig. 3). In the patients with an elevated LDL level before treatment, the LDL level was lowered after administration of EPA for 12 and 18 months (Fig. 4). In all patients in the present study, no marked side effects such as liver dysfunction were seen after long-term administration of EPA.

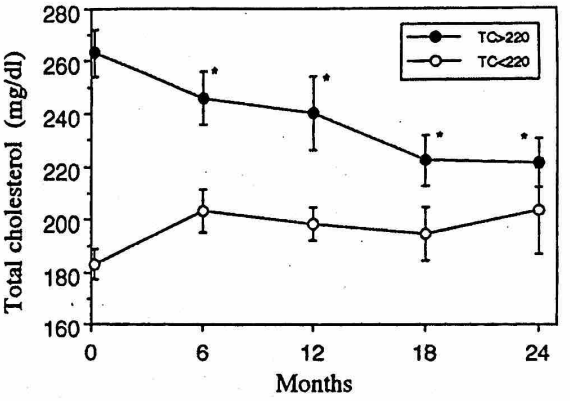

Fig. 2. Effects of administration of EPA on serum total cholesterol (TC) levels. Serum level of TC was measured during EPA treatment. Patients with pretreatment serum levels higher than the normal range (closed circles), and those with normal pretreatment serum levels (open circles). Asterisks denote significant differences ($p<0.05$) from the pretreatment values.

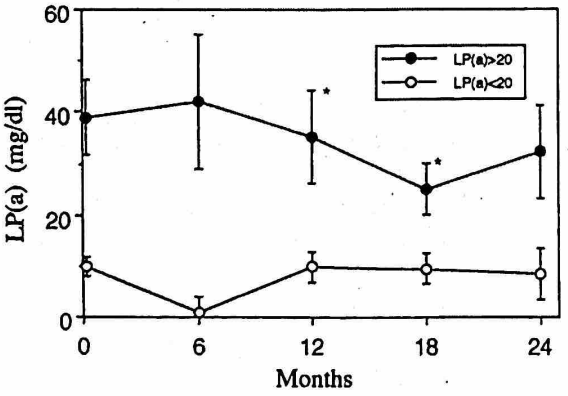

Fig. 1. Effects of administration of EPA on serum Lp(a) levels. Serum level of Lp (a) was measured during EPA treatment. Patients with pretreatment serum levels higher than the normal range (closed circles), and those with normal pretreatment serum levels (open circles). Each value is the mean±SE. Asterisks denote significant differences ($p<0.05$) from the pretreatment values.

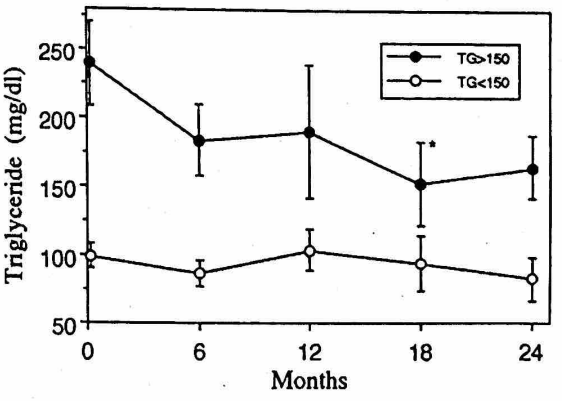

Fig. 3. Effects of administration of EPA on serum total triglyceride (TG) levels. Serum level of TG was measured during EPA treatment. Patients with pretreatment serum levels higher than the normal range (closed circles), and those with normal pretreatment serum levels (open circles). Asterisks denote significant differences ($p<0.05$) from the pretreatment values.

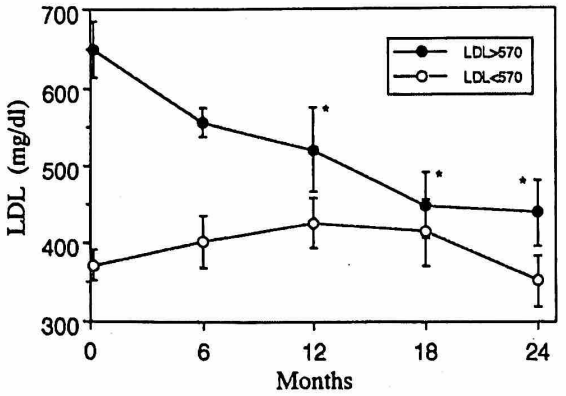

Fig. 4. Effects of administration of EPA on serum low-density lipoprotein (LDL) levels. Serum level of LDL was measured during EPA treatment. Patients with pretreatment serum levels higher than the normal range (closed circles), and those with normal pretreatment serum levels (open circles). Asterisks denote significant differences ($p<0.05$) from the pretreatment values.

Discussion

Lp(a) has been assumed to be an additional risk factor for atherosclerotic diseases (8). Therefore, reduction of the serum concentration of Lp(a) would be of great clinical interest. Recently, the effects of several drugs to reduce the effect of Lp(a) have been studied. Carlson *et al.* reported the pronounced Lp(a)-lowering effect of nicotinic acid in hyperlipidemic subjects (5). Vessby *et al.* studied the effects of cholestyramine (9) and Schmidt *et al.* investigated the effects of n-3 polyunsaturated fatty acids on Lp(a) (10). In this latter study, patients were given n-3 polyunsaturated fatty acids including EPA and DHA for 6 or 12 weeks, but no effect on Lp(a) was observed. In the present study, capsules containing 100% pure eicosapentaenoic acid were used. When analyzing the data, patients were divided into two groups according to the Lp(a) level before EPA administration. The level of Lp(a) in patients with a high level before administration decreased after 12 and 18 months of EPA administration. The mechanism of synthesis and degradation of Lp(a) is not clear at present, and there is no good explanation for the observed effect of EPA. Since Lp(a) contains an LDL component (apo-B) linked to apo(a) by a single disulfide bridge (11), the significant reduction of LDL after administration for 12 and 18 months is of interest. Intake of EPA might reduce hepatic synthesis of low-density lipoproteins and decrease the production of Lp(a) in the liver. We also studied the changes in the levels of TC, TG and LDL. The serum levels of TC, TG and LDL in the patients in which these levels were high before treatment were lowered significantly after administration of EPA for 6, 12, 18 and 24 months, for 18 months and for 12 and 18 months, respectively. Harris *et al.* reported that intake of n-3 fatty acids produced persistent reductions in TG levels, but not in TC or LDL levels (12), and Gries *et al.* reported that n-3 fatty acids could reduce the TG level after 6 months of treatment (13). The effect of EPA on TG in the present study was compatible with the results of these previous studies. However, the long-term effects of administration of highly purified EPA for more than 1 year have not been reported. The present study showed that the long-term administration of EPA reduced not only the Lp(a) level, but also the serum levels of TC, TG and LDL.

In conclusion, these findings indicate that long-term administration of EPA may lower the levels of Lp(a) and serum lipids such as triglyceride and LDL, and that this treatment is safe and beneficial for patients with various arterial diseases in terms of preventing progression of the disease.

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650	_2 | ‡a Thrombosis ‡0 https://id.nlm.nih.gov/mesh/D013927
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ATTACHMENT 7b

Atherosclerosis MeSH Descriptor Data 2021

MeSH Heading
Atherosclerosis
Tree Number(s)
C14.907.137.126.307
Unique ID
D050197
RDF Unique Identifier
http://id.nlm.nih.gov/mesh/D050197
Scope Note
A thickening and loss of elasticity of the walls of ARTERIES that occurs with formation of ATHEROSCLEROTIC PLAQUES within the ARTERIAL INTIMA.
Entry Term(s)
Atherogenesis
NLM Classification #
WG 550
Previous Indexing
Arteriosclerosis (1965-2005)
See Also
Chylomicron Remnants
Plaque, Atherosclerotic
Public MeSH Note
2006; see ARTERIOSCLEROSIS 1963-2005
History Note
2006; use ARTERIOSCLEROSIS 1963-2005
Date Established
2006/01/01
Date of Entry
2005/06/30
Revision Date
2010/06/25

ATTACHMENT 7c

Thrombosis MeSH Descriptor Data 2021

MeSH Heading
Thrombosis
Tree Number(s)
C14.907.355.830
Unique ID
D013927
RDF Unique Identifier
http://id.nlm.nih.gov/mesh/D013927
Annotation
general; prefer specifics; mural thrombus: if in the heart wall, coordinate IM with HEART DISEASES (IM); if in the wall of a blood vessel, coordinate IM with specific blood vessel (IM) or precoordinated blood vessel disease term (IM)
Scope Note
Formation and development of a thrombus or blood clot in the blood vessel.
Entry Term(s)
Blood Clot
Thrombus
See Also
Blood Coagulation
Thrombectomy
Date Established
1966/01/01
Date of Entry
1999/01/01
Revision Date
2016/05/31

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ATTACHMENT 8

(Yokoyama II)

THE LANCET

Volume 369 · Number 9567 · Pages 1055–1140 · March 31–April 6, 2007 www.thelancet.com

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Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis

MitsuhiroYokoyama, Hideki Origasa, Masunori Matsuzaki, Yuji Matsuzawa, Yasushi Saito, Yuichi Ishikawa, Shinichi Oikawa, Jun Sasaki, Hitoshi Hishida, Hiroshige Itakura, Toru Kita, Akira Kitabatake, Noriaki Nakaya, Toshiie Sakata, Kazuyuki Shimada, Kunio Shirato, for the Japan EPA lipid intervention study (JELIS) Investigators

Lancet 2007; 369: 1090–98

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Summary

Background Epidemiological and clinical evidence suggests that an increased intake of long-chain n-3 fatty acids protects against mortality from coronary artery disease. We aimed to test the hypothesis that long-term use of eicosapentaenoic acid (EPA) is effective for prevention of major coronary events in hypercholesterolaemic patients in Japan who consume a large amount of fish.

Methods 18 645 patients with a total cholesterol of 6·5 mmol/L or greater were recruited from local physicians throughout Japan between 1996 and 1999. Patients were randomly assigned to receive either 1800 mg of EPA daily with statin (EPA group; n=9326) or statin only (controls; n=9319) with a 5-year follow-up. The primary endpoint was any major coronary event, including sudden cardiac death, fatal and non-fatal myocardial infarction, and other non-fatal events including unstable angina pectoris, angioplasty, stenting, or coronary artery bypass grafting. Analysis was by intention-to-treat. The study was registered at clinicaltrials.gov, number NCT00231738.

Findings At mean follow-up of 4·6 years, we detected the primary endpoint in 262 (2·8%) patients in the EPA group and 324 (3·5%) in controls—a 19% relative reduction in major coronary events (p=0·011). Post-treatment LDL cholesterol concentrations decreased 25%, from 4·7 mmol/L in both groups. Serum LDL cholesterol was not a significant factor in a reduction of risk for major coronary events. Unstable angina and non-fatal coronary events were also significantly reduced in the EPA group. Sudden cardiac death and coronary death did not differ between groups. In patients with a history of coronary artery disease who were given EPA treatment, major coronary events were reduced by 19% (secondary prevention subgroup: 158 [8·7%] in the EPA group *vs* 197 [10·7%] in the control group; p=0·048). In patients with no history of coronary artery disease, EPA treatment reduced major coronary events by 18%, but this finding was not significant (104 [1·4%] in the EPA group *vs* 127 [1·7%] in the control group; p=0·132).

Interpretation EPA is a promising treatment for prevention of major coronary events, and especially non-fatal coronary events, in Japanese hypercholesterolaemic patients.

Introduction

Epidemiological and clinical evidence suggests a significant inverse association between long-term intake of long-chain n-3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and mortality associated with coronary artery disease.[1–7] Thus, the consumption of fish or fish-oil could protect against major events associated with coronary artery disease, especially fatal myocardial infarction and sudden cardiac death. Two large-scale secondary prevention trials, the Diet and Reinfarction Trial and the Gruppo Italiano per lo Studio della Sopravivenza nell' Infarto Miocardico-Prevenzione Trial, reported that increased consumption of fish or fish-oil supplements reduced coronary death in postinfarction patients.[8,9] No randomised trials have examined the effects of n-3 polyunsaturated fatty acids on major coronary events in a high-risk, primary prevention population.

EPA ethyl ester, which is purified from n-3 polyunsaturated fatty acids present in fish oil, is approved by Japan's Ministry of Health, Labour, and Welfare as a treatment for hyperlipidaemia and peripheral artery disease. The biological functions of EPA include reduction of platelet aggregation,[10,11] vasodilation,[12,13] antiproliferation,[14] plaque-stabilisation,[15] and reduction in lipid action.[16,17] Therefore the preventive effects of EPA on major cardiovascular events are of both clinical interest and therapeutic importance.

Primary and secondary prevention trials have proved that cholesterol-lowering treatment with inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase—statins—reduces the risk of all-cause mortality and major cardiovascular events in patients with a wide range of cholesterol concentrations, whether or not they have had coronary artery disease.[18–21] Thus, statins are now established as the first-line treatment for hyperlipidaemia.[22] Preliminary data for treatment with a combination of n-3 polyunsaturated fatty acids and statins have shown beneficial effects on the lipid profiles of patients with a mixed type of hyperlipidaemia;[23–25] however, no major long-term inter-