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***APOE* genotype modifies the association between plasma measured omega-3 fatty acids and plasma lipids in the Multi-Ethnic Study of Atherosclerosis (MESA)**

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Abstract

Objective—The benefits of fish oil fatty acids eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) on plasma lipid profiles have been inconsistent but may partially depend on individual Apolipoprotein E (*APOE*) genotypes. We aimed to determine whether *APOE* genotype modifies the association of lipid profile characteristics with plasma EPA and DHA levels.

Methods—*APOE* genotype was determined in this cross-sectional analysis of 2340 Multi-Ethnic Study of Atherosclerosis (MESA) participants. Relative plasma phospholipid EPA and DHA levels, plasma lipids, and lipoprotein subclass particle sizes and concentrations were measured.

Results—Significant gene-EPA interactions were found with HDL-C, and particle concentrations of large and total HDL ($p_{\text{interaction}} = 0.0002, 0.006, \text{ and } 0.007$, respectively). The above lipid targets were positively associated with EPA in the E2 groups, whereas negative trends were observed among the E4 participants. Gene-DHA interactions were noted for small LDL particle concentrations alone ($p_{\text{interaction}} = 0.01$), where a positive trend was found among E4 but not E2 or E3 participants.

Conclusions—These results indicate a significant contribution of the *APOE* genotype to the EPA-lipid profile relationship; however, the results do not explain the differences in previous findings regarding LDL-C, triglycerides or total cholesterol. Future investigators examining the

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effects of EPA on HDL-C or lipoprotein characteristics may consider including *APOE* genotype in their analyses.

Keywords

APOE genotype; eicosapentaenoic acid (EPA); docosahexaenoic acid (DHA); plasma lipids; lipoproteins

Introduction

The cardiovascular health benefits of fish consumption and fish oil supplementation have been examined in a host of nutritional and epidemiological studies [1-10] and are largely attributed to the marine omega-3 fatty acids (FAs), namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Indeed, EPA and DHA have been reported to reduce arrhythmia [11-13], thrombosis [14, 15], inflammation [9], resting heart rate and systolic and diastolic blood pressure [16,17] as well as beneficially influence blood lipids and lipoproteins [5, 18]. Of these, the relationships of EPA/DHA with blood lipids including total cholesterol, LDL-C, and HDL-C are largely controversial with significant variations found between study populations.

To our knowledge, a limited number of reports have found significant associations of plasma and membrane EPA/DHA with blood lipids. Generally, it has been found that EPA positively associates with HDL-C [19-26] and negatively associates with triglycerides (TGs) [19, 21, 22, 24, 26]; though some studies have found no associations between EPA and TGs [20, 23]. Similarly, associations between DHA and TGs, HDL-C and LDL-C have been observed [20, 21, 22, 24, 26]; again, null findings have also been reported [23, 25]. Though the reason(s) for the above discordance is unclear, there are likely multiple factors involved including, but not limited to, diet, race, baseline characteristics of the study populations, and genetic factors. The present study examines the potential contribution of one of these variables, the Apolipoprotein E (*APOE*) genotype.

APOE is among the most extensively studied genes in the context of cardiovascular disease, and it has been found that *APOE* genotypes E2, E3 and E4 modify the cholesterol and lipoprotein responses to dietary fat intake [27-29]. For example, it has generally been shown that carriers of the *APOE* E4 genotype demonstrate a more robust reduction in LDL-C in response to reduced saturated fat intake, although results are highly inconsistent among studies [27-29]. Though a limited number of studies have examined whether the *APOE* genotype contributes to the lipid response to fish oil fatty acids, they have yielded inconsistent results—with two studies supporting an *APOE*-dependent effect [30, 31], and a third reporting the null finding [32].

The present study examines whether the *APOE* genotype interacts with fish oil FAs and lipid/lipoprotein profile in a large scale cross-sectional analysis of 2,340 free-living participants of the Multi Ethnic Study of Atherosclerosis (MESA). To more accurately characterize lipid profile, lipoprotein particle sizes and concentrations have been included, in addition to the commonly used measures of total cholesterol, LDL-C, HDL-C, and triglycerides. We avoided the inherent problems of assessing EPA and DHA dietary intakes by directly measuring their plasma levels in the phospholipid fraction—known to reflect both dietary intake [33] and cell membrane composition [34].

MATERIALS AND METHODS

2.1. Population

The design of the MESA study was previously described [35], and information about the MESA protocol is available at www.mesa-nhlbi.org. Briefly, 6814 men and women between the ages of 45 and 84 years without clinical evidence of cardiovascular disease were recruited from 6 communities in the US. Subjects who self-reported their race/ethnicity group as White or European-American, Black or African-American, Spanish/Hispanic/Latino, or Chinese-American were potentially eligible. Institutional Review Board approval was obtained at all MESA sites, and all participants gave informed consent.

The current study considered a subpopulation of 2,880 adults from the MESA cohort with approximately equal representation from four racial/ethnic groups (African-Americans, European-Americans, Chinese-Americans and Hispanics). All study participants gave informed consent (MESA Genetics Candidate Gene Evaluation Cohort). In our analyses, we further excluded those taking lipid-lowering drugs (n= 634) or missing data (n=54) as well as participants with the E2/E4 of *ApoE* genotype. The final sample consisted of 2340 participants (554 African-Americans, 601 Chinese-Americans, 589 European-Americans, and 596 Hispanic-Americans).

2.2 Plasma lipids and lipoprotein measurements

Plasma triglyceride, total cholesterol, HDL-C and LDL-C concentrations were measured as described previously [36]. LDL and HDL subclass particle concentrations were determined in the MESA study using nuclear magnetic resonance (NMR) spectroscopy and the LipoProfile-3 algorithm at LipoScience Inc., North Carolina[37]. Particle concentrations were determined for 2 LDL subclasses [small LDL (18.0 to 20.5 nm) and large LDL (20.5 to 23.0 nm)] and 3 HDL subclasses [small HDL (7.3-8.2 nm), medium HDL (8.2-9.4 nm), and large HDL (9.4-14.0 nm)]. Total LDL, and HDL particle concentrations as well as weighted-average particle sizes were calculated.

2.3. Plasma phospholipid fatty acid measurement

Phospholipid fatty acids were extracted from EDTA plasma using the method previously described by Cao et al [38]. Briefly, lipids were extracted from the plasma using a chloroform/methanol extraction method and the cholesterol esters, triglycerides, phospholipids and free fatty acids were separated by thin layer chromatography. Fatty acids from the phospholipids were converted to methyl esters and detected by gas chromatography that is configured for a single capillary column with a flame ionization detector. The fatty acids detected were expressed as a percent of total fatty acids. The following representative CVs were obtained from intra-laboratory quality control testing (n=20): EPA, 3.3% and DHA, 2.7%.

2.4. DNA Isolation and Genotyping

Genomic DNA was extracted from peripheral leukocytes isolated from packed cells of anticoagulated blood using a commercially available DNA isolation kit (Puregene®, Qiagen Instrument Service, Germantown, MD). DNA was analyzed for *APOE* genotypes (E2, rs7412, and E4, rs429358) using Applied Biosystems TaqMan SNP system (ABI# C_904973_10 and C_3084793_20, respectively). Based on the *APOE* genotype, participants were classed into 3 groups: E2 (E2/E2 and E2/E3 genotypes), E3 (E3/E3 genotype) and E4 (E3/E4 and E4/E4 genotypes), and individuals with the E2/E4 genotype were excluded from the analysis, as the E2 and E4 alleles have been shown with opposite effects on plasma lipids and lipoproteins [39-41].

2.5. Statistical Analysis

SAS, version 9.2 (SAS Institute, Cary, NC) was used to perform all data analysis. Descriptive statistics including mean (standard deviation) and frequencies across *APOE* genotypes were determined. Linear regression analysis with robust standard errors were used to evaluate the effect of plasma phospholipid fatty acids on measures of lipids and lipoproteins and the modifying effect of *APOE* genotype on the fatty acid-lipid association. *APOE* genotype, fatty acids, and their interactions were the primary predictor of interest. *APOE* genotypes were coded as categorical for the three genotype groups (E2, E3, and E4). Other covariates included age, sex, self-reported ethnic group, field center, education, smoking status, alcohol use, total intentional exercise, BMI, and diabetes; triglycerides was also used as a covariate for outcomes other than triglycerides. Additionally, the first five principal components from analysis of ancestry informative markers (AIMs) were included to adjust for population stratification. To capture non-linear relationship between lipid and fatty acid, a squared term of fatty acid was also included in the model. Higher orders of quadratic terms were also tested, but not significant for most of lipid profiles. The fatty acids-lipids associations and *APOE*-fatty acid interactions were considered statistically significant if $p < 0.05$.

RESULTS

APOE genotypes (E2, E3 and E4) of 2340 study participants were distributed as follows: 0.77% E2/E2 ($n=18$), 13.46% E2/E3 ($n=315$), 62.35% E3/E3 ($n=1459$), 21.75% E3/E4 ($n=509$), and 1.67% E4/E4 ($n=39$). The allele frequencies did not deviate significantly from those predicted by the Hardy-Weinberg equilibrium. Baseline demographic, lifestyle and clinical characteristics of the study population are shown in Table 1.

The adjusted mean levels of plasma lipids, lipoproteins, and plasma phospholipid fatty acids by *APOE* groups are presented in Table 2. It was found that the E2 group had a relatively atheroprotective lipid profile, whereas the E4 group had a more atherogenic lipid profile compared to E3 homozygotes. Specifically, E2 participants had lower levels of total cholesterol, LDL-C, and LDL particle (small, large and total) concentrations as well as higher levels of HDL-C and medium and total HDL particle concentrations compared to either E3 or E4 groups. E4 participants showed a more atherogenic phenotype with higher mean levels of small and total LDL particle concentrations, lower HDL-C levels, smaller mean LDL particle size, as well as lower medium and total HDL particle concentrations compared to the E3 group (all $p < 0.017$). In contrast to plasma lipids/lipoproteins, mean levels of plasma phospholipid EPA or DHA did not differ among *APOE* groups. In addition, a moderate correlation ($r=0.64$) was observed between EPA and DHA.

The interactions between *APOE* genotypes and plasma phospholipid EPA or DHA were tested, and statistically significant interactions ($p < 0.05$) are presented in Tables 3 and 4. Slopes between plasma phospholipid fatty acids and plasma lipids/lipoprotein particles by *APOE* groups were estimated from linear combination of regression coefficients of the main and interaction terms. The slopes are expressed as average change in each outcome (mmol/L, nmol/L, $\mu\text{mol/L}$, or nm) for every 1% increase in fatty acid. Slopes were considered statistically significant with $p < 0.017$, adjusting for the multiple tests in the three genotype groups. As shown in Table 3, the associations between plasma phospholipid EPA and several plasma lipids/lipoproteins outcomes were modified by *APOE* genotype. Specifically, HDL-C and large HDL particle concentrations showed significant positive associations with EPA in those with the E2 allele ($p_{\text{interaction}}=0.0002$ and 0.006 , Fig. 1.A. and B.), but not in those with E3 or E4 genotypes. In addition, regarding to total HDL particle concentration, although the positive association with EPA in the E2 group did not reach statistical significance, it is significantly different from the negative total HDL-EPA association in the

E4 group ($p_{\text{interaction}}=0.007$, Fig.1.C.). A similar but weaker *APOE*-EPA interaction was found with mean HDL particle size ($p_{\text{interaction}}=0.03$, Figure not shown). Borderline significant *APOE*-EPA interactions were also found for small LDL and mean LDL particle size ($p_{\text{interaction}}=0.04$, and 0.05 respectively, figures not shown). Specifically, significant positive association between small LDL and EPA, and significant negative association between mean LDL particle size and EPA were found only in the E4 group, but not in E2 or E3 groups.

As shown in Table 4, a significant *APOE*-DHA interaction was observed for small LDL particle concentration ($p_{\text{interaction}}=0.01$, figure not shown). A positive association between plasma DHA and small LDL particle concentration was seen in the E4 group, and this was significantly different from the negative trends in the E2 and E3 groups. A suggestive ApoE-DHA interaction was found with medium HDL particle concentration ($p=0.05$, figure not shown), where the negative association between DHA and medium HDL particles in the E3 group was weaker than that in E2 and E4 groups.

For outcomes where no significant gene-fatty acid interactions were identified, the genotype-independent associations between fatty acids and plasma lipids/lipoproteins were tested (Table 5). EPA was positively associated with total Cholesterol (slope=0.14, $p<0.0001$), LDL-C (slope=0.12, $p=0.005$), total LDL particle concentration (slope=81.3, $p<0.0001$), and small HDL particle concentrations (slope=0.49, $p=0.01$), while it is negatively associated with medium HDL particle concentration (slope=-0.66, $p=0.008$). On the other hand, DHA was positively associated with small HDL particle concentration (slope=1.14, $p=0.0003$).

DISCUSSION

Among this multi-ethnic prospective study population of generally healthy adults, we observed that *APOE* genotype differentially modified the association of plasma phospholipid EPA with: 1) HDL-C levels; 2) large HDL particle concentrations; 3) total HDL particle concentrations; 4) HDL particle size; 5) small LDL particle concentrations; and 6) LDL particle size. *APOE* also modified the associations of plasma phospholipid DHA with small LDL and medium HDL particle concentrations. No relation between *APOE* and plasma phospholipid EPA or DHA was observed.

To date, two intervention studies have demonstrated influences of *APOE* on the plasma lipid response to high dose EPA and/or DHA [30, 31]. Olano-Martin et al. [31] showed reductions of total cholesterol (TC) following four weeks of EPA (3.3g/day), but only in E4 carriers. In addition, they found that DHA (3.7g/day) increased LDL-C and LDL mass over the same time period, again only in E4 carriers. No differences were observed in small LDL for either treatment, regardless of genotype. In contrast, Minihane et al. [30] found that EPA +DHA (6g/day) for 6 weeks resulted in a significant decrease in small LDL in individuals with an atherogenic lipid profile; notably, the magnitude of the decrease was greater in E4 carriers compared to E3 homozygotes. E4 carriers showed a modest but significant *increase* in TC compared to the modest decrease observed in E3 homozygotes. Finally, the FINGEN study reported a sex-treatment-genotype effect on lipid responses following 8 weeks of fish oil treatment, with the greatest triglyceride-lowering effect observed in E4 males [32]. Though no effect was observed for genotype alone, it should be noted that 1) a trend toward greater lipid responsiveness was found in E4 carriers, and; 2) FINGEN participants were given lower doses of EPA+DHA (0.7g or 1.8g/day) compared to the previous studies. As a dose-response was reported in the FINGEN study, it is reasonable to hypothesize that an *APOE* effect may have reached significance with greater EPA+DHA intake.

In contrast to these interventional studies that used fish oil interventions, the present analysis in a free-living study population showed *APOE* modified the relations between plasma phospholipid EPA and DHA with small LDL. These interactions with LDL may be partially explained by the effects of the *APOE* E4 genotype and EPA/DHA on LDL metabolism. First, EPA/DHA supplementation has been found to modestly increase plasma LDL-C—a phenomenon that is likely the result of increased lipoprotein lipase activity that converts VLDL remnants to LDL particles [42–44]. Couple this effect with that of ApoE protein—a ligand for the LDL-receptor present on chylomicrons, VLDL, and HDL, but not LDL [45]. Compared to the E3 and E2 isoforms, the ApoE E4 isoform preferentially binds to chylomicrons and VLDL, leading to higher concentration of ApoE on these triglyceride-rich particles [46, 47], and ultimately resulting in both increased competition with LDL for the LDL-receptor and delayed LDL clearance. This delay in LDL clearance allows for cholesterol ester transfer protein greater time to exchange LDL-cholesterol esters for VLDL-triglyceride. The resulting triglyceride-enriched, cholesterol ester-depleted LDL particles are then acted upon by hepatic lipase, forming small dense LDL particles. Altogether, the synergic effects EPA/DHA and the ApoE E4 isoform may partially explain the stronger associations of small LDL with EPA and DHA in E4 carriers.

In E2 carriers, we observed that high plasma phospholipid EPA was associated with higher levels of HDL-C and large HDL particle concentration—a potential atheroprotective influence. These findings are not observed by Minihane et al. [30], who reported that E2 carriers showed no significant change in total cholesterol or HDL-C following supplementation with fish oil. The discrepant findings may be explained by the relatively weak association in the present cross-sectional analysis and/or by a lack of power in the study by Minihane et al. [30]. Similar to the aforementioned findings with LDL, the interactions with HDL may be accounted for by the known effects of ApoE and EPA on HDL metabolism. n-3 fatty acids supplementation has been reported to influence hepatic uptake of HDL-C [48] as well as the process of reverse cholesterol transport [49, 50]. In addition, the ApoE protein has been shown to impact HDL clearance [51], promote cholesterol-rich core expansion in HDL [52], and facilitate cholesterol efflux from macrophages [53, 54]. Evidence suggests that apoE3 and apoE4 isoforms have differential effects on HDL metabolism [55]. Overall, ApoE and EPA may influence common pathways of HDL metabolism and partially explain the observed interactions with HDL-C and HDL particles.

Apart from the above interactions, we found genotype-independent associations among plasma phospholipid EPA/DHA and plasma lipids/lipoproteins. Notably, different plasma fractions as well as erythrocyte membrane EPA and DHA have been previously associated with TC, TGs, LDL-C and HDL-C. An initial study by Lindeberg et al. [19] reported that EPA levels in the plasma cholesterol ester fraction positively associated with HDL-C and negatively associated with triglyceride levels. Two subsequent studies using the plasma phospholipid fraction confirmed these findings and reported positive associations of EPA with TC and LDL-C [21, 22]. Similar associations with erythrocyte membrane EPA have also been reported among TC, LDL-C, HDL-C and TGs [24]. In contrast, the associations of EPA with TC and LDL-C were not found by Lindqvist et al. [23]. In addition, Sun et al. [26] only showed that plasma but not erythrocyte membrane EPA positively associated with HDL-C, although both negatively associated with TGs. Overall, EPA levels have been found to associate with HDL-C, LDL-C, and TC which are reflected by our findings. The negative correlation of EPA with TGs observed by most investigators was not found in the present analysis, though our statistical model was more conservative than previous cross-sectional studies by using a robust standard error and including square terms to capture non-linearity of associations.

Similar to EPA, DHA has been shown to associate with HDL-C, TC, and TG levels, though findings have been more inconsistent than those of EPA. Investigators have reported direct associations of DHA with TC and LDL-C [24] as well as HDL-C [21, 22]. Associations with TGs have been reported in both negative [24, 26] and positive [20] directions. In agreement with our findings, no associations of plasma DHA and blood lipids have been shown recently [23, 25]. Contrary to our hypothesis, we found no evidence that the above variations in study findings are attributable to difference in *APOE* genotype, as no interactions were observed with DHA and these lipids targets.

There are several strengths as well as limitations of the present study. First, EPA and DHA fatty acids were directly measured in the plasma phospholipid fraction, providing a more accurate and physiologically relevant assessment of omega-3 levels than food frequency questionnaires based on dietary recalls. However, it is important to acknowledge that the phospholipid fraction reflects the dietary fatty acid intake of the previous week, and are therefore less ideal than red blood cell membrane fatty acids, which reflect the diet in the past several weeks. Second, the present study is the largest to examine the interaction of *APOE* genotype between n-3 fatty acids and plasma lipids, thus ensuring adequate statistical power, particularly for those with the low allele frequency, E2 genotype. Despite the above strengths, the observational cross-sectional study design only allows determination of associations, but not the temporal relations of EPA/DHA with lipids/lipoproteins. Finally, although multiple adjustments were made within the statistical models, the potential for residual confounding remains.

In conclusion, the present analysis shows significant interactions among *APOE* and plasma phospholipid EPA/DHA on HDL-C as well as lipoprotein characteristics. As the two variant alleles of *APOE*, E2 and E4, make up a significant portion of the population, future studies may consider appropriately stratifying their populations or otherwise statistically adjusting for *APOE* genotype. A large-scaled interventional study with EPA and/or DHA supplementation may be warranted to confirm these findings and determine whether EPA and/or DHA may have unwanted clinical effects on lipoprotein characteristics in carriers of the E4 genotype. As we found no modification effect of *APOE* on EPA/DHA with LDL-C, TC, or TGs, other factors likely explain the inconsistencies in study findings.

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Highlights

We measured levels of plasma phospholipid EPA and DHA in 2340 participants from the Multi-Ethnic Study of Atherosclerosis that were without clinical evidence of cardiovascular disease.

Lipoprotein subclass particle concentrations were measured with nuclear magnetic resonance (NMR) and ApoE was genotyped for all participants.

Significant *APOE*-EPA interactions were found with HDL-C, and particle concentrations of large and total HDL.

An *APOE*-DHA interaction was found for small LDL particle concentrations

Figure 1.

Mean($\pm$ SE) levels of HDL-C (A), large HDL particle concentration (B) and total HDL particle concentration (C) are shown by *APOE* genotypes and tertiles of plasma EPA. Values were adjusted for age, sex, race, field center, ancestry informative marker principal components (PC1-PC5), education, smoking status, alcohol use, total intentional exercise, BMI, diabetes, and triglyceride.

Table 1

Unadjusted Baseline Characteristics of MESA participants

	All (n=2340)	APOE allele ^I		
		E2 (n=333)	E3 (n=1459)	E4(n=548)
Demographics				
Age, y (SD)	60.80 (10.22)	61.36 (10.38)	61.02 (10.27)	59.86 (9.97)
Sex, (% of male)	1097 (46.88)	156 (46.85)	702 (48.12)	239 (43.61)
Race, n (%)				
African-Americans	554 (23.68)	101 (30.33)	265 (18.16)	188 (34.31)
Chinese-Americans	601 (25.68)	91 (27.33)	416 (28.51)	94 (17.15)
European-Americans	589 (25.17)	91 (27.33)	359 (24.61)	139 (25.36)
Hispanic-Americans	596 (25.47)	50 (15.02)	419 (28.82)	127 (23.18)
Education, n (%) > high school	1410 (60.26)	212 (63.66)	852 (58.40)	346 (63.14)
Lifestyle Factors				
Total Intentional Exercise, Metabolic Equivalent, min/week (SD)	1486.76 (2205.96)	1339.88 (1644.74)	1439.71 (2257.24)	1701.25 (2349.21)
Current Smoker, n(%)	336 (14.36)	52 (15.62)	196 (13.43)	88 (16.60)
Current Alcohol, n(%)	1179 (50.75)	171 (51.82)	707 (48.73)	301 (55.54)
Clinical Factors				
BMI, kg/m²(SD)	27.70 (5.51)	27.54 (5.26)	27.51 (5.33)	28.32 (6.07)
Diabetes, n (%)	257 (10.98)	34 (10.21)	163 (11.17)	60 (10.95)

^I E2=genotypes E2/E2 and E2/E3, E3=genotype E3/E3, and E4=genotypes E3/E4 and E4/E4.

Table 2Mean ($\pm$ SE) level of plasma lipids, lipoproteins and phospholipid fatty acids across *APOE* genotypes¹

	<i>APOE</i> allele ²³		
	E2 (n=333)	E3 (n=1459)	E4(n=548)
Lipid profile			
Triglyceride, Geometric mean, mmol/L	1.36 $\pm$ 0.01	1.28 $\pm$ 0.01	1.34 $\pm$ 0.01
Total Cholesterol, mmol/L	4.82 $\pm$ 0.05 ^{b, c}	5.12 $\pm$ 0.02 ^a	5.15 $\pm$ 0.04 ^a
LDL-C, mmol/L	2.74 $\pm$ 0.04 ^{b, c}	3.13 $\pm$ 0.02 ^a	3.20 $\pm$ 0.03 ^a
HDL-C, mmol/L	1.36 $\pm$ 0.02 ^{b, c}	1.31 $\pm$ 0.01 ^{a, c}	1.26 $\pm$ 0.01 ^{a, b}
Lipoprotein characteristics			
Small LDL conc. (18-20.5 nm), nmol/L	450.3 $\pm$ 20.0 ^{b, c}	548.4 $\pm$ 9.4 ^{a, c}	621.8 $\pm$ 15.6 ^{a, b}
Large LDL conc. (20.5-23 nm), nmol/L	491.2 $\pm$ 13.1 ^{b, c}	608.8 $\pm$ 6.2 ^a	592.9 $\pm$ 10.3 ^a
Mean LDL particle size, nm	20.73 $\pm$ 0.03	20.76 $\pm$ 0.01 ^c	20.66 $\pm$ 0.02 ^b
Total LDL conc., nmol/L	1081.3 $\pm$ 18.5 ^{b, c}	1287.6 $\pm$ 8.77 ^{a, c}	1336.3 $\pm$ 14.4 ^{a, b}
Small HDL conc. (7.3-8.2 nm), μ mol/L	14.5 $\pm$ 0.3	14.6 $\pm$ 0.1	14.9 $\pm$ 0.2
Medium HDL conc. (8.2-9.4 nm), μ mol/L	14.4 $\pm$ 0.3 ^{b, c}	13.0 $\pm$ 0.2 ^{a, c}	12.2 $\pm$ 0.3 ^{a, b}
Large HDL conc. (9.4-14 nm), μ mol/L	6.1 $\pm$ 0.2	6.0 $\pm$ 0.1	5.7 $\pm$ 0.1
Mean HDL particle size, nm	9.24 $\pm$ 0.02	9.26 $\pm$ 0.01	9.25 $\pm$ 0.02
Total HDL conc., μ mol/L	34.9 $\pm$ 0.3 ^{b, c}	33.6 $\pm$ 0.2 ^{a, c}	32.7 $\pm$ 0.3 ^{a, c}
Plasma phospholipid omega-3 fatty acids			
EPA, %	0.93 $\pm$ 0.05	0.96 $\pm$.02	0.96 $\pm$ 0.04
DHA, %	4.08 $\pm$ 0.07	4.17 $\pm$ 0.03	4.11 $\pm$ 0.06

¹ Adjusted for age, sex, race, field center, ancestry informative marker principal components (PC1-PC5), education, smoking status, alcohol use, total intentional exercise, BMI, diabetes.

² E2=genotypes E2/E2 and E2/E3, E3=genotype E3/E3, and E4=genotypes E3/E4 and E4/E4.

³ Difference observed between groups (ppdiff < 0.017)

^a different from E2

^b different from E3

^c different from E4.

Table 3Associations of EPA with plasma lipids and lipoproteins across *APOE* genotypes^{1,2}

	E2 (n=333)	E3 (n=1459)	E4(n=548)	<i>p</i> -value interaction
	Slope (<i>p</i> -value)	Slope (<i>p</i> -value)	Slope (<i>p</i> -value)	
<i>Lipid profile</i>				
HDL-C (mmol/L)	0.074 (0.001)	0.026 (0.08)	-0.012 (0.32)	0.0002
<i>Lipoprotein characteristics</i>				
Small LDL conc. (nmol/L)	35.06 (0.08)	34.61 (0.03)	84.44 (< 0.001)	0.04
Mean LDL size (nm)	-0.01 (0.85)	-0.02 (0.41)	-0.07 (0.006)	0.05
Large HDL conc. (μmol/L)	0.89 (0.001)	0.26 (0.09)	-0.01 (0.95)	0.006
Mean HDL size (nm)	0.04 (0.22)	0.00 (0.92)	-0.04 (0.09)	0.03
Total HDL conc. (μmol/L)	0.67 (0.03)	0.23 (0.38)	-0.28 (0.29)	0.007

¹ Values are the slope of DHA on each plasma lipid or lipoprotein in each *APOE* genotype group (p-values in parentheses). The p-value for interaction is calculated based on test for equality for the three *APOE* genotype groups..

² Adjusted for age, sex, race, field center, ancestry informative marker principal components (PC1-PC5), education, smoking status, alcohol use, total intentional exercise, BMI, diabetes. Triglycerides was included as a covariate for outcomes other than triglycerides

Table 4Associations of DHA with plasma lipids and lipoproteins across *APOE* genotypes^{1,2}

	E2 (n=333)	E3 (n=1459)	E4(n=548)	<i>p</i> -value interaction
	Slope (<i>p</i> -value)	Slope (<i>p</i> -value)	Slope (<i>p</i> -value)	
<i>Lipoprotein characteristics</i>				
Small LDL conc. (nmol/L)	-2.99 (0.90)	-3.37 (0.88)	27.34 (0.25)	0.01
Medium HDL conc. (μmol/L)	-1.46 (< 0.001)	-1.09 (0.004)	-1.48(< 0.001)	0.05

¹Values are the slope of EPA on each plasma lipid or lipoprotein in each *APOE* genotype group (p-values in parentheses). The p-value for interaction is calculated based on test for equality for the three *APOE* genotype groups.

²Adjusted for age, sex, race, field center, ancestry informative marker principal components (PC1-PC5), education, smoking status, alcohol use, total intentional exercise, BMI, diabetes. Triglycerides was included as a covariate for outcomes other than triglycerides

Table 5Associations between plasma EPA and DHA with lipid profile and lipoprotein characteristics¹²³

	Plasma EPA	Plasma DHA
	slope (<i>p</i>)	slope (<i>p</i>)
Lipid profile		
Log Triglyceride (mmol/L)	² ns	ns
Total Cholesterol (mmol/L)	0.14 (<0.001)	ns
LDL-C (mmol/L)	0.12 (0.005)	ns
HDL-C (mmol/L)	<i>Interaction</i>	ns
Lipoprotein characteristics		
Small LDL conc. (nmol/L)	<i>Interaction</i>	<i>Interaction</i>
Large LDL conc. (nmol/L)	ns	ns
Mean LDL size (nm)	<i>Interaction</i>	ns
Total LDL conc. (nmol/L)	8 1.3 (<0.001)	ns
Small HDL conc. (μmol/L)	0.49 (0.01)	1.14 (<0.001)
Med. HDL conc. (μmol/L)	-0.66 (0.008)	<i>Interaction</i>
Large HDL conc. (μmol/L)	<i>Interaction</i>	ns
Mean HDL size (nm)	<i>Interaction</i>	ns
Total HDL conc. (μmol/L)	<i>Interaction</i>	ns

⁴ns = not significant¹The association of plasma phospholipids with lipid or lipoprotein is not presented where an interaction was observed.²Values are slopes of EPA or DHA on each plasma lipid or lipoprotein (p-values in parentheses).³Adjusted for age, sex, race, field center, ancestry informative marker principal components (PC1-PC5), education, smoking status, alcohol use, total intentional exercise, BMI, and diabetes. Triglycerides was included as a covariate for outcomes other than triglycerides