

Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study^{1–3}

Muriel J Caslake, Elizabeth A Miles, Bettina M Kofler, Georg Lietz, Peter Curtis, Christopher K Armah, Alan C Kimber, Jilly P Grew, Lesley Farrell, Julie Stannard, Frances L Napper, Alex Sala-Vila, Annette L West, John C Mathers, Christopher Packard, Christine M Williams, Philip C Calder, and Anne M Minihane

ABSTRACT

Background: The lipid-modulatory effects of high intakes of the fish-oil fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are well established and likely to contribute to cardioprotective benefits.

Objectives: We aimed to determine the effect of moderate EPA and DHA intakes (<2 g EPA+DHA/d) on the plasma fatty acid profile, lipid and apolipoprotein concentrations, lipoprotein subclass distribution, and markers of oxidative status. We also aimed to examine the effect of age, sex, and apolipoprotein E (*APOE*) genotype on the observed responses.

Design: Three hundred twelve adults aged 20–70 y, who were prospectively recruited according to age, sex, and *APOE* genotype, completed a double-blind placebo-controlled crossover study. Participants consumed control oil, 0.7 g EPA+DHA/d (0.7FO), and 1.8 g EPA+DHA/d (1.8FO) capsules in random order, each for an 8-wk intervention period, separated by 12-wk washout periods.

Results: In the group as a whole, 8% and 11% lower plasma triacylglycerol concentrations were evident after 0.7FO and 1.8FO, respectively ($P < 0.001$); significant sex \times treatment ($P = 0.038$) and sex \times genotype \times treatment ($P = 0.032$) interactions were observed, and the greatest triacylglycerol-lowering responses (reductions of 15% and 23% after 0.7FO and 1.8FO, respectively) were evident in *APOE4* men. Furthermore, lower VLDL-cholesterol ($P = 0.026$) and higher LDL-cholesterol ($P = 0.010$), HDL-cholesterol ($P < 0.001$), and HDL2 ($P < 0.001$) concentrations were evident after fish-oil intervention.

Conclusions: Supplements providing EPA+DHA at doses as low as 0.7 g/d have a significant effect on the plasma lipid profile. The results of the current trial, which used a prospective recruitment approach to examine the responses in population subgroups, are indicative of a greater triacylglycerol-lowering action of long-chain *n*–3 polyunsaturated fatty acids in males than in females. *Am J Clin Nutr* 2008;88:618–29.

INTRODUCTION

Although there are some inconsistencies in the literature, a large body of epidemiologic data and evidence from randomized controlled trials (RCTs) has shown the cardioprotective actions of the fish-oil *n*–3 fatty acids (FAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (1–10). Reductions in relative risks of 20% to 50% in total and cardiovascular mortality and sudden death are frequently reported. At intakes of 0.5–1.5 g

EPA+DHA/d, the benefits have been attributed largely to a reduction in acute coronary events mediated through the antiarrhythmic action of EPA+DHA (11) or, more recently, to an effect on plaque morphology and stability (12).

At intakes of >2 g EPA+DHA/d, additional cardioprotective benefits such as antithrombotic actions and a positive effect on vascular reactivity, blood pressure, plasma lipid concentrations, and lipoprotein subclass distribution have been reported (13–17). The hypotriacylglycerolemic action of these relatively high fish-oil *n*–3 FA intakes is well recognized; the degree of triacylglycerol (TAG) lowering is comparable to the response observed with commonly used pharmacologic treatments such as fibrates. The fact that EPA+DHA have strong TAG-lowering action has led to the recommendation by expert bodies (eg, American Heart Association) for their use as an alternative to pharmacologic treatments such as fibrates (18). Although the blood lipid-modulating effects of intakes of EPA or DHA (or both) of <2 g/d were examined in a limited number of RCTs, most of these RCTs arguably were underpowered to detect more modest changes in the blood lipid profile.

Furthermore, as highlighted in a recent systematic review (8), data on the effects of EPA and DHA on cardiovascular disease (CVD) outcomes in different population subgroups are limited. The lipid response to fish-oil supplementation is known to be highly heterogeneous both within and between studies. In a previous study, our group (19) reported a group mean reduction of

¹ From the Department of Vascular Biochemistry, Faculty of Medicine, University of Glasgow, Glasgow, United Kingdom (MJC, LF, and CP); the Institute of Human Nutrition, School of Medicine, University of Southampton, Southampton, United Kingdom (EAM, AS-V, ALW, FLN, and PCC); the Hugh Sinclair Human Nutrition Group, School of Chemistry, Food Biosciences and Pharmacy (BMK, CKA, JPG, CMW, and AMM), and the Quantitative Biology and Applied Statistics Section, School of Biological Sciences (ACK), University of Reading, Reading, United Kingdom; and the Human Nutrition Research Centre, School of Clinical Medical Sciences, Newcastle University, Newcastle, United Kingdom (GL, PC, JS, and JCM).

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³ Reprints not available. Address correspondence to AM Minihane, Hugh Sinclair Human Nutrition Group, School of Chemistry, Food Biosciences and Pharmacy, University of Reading, Reading RG6 6AP, United Kingdom. E-mail: a.m.minihane@reading.ac.uk.

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35% in fasting TAG and an increase of 7.1% in fasting LDL-cholesterol concentrations after supplementation for 6 wk with 3 g EPA + DHA/d, as compared with the control oil (CO). However, these mean responses represented ranges of -114% to 61% in TAG concentrations and of -49% to 87% in LDL-cholesterol concentrations in the 55 participants (19). It is likely that factors such as health status, medication use, background diet, age, sex, baseline lipid concentrations, and genetic variability account for this highly heterogeneous blood lipid response to fish-oil intervention, but the relative effect of these factors is unknown.

The apolipoprotein E (APOE) genotype (ϵ allele) represents the most widely investigated genotype with respect to genetic influence on blood lipid concentrations and their response to dietary fat manipulation (20). Carriers of the $\epsilon 4$ allele, which represent $\approx 22\%$ of whites, are believed to be most responsive to changes in dietary total and saturated fat and cholesterol intakes (20). Given this, and the central role of apolipoprotein (apo) E (apoE) in lipoprotein metabolism, the APOE genotype (which has $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ as common variants) represents an obvious potential genetic modulator of the fasting lipoprotein response to fish-oil intervention.

Our previous study observed a significant effect of APOE genotype on both the LDL-cholesterol and TAG responses to fish-oil supplementation (19): LDL was markedly increased in the carriers of the $\epsilon 4$ allele, which suggested that the relatively common observation of elevated LDL cholesterol may be most evident in those with this genotype.

The ability to draw definitive conclusions from this earlier study was limited by the facts that it was conducted in subjects with an atherogenic lipoprotein phenotype and that it lacked adequate statistical power to examine genotype-phenotype associations. Therefore, in the present study, the FINGEN Study, prospective recruitment according to sex, age, and APOE genotype was used in a normolipidemic population to evaluate the effect of these factors on lipid responses to fish-oil supplementation by using EPA+DHA intakes that are achievable by the general population through an increase in the consumption of oily fish.

SUBJECTS AND METHODS

Volunteers

The FINGEN Study is a 4-center trial that was conducted at the universities of Glasgow, Newcastle, Reading, and Southampton in the United Kingdom between June 2003 and Sept 2005. The aim of the prospective recruitment was to recruit a total of 330 persons. The target was for 270 persons to complete the study, stratified according to APOE genotype, sex, and age as follows: 1) 90 persons in each of the E2 (E2/E2 and E2/E3), E3/E3 and E4 (E3/E4 and E4/E4) subgroups (E2/E4 persons were not included in the study); 2) 135 men and 135 women; 3) $n = 45$ in each of the apoE2 male, apoE2 female, apoE3 male, apoE3 female, apoE4 male, and apoE4 female subgroups; and 4) $n = 54$ in each of the age groups of 20–29, 30–39, 40–49, 50–59, and 60–69 y.

The study group size was estimated by least standardized difference by using expected changes in plasma LDL-cholesterol and TAG concentrations as the primary phenotypic outcomes. On the basis of a 5% significance level, a power of 80% for

detecting main effect, and 2-factor interactions, with a standardized size effect of ≥ 0.6 , a sample size of 45 per genotype or sex subgroup was required.

The volunteers were generally fit and healthy. Exclusion criteria for participation in the study were diagnosed diabetes or fasting glucose concentrations of >6.5 mmol/L; liver or other endocrine dysfunction; a myocardial infarction in the previous 2 y; hypolipidemic therapy or any other medication known to interfere with lipid metabolism; consumption of FA supplements or oily fish >1 time/wk; current use of a weight-reducing diet; body mass index (in kg/m^2) of <18.5 or >30 ; or fasting total cholesterol (TC) and TAG concentrations of >8.0 and 3.0 mmol/L, respectively.

All subjects provided written informed consent before participation in the study. The study was approved by the research ethics committee at each of the four universities involved in the present study.

Study design

The study was a double-blind, placebo-controlled, dose-response crossover study consisting of 3 intervention arms of 8-wk duration each and separated by 12-wk washout intervals. During the intervention periods, participants consumed either A) the CO, B) 0.7 g EPA+DHA/d (0.7FO), or C) 1.8 g EPA+DHA/d (1.8FO) in random order. The randomization of subjects to treatment order (ABC, ACB, BCA, BAC, CAB, or CBA) was achieved by using a computer-generated random-number table. Fasting (12-h fast) blood samples were collected at the beginning and end of each intervention arm, and participants were asked to refrain from alcohol consumption and organized exercise and to consume the same low-fat (<10 g fat) meal of their choice as their main evening meal before each clinical visit. On 2 occasions during the study, participants were also asked to complete a food-frequency questionnaire (FFQ) to characterize background habitual diet.

Study capsules

Participants were asked to consume a total of four 0.8-g capsules/d, 1 each with breakfast and lunch and 2 with their evening meal. The composition of the capsules (Ocean Nutrition, Bedford, Canada) is given in **Table 1**. The CO was an 80:20 blend of palm oil and soybean oil, which provides a mixture containing palmitic, oleic, and linoleic acids at a ratio of $\approx 3:4:2$ and an FA composition comparable to the average adult diet in the United Kingdom (21). The fish-oil capsules provided 0.7 g or 1.8 g EPA+DHA/d as ethyl esters, and 95–99% and 1–5% of the long-chain $n-3$ polyunsaturated FAs (LC $n-3$ PUFA) were derived from anchovy oil and sardine oil, respectively, for a DHA:EPA ratio of 1.5:1, which represents the ratio in commonly consumed oily fish. The capsules were analyzed on 4 occasions during the 2-y study, and no loss of EPA or DHA with storage was evident (data not shown). All oils contained a mixed tocopherol concentrate ($>70\%$ total tocopherol, of which $>80\%$ consists of β -, γ -, and δ -tocopherols) at a concentration of 2 mg/g oil. Compliance with treatment was determined by using capsule count and plasma phosphatidylcholine (PC) FA composition.

APOE genotyping

APOE genotyping using screening blood samples was determined by using a derivation of the technique of Hixon and



TABLE 1
Fatty acid composition of the study oils¹

Fatty acid	Fatty acid		
	Control oil	0.7FO	1.8FO
	<i>g/100 g total fatty acids</i>		
Myristic acid (14:0)	0.8 ± 0.0 ²	0.9 ± 0.0	0.8 ± 0.0
Palmitic acid (16:0)	29.9 ± 0.2	20.8 ± 0.4	5.4 ± 0.3
Stearic acid (18:0)	4.3 ± 0.0	4.2 ± 0.1	3.6 ± 0.0
Oleic acid (18:1n-9)	40.7 ± 0.3	27.9 ± 0.4	7.7 ± 0.3
Linoleic acid (18:2n-6)	21.0 ± 0.1	12.6 ± 0.0	1.7 ± 0.0
Linolenic acid (18:3n-3)	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
Arachidonic acid (20:4n-6)	ND	0.6 (0.0)	1.4 (0.0)
Eicosapentaenoic acid (20:5n-3)	ND	9.2 ± 0.2	23.0 ± 0.3
Docosapentaenoic acid (22:5n-3)	ND	2.8 ± 0.1	7.0 ± 0.1
Docosahexaenoic acid (22:6n-3)	ND	12.8 ± 0.5	32.8 ± 0.8

¹ n = 5. ND, not detectable; 0.7FO, 0.7 g eicosapentaenoic acid (EPA) + docosahexaenoic (DHA)/d; 1.8FO, 1.8 g EPA+DHA/d.

² $\bar{x} \pm SD$ (all such values).

Vernier (22). DNA was isolated from the buffy coat layer of 10 mL of blood drawn into tubes containing EDTA with the use of a Qiagen DNA Blood Mini Kit (Qiagen Ltd, Crawley, United Kingdom). Polymerase chain reaction was conducted by using the ApoE sense primer—5′-aca gaa ttc gcc ccg gcc tgg tac ac-3′—and the ApoE antisense primer—5′-taa gct ggc cac ggc tgt cca agg a-3′. After digestion with the *HhaI* restriction endonucleases, the resultant fragments were separated by gel electrophoresis on a 10% polyacrylamide gel (BioRad, Hemel Hempstead, United Kingdom), and the *APOE* genotype was identified from the fragment pattern as described previously (19).

Biochemical analysis

Although the study was conducted at 4 individual centers, analyses were centralized to prevent an effect of intercenter variation on the study outcomes. Analyses of plasma TAG, TC, LDL cholesterol, HDL cholesterol, VLDL cholesterol, oxidized LDL (oxLDL), glucose, and insulin and LDL and HDL density profiling were undertaken at Glasgow; plasma nonesterified FA (NEFA), apoE, apoB, and apoA1 concentrations were measured at Reading; plasma phosphatidylcholine FA composition was measured at Southampton; and analysis of the FFQ was conducted at Newcastle.

Blood was drawn from the antecubital vein into tubes containing potassium-EDTA for measurement of lipid, apolipoprotein, and glucose and into tubes containing lithium heparin for analysis of insulin and plasma PC FAs. Within 30 min of withdrawal, the blood was centrifuged at 3000 rpm for 10 min at 4 °C, and the plasma was stored at -80 °C until it was analyzed. Before the freezing of the plasma to be used for the determination of apoE, apoB, and apoA1 concentrations, a protease inhibitor and an antibiotic-containing preservative were added (5% vol/vol) (23).

Analysis of plasma lipid, apolipoprotein, glucose, and insulin

Plasma TC and TAG concentrations were quantified on the Hitachi 717 analyzer by using commercially available enzymatic colorimetric kits (Roche, Burgess Hill, United Kingdom). Plasma VLDL, LDL-, and HDL-cholesterol concentrations were

measured according to the Lipid Research Clinics Program Manual of Laboratory Operations (24).

Plasma NEFA, glucose, and apoE, apoB, and apoA1 concentrations were measured by using automated enzymatic colorimetric techniques (NEFA: Instrumentation Laboratory Ltd, Warrington, United Kingdom; glucose: Roche Diagnostics Ltd, Warrington, United Kingdom) for apolipoprotein analyses on an iLAB 600 autoanalyzer (Instrumentation Laboratory Ltd). Plasma insulin was quantified by using a 2-site enzyme-linked immunosorbent assay (Mercodia AB, Uppsala, Sweden).

To provide surrogate markers of insulin sensitivity, the homeostatic model assessment (HOMA) and the revised quantitative insulin sensitivity check index (RQUICKI) were calculated from the fasting glucose, insulin, and NEFA data according to the following equations (25):

$$\text{HOMA} = [\text{glucose (mmol/L)} \times \text{insulin } (\mu\text{U/mL})] / 22.5 \quad (1)$$

and

$$\text{RQUICKI} = 1 / [\log \text{insulin } (\mu\text{U/mL}) + \log \text{glucose (mg/dL)} + \log \text{NEFA (mmol/L)}] \quad (2)$$

LDL and HDL subclass analysis

The combined LDL-HDL fraction of density 1.019–1.21 g/mL was isolated by ultracentrifugation at 15 °C for 24 h in a Beckman fixed-angle rotor (Beckman Instruments, High Wycombe, United Kingdom). Particle sizes of LDL and HDL were determined by using nondenaturing gradient gel electrophoresis on 2–30% polyacrylamide gel (Alamo Gels Inc, San Antonio, TX). LDL was divided into I, II, and III subfractions by using in-house standards that had been prepared by density-gradient ultracentrifugation.

HDL was divided into subfractions 2a, 2b, 3a, 3b, and 3c by using high molecular standards (Amersham, Chalfont St Giles, United Kingdom) according to previously determined size ranges (26). The relative proportion of each subfraction is reported as a percentage of total lipoproteins.

Plasma measures of oxidative stress

α -Tocopherol was quantified by using HPLC, according to a previously described method (27). Concentrations of oxLDL were determined by using a 2-site enzyme-linked immunosorbent assay (Mercodia AB) that is based on the quantification of modified apoB in the sample.

Plasma phosphatidylcholine fatty acid analysis

The FA composition of the plasma PC fraction was determined by using previously described methods (28). Lipid extraction, PC isolation using solid-phase extraction, transmethylation, and methyl ester separation by gas phase chromatography were the principal steps involved.

Food-frequency questionnaire

The background diet of the study participants was assessed by using a modified version of the European Prospective Investigation into Cancer and Nutrition FFQ, which contained a total of 154 items. Eight additional questions relating to fish consumption also were included, and fish subgroups were classified according to Welch et al (29). The information from the FFQ

was converted into intakes of energy and nutrients by using portion size information and nutrient composition values from the 6th edition of McCance and Widdowson food tables (30).

Statistical analysis

Data are shown for participants who completed the study. Repeated-measures analysis was undertaken on the outcome data (end of treatment values) with the baseline (beginning of respective treatment period) values as covariates to determine the effect of treatment in the group as a whole and to examine the individual and combined effects of the design variables (ie, age, sex, and *APOE* genotype) on response to treatment and to test for period and sequence effects. For α -tocopherol and the ratio of α -tocopherol to TC, the model was poor because of heterogeneity according to age; therefore, age subgroups were analyzed separately as described below.

Because TAG concentrations are recognized determinants of HDL cholesterol and LDL and HDL subclass distribution, additional analysis was conducted by including TAG as a covariate to establish the TAG-independent effects of treatment on these outcome measures. All analyses were conducted with SAS software (version 9.1; SAS Institute, Cary, NC) and SPSS software (version 15; SPSS Inc, Chicago, IL); $P < 0.05$ was considered significant.

RESULTS

Volunteer baseline characteristics

Of the 801 persons who attended a screening visit, a total of 364 participated in the study; of this group, 312 completed the 3 intervention periods and all 6 clinical visits. To view a Consolidated Standards of Reporting Trials (CONSORT) flow diagram, see Figure S1 under "Supplemental data" in the current online issue. The characteristics of the volunteers at study entry are shown in **Table 2** for the group as a whole and for men ($n = 149$) and women ($n = 163$) separately. Of 87 subjects having an *E2* allele, 5 were homozygous for *E2/E2*, and the remaining 82 were *E2/E3*. In the subgroup with the *E4* allele, 13 and 101 were homozygous and heterozygous for *E4*, respectively. The expected differences between the sexes with respect to established CVD risk biomarkers [except TC ($P = 0.573$) and LDL cholesterol ($P = 0.152$)] were evident (Table 2).

Habitual energy and fat intakes

Although the men had significantly higher intakes of energy than did the women ($P = 0.005$), no significant differences in the percentage of total energy from fat ($P = 0.828$), saturated FA (SFA; $P = 0.920$), monounsaturated FA (MUFA; $P = 0.085$), or polyunsaturated FA (PUFA; $P = 0.983$); the ratio of PUFA to SFA (P:S) ($P = 0.864$); and oily fish intake ($P = 0.341$) were observed between the men and the women (Table 2).

Capsule count and fatty acid composition of plasma phosphatidylcholine

According to the records of returned capsules, compliance with treatment was generally high: 94.9%, 95.5%, and 95.2% of the CO, 0.7FO, and 1.8FO capsules were consumed, and no significant intertreatment difference was evident. Univariate analysis of variance indicated no significant effect of sex or

APOE genotype on compliance. However, there was a significant effect of age on compliance with the CO ($P = 0.036$) and 0.7FO ($P = 0.001$) treatments, and post hoc analysis indicated increasing compliance with increasing age.

The effect of treatment on the plasma PC FA composition is detailed in **Table 3**. No significant differences in the FA profiles were evident between the 3 baseline measurements, regardless of sequence, which shows the adequacy of the washout period used in this intervention trial. A significant effect of treatment on PC EPA ($P < 0.001$), docosapentaenoic acid (DPA) ($P < 0.001$), and DHA ($P < 0.001$) was evident, as was a dose response to fish oil, with 43% and 69% higher LC n-3 PUFA (EPA + DPA + DHA) after 0.7FO and 1.8FO, respectively. These changes were associated with significantly higher stearic acid ($P = 0.022$) and significantly lower oleic acid ($P < 0.001$), linoleic acid ($P < 0.001$), and γ -linolenic acid ($P < 0.001$) concentrations.

A significant sex \times treatment interaction was observed with respect to the changes in PC linoleic acid ($P = 0.001$), EPA ($P < 0.001$), DPA ($P < 0.001$), and total LC n-3 PUFA ($P < 0.001$) concentrations, with greater responsiveness of the PC FAs, and a significant dose response to fish oil evident only in females (**Figure 1A**). For example, after the 0.7FO and 1.8FO intervention, 19% and 24% greater increases in plasma PC total LC n-3 PUFA were seen in the female subjects than in the male subjects. Given that the female participants had a significantly lower body mass but received the same dose of fish oil, further analyses were conducted on body weight-adjusted data. As indicated in Figure 1B, there were no significant differences in the increase in EPA or total LC n-3 PUFA between the sexes after body-weight adjustment. However, 19% and 16% greater weight-adjusted increases in plasma PC DHA were observed in the men than in the women after 0.7FO ($P = 0.009$) and 1.8FO ($P = 0.026$) interventions, respectively. Subgroup comparison of premenopausal and postmenopausal female participants indicated no significant differences in plasma PC FA responsiveness according to menopausal status (data not shown).

Lipid profile

A significant treatment effect was evident for plasma TAG ($P < 0.001$): 8.0% and 11.2% CO-adjusted reductions were observed in the group as a whole in response to 0.7FO and 1.8FO intervention, respectively, and no significant dose effect was evident. A significant sex \times treatment interaction ($P < 0.038$) was observed; a greater hypotriacylglycerolemic response was seen in the male subjects than in the female subjects, and a significant dose response to treatment evident only in males (**Figure 2A**).

Although the trend toward greater responsiveness in *E4* persons was not statistically significant, there was a significant ($P = 0.032$) treatment \times sex \times genotype interaction; the greatest hypotriacylglycerolemic effects were evident in apoE4 males: 15% ($P = 0.004$) and 23% ($P < 0.001$) reductions in TAG concentrations were evident after the 8-wk 0.7FO and 1.8FO intervention periods, respectively, in the male *E4* subgroup (**Figure 2B**). This finding corresponds to a doubling of the response observed for the group as a whole ($n = 312$).

Although treatment had no significant effect on TC concentrations, both fish-oil doses resulted in modestly (3-4%) but significantly higher circulating LDL- ($P = 0.010$) and HDL- ($P < 0.001$) cholesterol concentrations. End-of-treatment values of

TABLE 2
Participant characteristics at baseline¹

	All participants (n = 312)	Men (n = 149)	Women (n = 163)	P ²
Genotype (n) ³				
E2	87	38	49	
E3	111	56	55	
E4	114	55	59	
Age group (n)				
20–29 y	50	21	29	
30–39 y	65	34	31	
40–49 y	68	34	34	
50–59 y	77	36	41	
60–70 y	52	24	28	
Age (y)	45.0 ± 0.7 ⁴	44.0 ± 1.1	45.8 ± 1.0	0.960
Weight (kg)	73.0 ± 0.8	81.9 ± 0.9	64.8 ± 0.8	<0.001
BMI (in kg/m ²)	25.2 ± 0.19	26.2 ± 0.2	24.3 ± 0.3	<0.001
Blood pressure (mm Hg)				
Systolic	123 ± 1	129 ± 1	118 ± 1	<0.001
Diastolic	74 ± 1	77 ± 1	72 ± 1	<0.001
Menopausal status				
Premenopausal			53%	
Perimenopausal			12%	
Postmenopausal			35%	
Plasma biochemistry				
TC (mmol/L)	5.12 ± 0.06	5.08 ± 0.08	5.15 ± 0.08	0.573
LDL-C (mmol/L)	3.22 ± 0.05	3.29 ± 0.07	3.15 ± 0.07	0.152
HDL-C (mmol/L)	1.42 ± 0.02	1.25 ± 0.02	1.59 ± 0.02	<0.001
TAG (mmol/L)	1.26 ± 0.03	1.43 ± 0.06	1.10 ± 0.03	<0.001
LDL3 (% total LDL)	33.4 ± 1.0	35.7 ± 1.6	31.4 ± 1.2	0.104
HDL3 (% total HDL)	35.3 ± 0.6	38.7 ± 0.9	32.2 ± 0.7	0.000
Plasma glucose (mmol/L)	5.14 ± 0.03	5.33 ± 0.06	4.96 ± 0.04	<0.001
Plasma insulin (μU/mL)	8.46 ± 0.23	9.29 ± 0.34	7.70 ± 0.29	<0.001
Energy intake (kJ/d)	10.1 ± 0.18	10.6 ± 0.25	9.7 ± 0.24	0.005
Habitual dietary fat intake				
Percentage of energy (%)	29.9 ± 0.3	29.7 ± 0.5	30.0 ± 0.5	0.828
P:S	0.41 ± 0.01	0.41 ± 0.01	0.40 ± 0.01	0.864
SFA (% of energy)	11.6 ± 0.18	11.6 ± 0.23	11.6 ± 0.26	0.920
MUFA (% of energy)	8.7 ± 0.11	8.9 ± 0.14	8.5 ± 0.17	0.085
PUFA (% of energy)	4.5 ± 0.08	4.5 ± 0.11	4.5 ± 0.11	0.983
Portions of oily fish/wk (n)	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	0.341
Units of alcohol/wk (n) ⁵	7.5 ± 0.4	10.7 ± 0.7	4.7 ± 0.4	<0.001

¹ TC, total cholesterol; -C, cholesterol; TAG, triacylglycerol; P:S, ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA); MUFA, monounsaturated fatty acids.

² P values were obtained by using either an independent-sample *t* test or a Mann-Whitney test.

³ E2, n = 5 E2/E2 + n = 82 E2/E3; E3, n = 111 E3/E3; E4, n = 13 E4/E4 + n = 101 E3/E4.

⁴ $\bar{x} \pm \text{SEM}$ (all such values).

⁵ 1 UK unit = 10 mL or 8 g pure alcohol.

3.28 ± 0.05, 3.38 ± 0.05, and 3.38 ± 0.06 mmol/L for LDL cholesterol and of 1.44 ± 0.02, 1.49 ± 0.02, and 1.50 ± 0.02 mmol/L for HDL cholesterol were seen after the CO, 0.7FO, and 1.8FO interventions, respectively. Both fish-oil doses produced similar and significantly ($P = 0.026$) lower VLDL cholesterol concentrations. There was no evidence of a dose-response effect of the fish oil for any of the cholesterol fractions.

Fish-oil supplementation produced a shift in the HDL subclass distribution ($P < 0.001$). Furthermore, a sex × treatment interaction was evident: the increase in the percentage of HDL-2 in men was more than twice that in women, and the decrease in HDL-3 in men was twice that in women ($P < 0.001$) (data not shown; see Figure S2 under “Supplemental data” in the current online issue). Because TAG is recognized as a strong determinant

of LDL and HDL particle size, further statistical analysis was conducted to determine whether the effect of fish oil on the percentage of HDL3 was independent of TAG concentrations. In correlation analysis, a relatively weak but significant positive association between the absolute change in HDL3 (as a percentage of total HDL) and in TAG concentrations after treatment was evident ($r = 0.149$, $P < 0.001$). Subsequent repeated-measures analysis of covariance indicated that the effect of treatment on the HDL3 percentage remained significant ($P = 0.030$) after the inclusion of TAG concentration as a covariate.

Although there was a general trend toward an increase in apoA1 and apoB in response to fish-oil treatment, the small (1–2%) changes were not significant. In contrast, significantly higher plasma apoE concentrations were evident after 1.8FO



TABLE 3
Plasma phosphatidylcholine fatty acid composition before and after treatment¹

	Control oil						0.7FO		1.8FO		P		
	0 wk		8 wk		0 wk	8 wk	0 wk	8 wk	0 wk	8 wk	Treatment × genotype	Treatment × sex	Treatment × age
	% total fatty acids												
Myristic acid (14:0)	0.31 ± 0.01 ²	0.30 ± 0.01	0.33 ± 0.01	0.31 ± 0.01	0.33 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	NS	NS	NS	
Palmitic acid (16:0)	30.40 ± 0.10	30.41 ± 0.10	30.36 ± 0.10	30.57 ± 0.11	30.36 ± 0.10	30.42 ± 0.11	30.42 ± 0.11	30.60 ± 0.11	30.60 ± 0.11	NS	NS	NS	
Stearic acid (18:0)	14.01 ± 0.09	13.94 ± 0.09 ^a	14.02 ± 0.09	14.05 ± 0.09 ^b	14.02 ± 0.09	14.10 ± 0.10	14.10 ± 0.10	14.23 ± 0.10 ^b	14.23 ± 0.10 ^b	0.022	NS	NS	
Oleic acid (18:1n-9)	11.09 ± 0.08	11.09 ± 0.09 ^a	11.07 ± 0.08	10.51 ± 0.08 ^b	11.07 ± 0.08	11.02 ± 0.08	11.02 ± 0.08	10.32 ± 0.08 ^b	10.32 ± 0.08 ^b	<0.001	NS	NS	
Linoleic acid (18:2n-6)	23.40 ± 0.15	23.58 ± 0.16 ^a	23.24 ± 0.16	21.95 ± 0.16 ^b	23.24 ± 0.16	23.30 ± 0.16	23.30 ± 0.16	20.99 ± 0.16 ^c	20.99 ± 0.16 ^c	<0.001	NS	NS	
Dihomo-γ-linolenic acid (20:3n-6)	3.51 ± 0.05	3.49 ± 0.04 ^a	3.54 ± 0.05	2.99 ± 0.04 ^b	3.54 ± 0.05	3.57 ± 0.05	3.57 ± 0.05	2.70 ± 0.04 ^c	2.70 ± 0.04 ^c	<0.001	NS	NS	
Arachidonic acid (22:4n-6)	9.39 ± 0.10	9.38 ± 0.10	9.50 ± 0.10	8.65 ± 0.09	9.50 ± 0.10	9.49 ± 0.11	9.49 ± 0.11	8.31 ± 0.09	8.31 ± 0.09	NS	NS	NS	
EPA (20:5n-3)	1.60 ± 0.04	1.60 ± 0.04 ^a	1.66 ± 0.05	2.88 ± 0.06 ^b	1.66 ± 0.05	1.63 ± 0.05	1.63 ± 0.05	3.78 ± 0.07 ^c	3.78 ± 0.07 ^c	<0.001	<0.001 ⁴	NS	
DPA (22:5n-3)	1.09 ± 0.01	1.08 ± 0.01 ^a	1.10 ± 0.01	1.21 ± 0.01 ^b	1.10 ± 0.01	1.08 ± 0.02	1.08 ± 0.02	1.29 ± 0.02 ^c	1.29 ± 0.02 ^c	<0.001	<0.001 ³	NS	
DHA (22:6n-3)	4.41 ± 0.07	4.33 ± 0.07 ^a	4.38 ± 0.07	6.22 ± 0.07 ^b	4.38 ± 0.07	4.29 ± 0.07	4.29 ± 0.07	6.78 ± 0.08 ^c	6.78 ± 0.08 ^c	<0.001	NS	NS	
Total EPA+DPA+DHA	7.17 ± 0.11	7.06 ± 0.11 ^a	7.21 ± 0.12	10.31 ± 0.12 ^b	7.21 ± 0.12	7.06 ± 0.11	7.06 ± 0.11	11.92 ± 0.14 ^c	11.92 ± 0.14 ^c	<0.001	<0.001 ⁴	NS	

¹ 0.7FO, 0.7 g eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)/d; 1.8FO, 1.8 g EPA+DHA/d. Repeated-measures analysis was undertaken on the outcome data (end-of-treatment values) with the baseline (beginning-of-treatment) values as covariates to determine the effect of treatment in the group as a whole and to examine the individual and combined effects of the design variables (ie, age, sex, and APOE genotype) on response to treatment and to test for period and sequence effects. When a significant treatment effect was observed, post hoc analysis was conducted to determine which treatment groups were significantly different. Values in a row with different superscript letters are significantly different, $P < 0.017$.

² $\bar{x} \pm SD$ (all such values).

³ For men, control oil values were significantly higher than 0.7FO and 1.8FO, and there was no significant difference between 0.7FO and 1.8FO values. For women, the control oil, 0.7FO, and 1.8FO values differed significantly from each other.

⁴ Greater response in females.

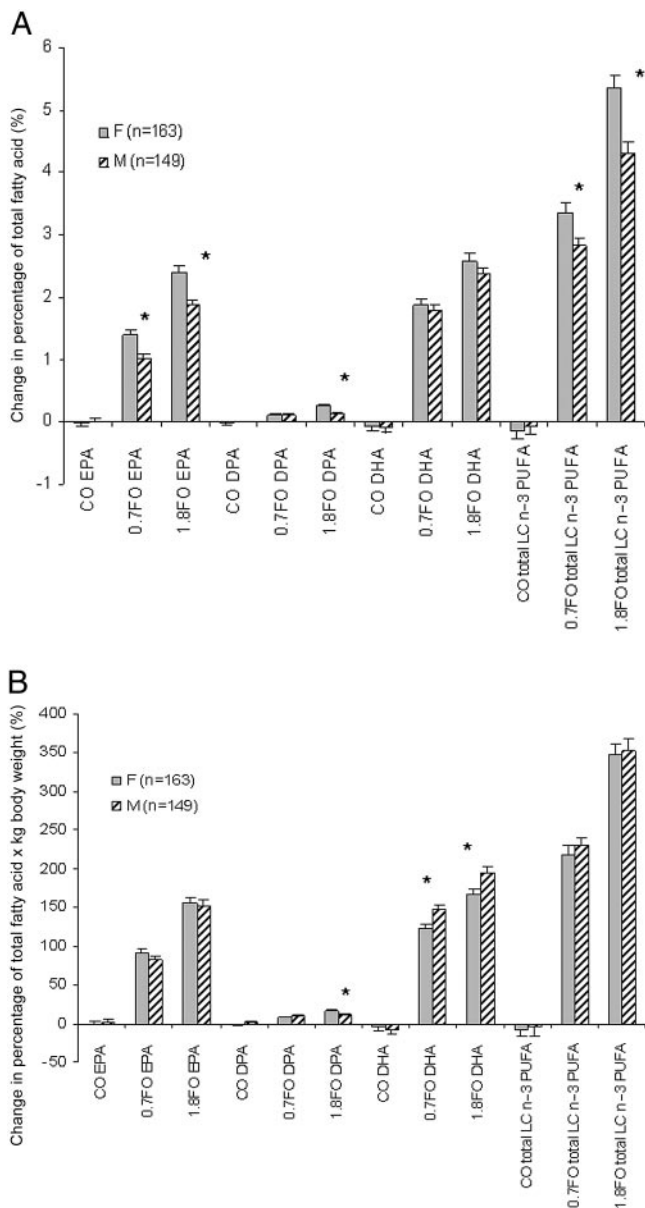


FIGURE 1. Change in plasma phosphatidylcholine long-chain (LC) n-3 polyunsaturated fatty acid (PUFA) enrichment according to treatment: effect of sex. CO, control oil; 0.7FO, 0.7 g eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)/d; 1.8FO, 1.8 g EPA + DHA/d; DPA, docosapentaenoic acid; LC, long-chain; F, females; M, males. A: uncorrected data. Treatment \times sex interaction for EPA, DPA, DHA, and total LC n-3 PUFA of <0.001 , <0.001 , NS, and <0.001 , respectively. B: data expressed as corrected for body mass [change in % of total fatty acid \times body wt (in kg)]. *Significant within-treatment effect of sex, $P < 0.05$.

than after CO and 0.7FO ($P = 0.002$); a sex \times treatment interaction ($P = 0.029$) and a significant effect of treatment were evident only in female subjects.

Markers of insulin sensitivity

There was no detectable effect of treatment on markers of insulin sensitivity, including plasma glucose, insulin, and NEFA concentrations (Table 4), or on homeostatic model assessment and the revised quantitative insulin sensitivity check index (data not shown).

Markers of oxidative status

Because the oxLDL assay quantifies the oxidation status of the apoB moiety and because it is therefore influenced by absolute apoB concentration, oxLDL data are expressed as the ratio of oxLDL to apoB (oxLDL:apoB). Furthermore, because plasma α -tocopherol concentrations are highly correlated with TC, α -tocopherol data are expressed as α -tocopherol:TC.

A significant treatment effect was evident for oxLDL:apoB; the response in the 1.8FO group was significantly ($P = 0.030$) different from that in the CO and 0.7FO groups. In the group aged

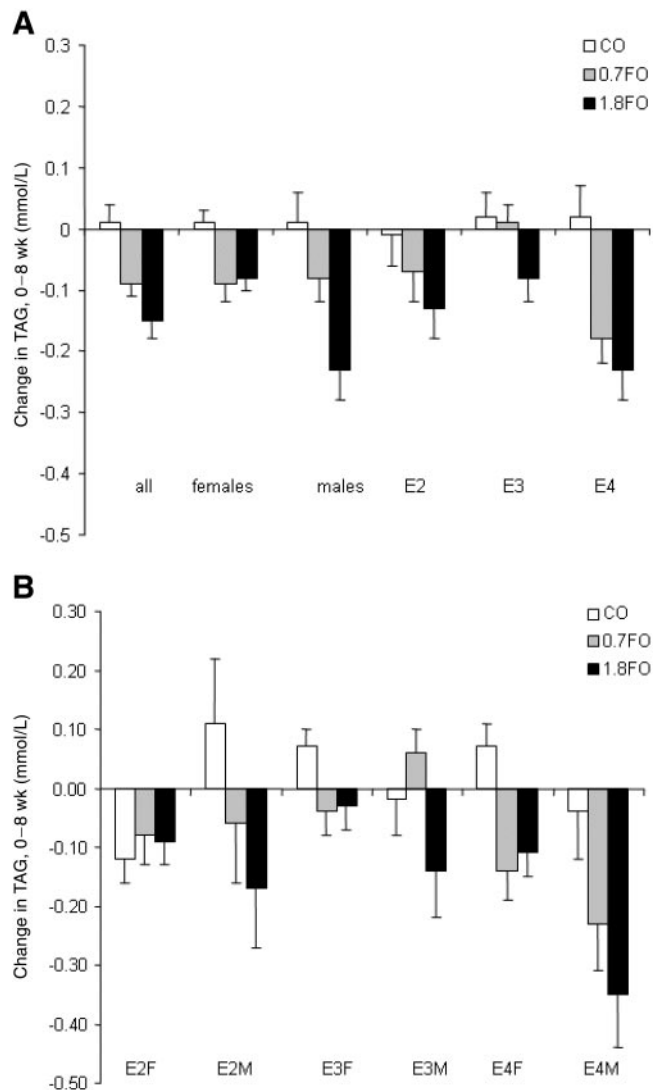


FIGURE 2. Plasma triacylglycerol (TAG) response to treatment: effect of sex and *APOE* genotype. CO, control oil; 0.7FO, 0.7 g eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)/d; 1.8FO, 1.8 g EPA + DHA/d; E2 = E2/E2 + E2/E3, $n = 87$; E3 = E3/E3, $n = 111$; E4 = E3/E4 + E4/E4, $n = 114$. Females (F), $n = 163$; males (M), $n = 149$. E2F, $n = 49$; E2M, $n = 38$; E3F, $n = 55$; E3M, $n = 56$; E4F, $n = 59$; E3M, $n = 55$. A: response to treatment in the group as a whole and by sex and genotype individually. With the use of repeated-measures analysis, a significant treatment effect ($P < 0.001$) and a significant treatment \times sex interaction ($P = 0.038$) were observed; a significant dose response was evident only in males. The trends toward greater responsiveness in E4 carriers were not statistically significant. B: genotype \times sex responses to treatment. With the use of repeated-measures analysis, a significant treatment \times sex \times genotype interaction was evident ($P = 0.032$).

TABLE 4
Response of plasma lipids, apolipoproteins, lipoprotein subclass distribution, and markers of oxidative stress to treatment¹

	Control oil						1.8FO						P		
	0 wk		8 wk		0 wk		8 wk		0 wk		8 wk		Treatment × genotype	Treatment × sex	Treatment × age
	0 wk	8 wk	0 wk	8 wk	0 wk	8 wk	0 wk	8 wk	0 wk	8 wk					
Triacylglycerols (mmol/L)	1.27 ± 0.04 ²	1.28 ± 0.04 ^a	1.25 ± 0.04	1.17 ± 0.03 ^b	1.28 ± 0.04	1.13 ± 0.03 ^b	<0.001	NS	<0.038 ³	NS	NS				
Total cholesterol (mmol/L)	5.11 ± 0.06	5.18 ± 0.06	5.11 ± 0.06	5.26 ± 0.06	5.14 ± 0.06	5.26 ± 0.06	NS	NS	NS	NS	NS				
LDL cholesterol (mmol/L)	3.23 ± 0.05	3.28 ± 0.05 ^a	3.26 ± 0.05	3.38 ± 0.05 ⁶	3.26 ± 0.05	3.38 ± 0.06 ^b	0.010	NS	NS	NS	NS				
HDL cholesterol (mmol/L)	1.44 ± 0.02	1.44 ± 0.02 ^a	1.43 ± 0.02	1.49 ± 0.02 ^b	1.44 ± 0.02	1.50 ± 0.02 ^b	<0.001	NS	NS	NS	NS				
VLDL cholesterol (mmol/L)	0.44 ± 0.02	0.46 ± 0.02 ^a	0.43 ± 0.02	0.39 ± 0.02 ^b	0.45 ± 0.02	0.38 ± 0.02 ^b	0.026	NS	NS	NS	NS				
LDL1 (% of total LDL)	19.0 ± 0.7	18.7 ± 0.7	19.4 ± 0.7	19.8 ± 0.7	19.0 ± 0.6	20.0 ± 0.7	NS	NS	NS	NS	NS				
LDL2 (% of total LDL)	49.0 ± 0.8	49.3 ± 0.8	48.3 ± 0.7	48.8 ± 0.7	48.4 ± 0.8	48.7 ± 0.7	NS	NS	NS	NS	NS				
LDL3 (% of total LDL)	31.8 ± 0.9	31.9 ± 0.8	32.2 ± 0.9	31.4 ± 0.8	32.3 ± 0.9	31.1 ± 0.8	NS	NS	NS	NS	NS				
HDL2 (% of total HDL)	63.6 ± 0.6	63.2 ± 0.6 ^a	62.9 ± 0.6	65.0 ± 0.5 ^b	63.3 ± 0.6	65.1 ± 0.6 ^b	<0.001	NS	<0.001 ³	NS	NS				
HDL3 (% of total HDL)	36.5 ± 0.6	36.8 ± 0.6 ^a	37.1 ± 0.6	35.0 ± 0.5 ^b	36.6 ± 0.6	35.0 ± 0.6 ^b	<0.001	NS	<0.001 ³	NS	NS				
apoA1 (μg/mL)	1326 ± 12	1341 ± 13	1323 ± 12	1337 ± 13	1331 ± 12	1344 ± 14	NS	NS	NS	NS	NS				
apoB (μg/mL)	807 ± 10	812 ± 11	810 ± 10	827 ± 10	813 ± 11	828 ± 11	NS	NS	NS	NS	NS				
apoE (μg/mL)	35.6 ± 0.7	36.4 ± 0.7 ^a	35.6 ± 0.7	36.5 ± 0.7 ^{a,b}	36.0 ± 0.7	36.7 ± 0.7 ^b	0.002	NS	0.029	NS	NS				
Glucose (mmol/L)	5.15 ± 0.03	5.52 ± 0.03	5.12 ± 0.03	5.16 ± 0.03	5.10 ± 0.03	5.18 ± 0.03	NS	NS	NS	NS	NS				
Insulin (μU/mL)	9.02 ± 0.32	7.51 ± 0.24	8.77 ± 0.25	7.65 ± 0.25	8.91 ± 0.26	7.77 ± 0.25	NS	NS	NS	NS	NS				
NEFA (umol/L)	404 ± 10	410 ± 10	409 ± 10	398 ± 10	391 ± 9	381 ± 11	NS	NS	NS	NS	NS				
oxLDL:apoB (U/L:μg/mL)	0.0816 ± 0.0010	0.0808 ± 0.0010 ^a	0.0822 ± 0.010	0.0801 ± 0.0010 ^a	0.0820 ± 0.0010	0.0820 ± 0.0011 ^b	0.030	NS	NS	NS	NS				
α-Tocopherol:TC (μmol:mmol)															
20–49 y old	10.24 ± 0.29	10.39 ± 0.61	11.26 ± 0.83	10.51 ± 0.65	10.77 ± 0.55	10.48 ± 0.55	NS	NS	NS	NS	NS				
50–59 y old	9.89 ± 1.08	8.94 ± 0.46 ^a	9.25 ± 0.57	8.59 ± 0.51 ^b	9.14 ± 0.64	8.54 ± 0.54 ^b	0.003	NS	NS	NS	NS				
60–70 y old	8.54 ± 0.39	8.62 ± 0.43 ^a	8.61 ± 0.45	8.49 ± 0.42 ^{a,b}	8.63 ± 0.43	8.12 ± 0.43 ^b	0.004	0.018 ⁴	NS	NS	NS				

¹ 0.7FO, 0.7 g eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)/d; 1.8FO, 1.8 g EPA+DHA/d; apo, apolipoprotein; NEFA, nonesterified fatty acids; oxLDL, oxidized LDL cholesterol; TC, total cholesterol. Repeated-measures analysis was undertaken on the outcome data (end-of-treatment values) with the baseline (beginning-of-treatment) values as covariates to determine the effect of treatment in the group as a whole and to examine the individual and combined effects of the design variables (ie, age, sex, and APOE genotype) on the response to treatment and to test for period and sequence effects. When a significant treatment effect was observed, posthoc analysis was conducted to determine which treatment groups were significantly different. Values in a row with different superscript letters are significantly different, $P < 0.017$.

² $\bar{x} \pm \text{SEM}$ (all such values).

³ In the men, the control oil, 0.7FO, and 1.8FO values were significantly different from each other. In the women, 0.7FO and 1.8FO values differed significantly from the control oil values, and 0.7FO and 1.8FO values did not differ significantly. A significant sex × genotype × treatment interaction also was evident ($P = 0.032$), and there was evidence of a greater effect of genotype on women than on men.

⁴ No effect of treatment was evident in E4 carriers.

60–70 y, an effect of treatment was evident for the α -tocopherol:TC response; a modestly lower ratio was seen in response to 1.8FO than in response to CO ($P = 0.04$). A significant ($P = 0.018$) treatment \times genotype interaction was also observed in this age group; there was little evidence of a fish-oil dose response in apoE2 and E4 subjects, but there was a significantly lower ratio in response to 1.8FO than in response to 0.7FO in the E3 subgroup.

DISCUSSION

To the best of our knowledge, the FINGEN Study is the most comprehensive investigation of the effect of moderate-dose fish-oil supplementation (<2 g EPA+DHA/d) on plasma lipid and lipoproteins and the first to investigate systematically the effect of age, sex, and *APOE* genotype on the response to modest fish-oil supplementation. In the subjects of the present study as a whole, administration of 0.7 or 1.8 g EPA+DHA/d resulted in an absolute change of 3.10% or 3.86% of total plasma PC FAs as LC n–3 PUFA, which equates to 43% and 69% increases from baseline for the 2 doses, respectively. The results of the current study were compared with those of previously reported trials conducted by our group (28, 31–34), as indicated in **Figure 3**, which plots EPA+DHA intakes against the increase from baseline in plasma phospholipid EPA+DHA content. Despite large inherent differences between these studies in terms of the supplementation periods (from 8 wk to 6 mo), age and sex mixes, and the DHA:EPA of the fish-oil supplements used (from 0.2 to 1.6), a highly significant correlation ($P < 0.001$) is evident. Regression analysis indicates an increase of ≈ 1.08 in the percentage of total FAs (EPA+DHA) for each 1-g increase in intake. Currently, because of the inherent difficulty in accurately quantifying EPA+DHA intakes from available dietary assessment methods, there is great interest in the potential use of the LC n–3 PUFA content of total lipid or lipid fractions from plasma, blood

cells, or accessible body tissues, such as adipose tissue, as biomarkers of short- or long-term intake. The current analysis highlights the potential usefulness of plasma phospholipid FA content as a biomarker of recent EPA+DHA intake. However, it should be noted that the strength of the association is in part attributable to the wide range of intakes for the studies included in Figure 3 (ie, 0.70–4.95 g EPA+DHA/d), which exceeds the normal ranges of intakes of these FAs in most Western populations. When the analysis was repeated including only those studies that administered more modest, dietarily achievable EPA+DHA intakes of <2.0 g/d, the strength of the correlation coefficient fell from 0.91 to 0.58, although it remained highly significant ($P = 0.006$) (data not shown).

The enrichment of EPA and total LC n–3 PUFA in plasma PC after fish-oil supplementation was significantly greater in females than in males, which could not be attributed to any differences in baseline LC n–3 PUFA status or compliance with treatment, and which highlights the need to consider sex when plasma phospholipid composition is used as a biomarker of LC n–3 PUFA intakes. However, this effect was ameliorated when these enrichment data were adjusted for the body mass of the volunteers, which suggests that the apparent sex difference in plasma EPA response was attributable to the greater dose per unit of body weight in females rather than to any physiologic effect on EPA metabolism. In contrast, there was a significantly greater weight-adjusted increase in plasma PC DHA in the men than in the women in response to fish-oil treatment.

Sex differences in ability to synthesize EPA and DHA from the parent essential n–3 PUFA, α -linolenic acid, have been observed previously; the capacity in premenopausal women is greater than that in men (35, 36). Our novel observation of greater enrichment of the weight-adjusted plasma PC pool of DHA suggests an effect of sex on DHA metabolism and partitioning. This finding is worthy of further investigation, because it has implications for the use of tissue concentrations as markers of status or intake in mixed-sex groups and also provides evidence of sex-mediated differences in tissue metabolism of LC n–3 PUFA, which may affect the responsiveness to supplementation. The current study provides some evidence to support the latter: the degree of differential responsiveness of plasma lipids and apolipoproteins to fish-oil intervention was influenced by sex.

Overall intakes of 0.7 and 1.8 g EPA+DHA/d resulted in 8% and 11% reductions in fasting TAG concentrations, respectively, and there was no significant dose response. Most previous placebo-controlled studies using doses comparable to the 1.8FO intake (1.5–1.8g EPA+DHA/d) failed to detect any significant effect of fish-oil intervention (37–40). However, closer examination of the data indicates a nonsignificant 7–18% fish oil-induced reduction in TAG concentrations in these studies; the lack of significance reflects the fact that TAG may have not have represented the primary study outcome, and the studies were underpowered ($n = 12$ –20/group) to detect more subtle (<20%) reductions in TAG concentrations. Retrospective power calculations based on the baseline fasting TAG concentrations in the current study of healthy subjects and a previous study of mildly hypertriglycerolemic persons (19), in which the investigators found mean \pm SD TAG concentrations of 1.24 ± 0.65 and 2.48 ± 0.88 mmol/L, respectively, indicate that group sizes of 231 and 98 would be necessary to detect 10% reductions in circulating TAG concentrations in response to modest fish-oil supplementation. Therefore, it is likely that a lack of study power

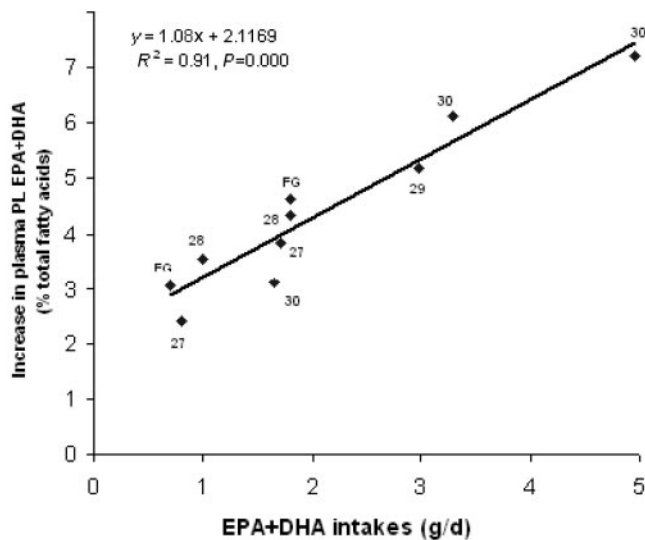


FIGURE 3. Relation between intakes of eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) and plasma phospholipid (PL) EPA+DHA enrichment. The data included in the analysis were derived from the current study and 5 previous trials conducted by our group (28, 31–34). The numbers on the figure relate to the study reference, and “FG” represents the current study (the FINGEN Study).

rather than a lack of a real hypotriacylglycerolemic response may be responsible for previously reported nonsignificant effects of doses of 1–2 g EPA+DHA/d on fasting TAG concentrations.

To date, few studies have examined the effect of EPA+DHA intakes of <1 g/d on TAG concentrations. In the current study, we report an 8% reduction in TAG in the group as a whole after consumption of 0.7 g EPA+DHA/d for 8 wk. A comparable modest but nevertheless significant placebo-corrected 4.8% reduction in TAG was observed in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico trial after supplementation with 0.9 g EPA+DHA/d for 26 wk (41).

We observed an indication of greater responsiveness to fish oil in males. Because the data were corrected for baseline concentration, this greater responsiveness in males is not simply reflective of higher fasting concentrations. Although the response to the 0.7 g EPA+DHA/d dose was similar in men and women, the lowering of TAG concentrations in response to the 1.8-g EPA+DHA/d dose in males was \approx 3 times that in females. This effect may be due to greater partitioning of EPA+DHA toward storage pools in females than in males and to a greater metabolism and utilization in males than in females, both of which differences are associated with the hypotriacylglycerolemic response. The TAG-lowering action of fish-oil FAs has been attributed, largely, to the activation of a number of hepatic transcription factors, which results in an up-regulation of expression of the genes encoding a number of β -oxidizing enzymes and a down-regulation of genes encoding lipogenic enzymes, which ultimately result in a smaller FA pool in the liver for VLDL synthesis and in lower VLDL-TAG secretion (42, 43).

The TAG-lowering effect of EPA+DHA is known to be mediated in part by apoE, because no hypotriacylglycerolemic effects are seen in apoE knockout rodents (44, 45). On the basis of that observation, of the central role of apoE in VLDL assembly and secretion into the circulation, and of its role as a high-affinity ligand for the hepatic removal of TRL remnants, it can be predicted that *APOE* genotype may influence the responsiveness to TAG lowering by fish oil. Although no significant effect of genotype was evident, there was a trend toward greater responsiveness in carriers of the ϵ 4 allele: a significant sex \times genotype \times treatment interaction was seen, and 15% and 23% reductions in TAG were evident in response to 0.7FO and 1.8FO in male ϵ 4 carriers, respectively. The selective affinity of the E4 protein isoform for VLDL (46), in contrast with the E2 and E3 isoforms, which have a preference for the more lipid-poor large HDL protein, may help explain the apparently greater TAG lowering in E4 persons.

Furthermore, consistent with our previous study (47), modest intakes of EPA+DHA resulted in higher circulating apoE concentrations. The effect was not *APOE* genotype-dependent and was evident only in females. Given the dual role of apoE as an inhibitor of VLDL synthesis (and therefore of TAG secretion into the circulation) and in TAG removal, the contribution of these modest changes in apoE to the overall TAG lowering observed and to the sex-specific differences is difficult to interpret.

In addition, fish-oil treatment resulted in higher HDL concentrations and a higher proportion of larger HDL2 particles, both of which are consistent with previous studies (48). Because HDL size is greatly dependent on circulating TAG concentrations (through the system of neutral lipid exchange), the contribution of TAG lowering to the significant increases in HDL size was examined. A weak but significant association between changes

in TAG and HDL size in response to treatment was evident. However, a significant effect of treatment remained after correction of the model for TAG changes, which indicates that there are TAG-independent effects of fish oils on HDL size. We speculate that these effects may be due, at least in part, to the reported effect of fish oils on hepatic lipase activity (49), which would result in less lipolysis of TAG-enriched HDL₂ particles into the smaller HDL₃ particles. As with TAG lowering, we observed a greater effect on HDL size in males: the reductions in response to fish-oil intervention were 8–11% and 2–4% in males and females, respectively. The greater TAG lowering in males, together with a potentially greater effect of EPA+DHA on hepatic lipase gene expression, is likely to be responsible for this greater positive effect on HDL size in men.

Two commonly cited, potentially deleterious effects of high-dose (>3 g EPA+DHA/d) fish-oil intervention are an LDL cholesterol-raising effect (15, 19) and an effect on whole-body oxidative status (50). However, the effects, if any, of lower intakes of fish oil on these markers of CVD risk are largely unknown. In the current study, the 2–3% increases in LDL cholesterol were much lower than those reported with higher intakes of EPA+DHA (15, 19), and it is unlikely that they are of much clinical significance. Increases in LDL cholesterol after EPA+DHA intervention have been attributed to the known effect of EPA and DHA on VLDL-TAG output with respect to a shift from the larger VLDL1 to the smaller TAG-depleted VLDL2 particle, which acts as a precursor for LDL synthesis (51). In contrast with our previous study, in which an effect of genotype on the LDL cholesterol response was evident—7% increases were observed in the group as a whole, and 3%, 1%, and 16% increases were observed in E2, E3, and E4 subgroups, respectively, after 3 g EPA+DHA/d (19)—there was no significant effect of genotype in the current study. Taken together, these data suggest that the effect of *APOE* genotype on the LDL-cholesterol response may be dose dependent.

Although a significant effect of treatment on oxLDL concentrations was observed, closer examination of the data shows that the significant between-group differences are due to lower concentrations in the placebo and 0.7FO groups and that there was no effect of treatment in the 1.8FO group. The effect on circulating α -tocopherol appears to be age dependent, and there was no effect of treatment in the age categories of <50 y. The greatest reductions were in response to CO in the group aged 50–59 y, and the significant (6%) reduction in the group aged 60–70 y after 1.8FO supplementation is unlikely to be of wide clinical significance. Overall, although some significance was achieved, and although data on urinary isoprostane (considered one of the most robust currently available biomarkers of oxidative status) are not available, the results of the current trial indicate that <2 g EPA+DHA/d intervention has little effect on oxidative status. This finding is in agreement with a review article in the area of study (52).

In conclusion, in contrast to what had been previously thought, the plasma lipid-modulating effects of LC n–3 PUFA are evident at doses as low as 0.7 g/d. At a population level, the overall clinical significance of the 0–10% TAG, cholesterol, and HDL and LDL size changes may be modest, but, for certain persons, such as males with an *APOE4* genotype (\approx 11% of whites), the lipid-modulatory effects observed at these EPA-DHA doses are likely to have a significant effect on CVD risk. Furthermore, in an era when we are moving away from generic dietary advice



toward a more personalized approach to nutritional advice (53), there is a great need to establish the various responses to treatment in population subgroups, as has been conducted in the current trial.

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The authors' responsibilities were as follows—MJC, GL, ACK, JCM, CP, CMW, PCC, and AMM (the study management group): were responsible for designing the study and supervising all aspects of the reported work; EAM, BMK, PC, CKA, JPG, LF, JS, and FLN: recruited and screened volunteers, carried out the intervention, and collected the blood samples and anthropometric, questionnaire, and compliance data; EAM, BMK, PC, AS-V, ALW, and CKA: laboratory analysis; PC: dietary analysis; ACK and AMM: statistical analysis; AMM: wrote the draft of the manuscript; and all authors: contributed to the final version of the manuscript. CMW is a consultant to Pepsico UK and Unilever Plc. PCC is a consultant to Equazen, Royal Dutch Unilever, and Mead Johnson Nutritionals and accepts speaking fees from Solvay Healthcare, Solvay Pharmaceuticals, B Braun Melsungen, and Fresenius Kabi. None of the other authors had a personal or financial conflict of interest.

REFERENCES

- Bang HO, Dyerberg J. Plasma lipids and lipoproteins in Greenlandic west coast Eskimos. *Acta Med Scand* 1972;192:85–94.
- Bucher HC, Hengstler P, Schindler C, Meier G. n-3 Polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *Am J Med* 2002;112:298–304.
- He K, Song Y, Daviglus ML, et al. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. *Circulation* 2004;109:2705–11.
- Hooper L, Thompson RL, Harrison RA, et al. Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. *BMJ* 2006;332:752–60.
- Konig A, Bouzan C, Cohen JT, et al. A quantitative analysis of fish consumption and coronary heart disease mortality. *Am J Prev Med* 2005;29:335–46.
- Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2003;23:e20–30.
- Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. *JAMA* 2006;296:1885–99.
- Wang C, Harris WS, Chung M, et al. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *Am J Clin Nutr* 2006;84:5–17.
- Whelton SP, He J, Whelton PK, Muntner P. Meta-analysis of observational studies on fish intake and coronary heart disease. *Am J Cardiol* 2004;93:1119–23.
- Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* 2007;369:1090–8.
- Leaf A. Omega-3 fatty acids and prevention of arrhythmias. *Curr Opin Lipidol* 2007;18:31–4.
- Thies F, Garry J, Yaqoob P, et al. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet* 2003;361:477–85.
- Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. *Atherosclerosis* 2006;189:19–30.
- Chin JP, Gust AP, Nestel PJ, Dart AM. Marine oils dose-dependently inhibit vasoconstriction of forearm resistance vessels in humans. *Hypertension* 1993;21:22–8.
- Harris W. n-3 Fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr* 1997;65(suppl):1645S–54S.
- Mori TA. Omega-3 fatty acids and hypertension in humans. *Clin Exp Pharmacol Physiol* 2006;33:842–6.
- Woodman RJ, Mori TA, Burke V, et al. Effects of purified eicosapentaenoic acid and docosahexaenoic acid on platelet, fibrinolytic and vascular function in hypertensive type 2 diabetic patients. *Atherosclerosis* 2003;166:85–93.
- Lichtenstein AH, Appel LJ, Brands M, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* 2006;114:82–96.
- Minihane AM, Khan S, Leigh-Firbank EC, et al. ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype. *Arterioscler Thromb Vasc Biol* 2000;20:1990–7.
- Minihane AM, Jofre-Monseny L, Olano-Martin E, Rimbach GH. Apo-lipoprotein E genotype, cardiovascular risk and responsiveness to dietary fat manipulation. *Proc Nutr Soc* 2007;66:183–97.
- Henderson L, Gregory J, Irving K, Swan G. The National Diet & Nutrition Survey: adults aged 19 to 64 years. London, United Kingdom: HMSO, 2003.
- Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *HhaI*. *J Lipid Res* 1990;31:545–8.
- Edelstein C, Scanu M. Precautionary measures for collecting blood destined for lipoprotein isolation. *Methods Enzymol* 1986;128:151–5.
- Lipid Research Clinics Program manual of laboratory operations. Washington, DC: DHEW Publications, 1975.
- Perseghin G, Caumo A, Caloni M, Testolin G, Luzi L. Incorporation of the fasting plasma FFA concentration into QUICKI improves its association with insulin sensitivity in nonobese individuals. *J Clin Endocrinol Metab* 2001;86:4776–81.
- Blanche PJ, Gong EL, Forte TM, Nichols AV. Characterization of human high-density lipoproteins by gradient gel electrophoresis. *Biochim Biophys Acta* 1981;665:408–19.
- Aebischer P, Schierle J, Schuep W. Simultaneous determination of retinol, tocopherols, carotene, lycopene, and xanthophylls in plasma by means of reversed-phase high-performance liquid chromatography. *Methods Enzymol* 1999;299:348–62.
- Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA, Calder PC. Dietary supplementation with eicosapentaenoic acid, but not with other long-chain n-3 or n-6 polyunsaturated fatty acids, decreases natural killer cell activity in healthy subjects aged >55 y. *Am J Clin Nutr* 2001;73:539–48.
- Welch AA, Lund E, Amiano P, et al. Variability of fish consumption within the 10 European countries participating in the European Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr* 2002;5:1273–85.
- McCance and Widdowson's the composition of foods: summary edition. 6th ed. London, United Kingdom: The Royal Society of Chemistry, 2002.
- Finnegan YE, Minihane AM, Leigh-Firbank EC, et al. Plant- and marine-derived n-3 polyunsaturated fatty acids have differential effects on fasting and postprandial blood lipid concentrations and on the susceptibility of LDL to oxidative modification in moderately hyperlipidemic subjects. *Am J Clin Nutr* 2003;77:783–95.
- Grimble RF, Howell WM, O'Reilly G, et al. The ability of fish oil to suppress tumor necrosis factor α production by peripheral blood mononuclear cells in healthy men is associated with polymorphisms in genes that influence tumor necrosis factor α production. *Am J Clin Nutr* 2002;76:454–9.
- Miles EA, Banerjee T, Calder PC. The influence of different combinations of gamma-linolenic, stearidonic and eicosapentaenoic acids on the fatty acid composition of blood lipids and mononuclear cells in human volunteers. *Prostaglandins Leukot Essent Fatty Acids* 2004;70:529–38.
- Rees D, Miles E, Banerjee T, et al. Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. *Am J Clin Nutr* 2006;83:331–42.
- Burdge GC. Metabolism of alpha-linolenic acid in humans. *Prostaglandins Leukot Essent Fatty Acids* 2006;75:161–8.
- Burdge GC, Calder PC. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev* 2005;45:581–97.
- Brown AJ, Roberts DC. Moderate fish oil intake improves lipemic response to a standard fat meal. A study in 25 healthy men. *Arterioscler Thromb* 1991;11:457–66.



38. Finnegan YE, Howarth D, Minihane AM, et al. Plant and marine derived (n-3) polyunsaturated fatty acids do not affect blood coagulation and fibrinolytic factors in moderately hyperlipidemic humans. *J Nutr* 2003;133:2210-3.
39. Oosthuizen W, Vorster HH, Jerling JC, et al. Both fish oil and olive oil lowered plasma fibrinogen in women with high baseline fibrinogen levels. *Thromb Haemost* 1994;72:557-62.
40. Valdini AF, Glenn MA, Greenblatt L, Steinhardt S. Efficacy of fish oil supplementation for treatment of moderate elevation of serum cholesterol. *J Fam Pract* 1990;30:55-9.
41. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* 1999;354:447-55.
42. Harris WS, Bulchandani D. Why do omega-3 fatty acids lower serum triglycerides? *Curr Opin Lipidol* 2006;17:387-93.
43. Jump DB, Botolin D, Wang Y, Xu J, Christian B, Demeure O. Fatty acid regulation of hepatic gene transcription. *J Nutr* 2005;135:2503-6.
44. Asset G, Bauge E, Fruchart JC, Dallongeville J. Lack of triglyceride-lowering properties of fish oil in apolipoprotein e-deficient mice. *Arterioscler Thromb Vasc Biol* 2001;21:401-6.
45. Zampolli A, Bysted A, Leth T, Mortensen A, De Caterina R, Falk E. Contrasting effect of fish oil supplementation on the development of atherosclerosis in murine models. *Atherosclerosis* 2006;184:78-85.
46. Dong LM, Weisgraber KH. Human apolipoprotein E4 domain interaction. Arginine 61 and glutamic acid 255 interact to direct the preference for very low density lipoproteins. *J Biol Chem* 1996;271:19053-7.
47. Buckley R, Shewring B, Turner R, Yaqoob P, Minihane AM. Circulating triacylglycerol and apoE levels in response to EPA and docosahexaenoic acid supplementation in adult human subjects. *Br J Nutr* 2004;92:477-83.
48. Tholstrup T, Hellgren LI, Petersen M, et al. A solid dietary fat containing fish oil redistributes lipoprotein subclasses without increasing oxidative stress in men. *J Nutr* 2004;134:1051-7.
49. Harris WS, Lu G, Rambjor GS, et al. Influence of n-3 fatty acid supplementation on the endogenous activities of plasma lipases. *Am J Clin Nutr* 1997;66:254-60.
50. Dolores M, Buckley R, Minihane A, Yaqoob P. Effects of oils rich in eicosapentaenoic and docosahexaenoic acids on the oxidisability and thrombogenicity of low density lipoprotein. *Atherosclerosis* 2004;175:333-43.
51. Lu G, Windsor SL, Harris WS. Omega-3 fatty acids alter lipoprotein subfraction distributions and the in vitro conversion of very low density lipoproteins to low density lipoproteins. *J Nutr Biochem* 1999;10:151-8.
52. Mori TA. Effect of fish and fish oil-derived omega-3 fatty acids on lipid oxidation. *Redox Rep* 2004;9:193-7.
53. Joost HG, Gibney MJ, Cashman KD, et al. Personalised nutrition: status and perspectives. *Br J Nutr* 2007;98:26-31.

