International Journal for

Vitamin and Nutrition Research

Clinical Nutrition

Experimental and General Nutrition

Nutritional Epidemiology

Interaction of Foodstuffs

Deficiency Syndromes

Pharmacology of Nutrition

Nutritionally Induced Diseases

Food Chemistry

Dietetics

Analytical Methods Related to Biological Problems

Volume 62
Number 1
1992

Hogrefe & Huber Publishers
Bern · Toronto · Seattle WA

International Journal for
Vitamin and Nutrition Research

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 in the 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.
Please pay strict attention to the *Directions to Contributors* (see second page) for preparations of the manuscripts.

The *International Journal for Vitamin and Nutrition Research* is published by Hogrefe & Huber Publishers, Toronto, Seattle, Bern, Göttingen. It appears in four issues yearly which make up a volume.
Subscriptions are available on a volume basis only. Yearly subscription rates: Swiss Francs 169.—, or US $ 117.00.
Advertisements: Please direct inquiries regarding the placing of advertisements in this journal to: Journals Advertising Coordinator, Hogrefe & Huber Publishers, Länggass-Strasse 76, CH-3000 Bern 9, Switzerland.

This journal is regularly listed in Current Contents and Index Medicus, indexed in AGRICOLA and CABS (Current Awareness in Biological Sciences)

HOGREFE & HUBER PUBLISHERS

European Address:	Länggass-Strasse 76 CH-3000 Bern 9, Switzerland
American Address:	P.O. Box 2487 Kirkland WA 98083-9968
Canadian Address:	14 Bruce Park Avenue Toronto, Ontario M4P 2S3, Canada

Internat. J. Vit. Nutr. Res. *62* (1992) 256–260
Received for publication November 12, 1991

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

The Second Department of Internal Medicine, Osaka University Medical School, Fukushima, Fuskushima-ku, Osaka 553, Japan

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).

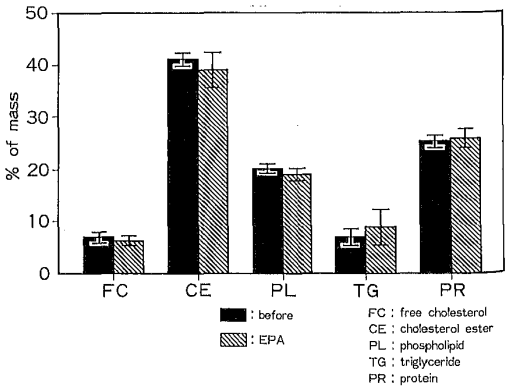

Figure 1: Chemical compositions of LDL before and after EPA administration.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.