Association of oily fish intake, sex, age, BMI, and *APOE* genotype with plasma long chain n-3 fatty acid composition [1-3]

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[4]Abbreviations:

LC n-3 PUFA, Long chain omega-3 polyunsaturated fatty acids; EPA, Eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA, Docosahexaenoic acid; APOE, Apolipoprotein E; BMI, body mass index; PC, Phosphatidylcholine; NEFAs, Non-esterified fatty acids; CEs, Cholesteryl esters; TGs, Triacylglycerols; FFQ, Food frequency questionnaire; FAMEs, Fatty

acid methyl esters; GLM, General linear model; SEM, Standard error mean; LDL, Low-density lipoprotein; LDLRs, Low-density lipoprotein receptors; HDLs, High density lipoproteins; LDLC, LDL-cholesterol.

Running title: Determinants of fatty acid status

Abstract

Omega-3 fatty acids are associated with better cardiovascular and cognitive health. However, the concentration of EPA, DPA and DHA in different plasma lipid pools differs and factors influencing this heterogeneity are poorly understood. Our aim was to evaluate the association of oily fish intake, sex, age, BMI and *APOE* genotype with concentrations of EPA, DPA and DHA in plasma PC, NEFAs, CEs and TGs. Healthy adults (148 male, 158 female, age 20-71 years) were recruited according to *APOE* genotype, sex and age. Fatty acid composition was determined by gas chromatography. Oily fish intake was positively associated with EPA in PC, CEs and TGs, DPA in TGs, and DHA in all fractions ($P \leq 0.008$). There was a positive association between age and EPA in PC, CEs and TGs, DPA in NEFAs and CEs, and DHA in PC and CEs ($P \leq 0.034$). DPA was higher in TGs in males than females ($P < 0.001$). There was a positive association between BMI and DPA and DHA in TGs ($P < 0.006$ and 0.02, respectively). *APOE* genotype*sex interactions were observed: the *APOE*4 allele associated with higher EPA in males ($P = 0.002$), and there was also evidence for higher DPA and DHA ($P \leq 0.032$). In conclusion, EPA, DPA and DHA in plasma lipids are associated with oily fish intake, sex, age, BMI, and *APOE* genotype. Such insights may be used to better understand the link between plasma fatty acid profiles and dietary exposure and may influence intake recommendations across population subgroups.

Keywords: apolipoprotein E (*APOE*) genotype; oily fish intake; omega 3 status; n-3 long chain polyunsaturated fatty acids; eicosapentaenoic acid (EPA); docosahexaenoic acid (DHA); fatty acid status; blood lipids.

Introduction

There is convincing evidence that higher intakes of the marine long chain n-3 PUFAs (LC n-3 PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are beneficial to cardiovascular and cognitive health, acting through a number of biological mechanisms, and that the concentration of EPA and DHA present in blood and tissue lipids is correlated positively with these effects [1-5]. Oily fish are a good source of EPA and DHA; therefore, national and international authorities recommend regular consumption of oily fish such as salmon, mackerel, kippers, sardines, herring, trout and fresh tuna, in order to provide approximately 500 mg EPA+DHA per day [6], with higher intakes of LC n-3 PUFAs recommended for those with diagnosed cardiovascular disease [7]. However, the associations between intake and blood and tissue status, and therefore physiological benefits, are highly variable [8], and the factors influencing this heterogeneity are not well understood. A greater knowledge of determinants of LC n-3 PUFA status could lead to the development of more robust, and perhaps subgroup specific, recommendations for EPA and DHA intake.

In addition to intake of the specific LC n-3 PUFAs and their precursors, the heterogeneity in habitual EPA, docosapentaenoic acid (DPA) and DHA concentrations may be influenced by differences in fatty acid metabolism between sexes; females are reported to synthesise EPA, DPA and DHA from shorter chain n-3 fatty acids more readily than males [9-13]. Lipid metabolism alters with age and becomes dysregulated in obesity, and EPA and DHA concentrations have been reported to be affected by increasing BMI [12 14] as well as with age [10-12]. Apolipoprotein E (*APOE*) genotype is associated with altered lipid metabolism and transport, with differential responses in *APOE*4 carriers relative to non-carrier groups [12 14]. Recent reports highlight the importance of *APOE* genotype in the response of EPA and DHA to supplementation and have indicated interactions between genotype and BMI [14]. In addition, the concentrations of LC n-3 PUFAs in individual lipid pools within blood (and in other tissues) differs [15]. However, despite these insights from the published literature, the influence of oily fish intake, along with sex, age, BMI and *APOE* genotype on EPA, DPA and DHA concentrations in different plasma pools has not been examined systematically. Using samples from the FINGEN study [4], where participants were prospectively recruited based on a number of these variables (sex, age, and *APOE* genotype), we have conducted such an analysis in a large number of participants to evaluate the independent and interactive impact of a number of potential determinants (oily fish intake, sex, age, BMI and *APOE* genotype) on EPA, DPA and DHA concentrations in the main plasma lipid fractions.

Participants and methods

The FINGEN study was a multi-centre trial conducted at the Universities of Glasgow, Newcastle, Reading and Southampton in the United Kingdom. Three hundred and twelve participants were recruited prospectively on the basis of *APOE* genotype (87 were *APOE*2 homozygotes or *APOE*2/*APOE*3, 111 were homozygous for *APOE3*, and 114 were *APOE*4/APOE3 or APOE4 homozygotes), sex (149 male and 163 female) and age (20 to 71 years, with approximately equal numbers in each of the 5 decades) [4]. Data from 306 participants were included in the current analysis, with the numbers in each subgroup detailed in **Supplemental Table 1** and **Supplemental Table 2**. Exclusion criteria included: diagnosed endocrine dysfunction including diabetes or fasting glucose concentration > 6.5 mmol/L, myocardial infarction in the previous 2 years, the use of medication that may interfere with lipid metabolism, fasting total cholesterol of > 8.0 mmol/L or TG of > 3.0 mmol/L, a BMI of < 18.5 or > 36.0 kg/m^2, or currently following a weight loss diet. Individuals taking n-3 fatty acid supplements were also excluded. The study was approved by the research ethics committee at each of the participating centres and written informed consent was obtained from all subjects prior to participation.

Study design

The FINGEN study was a randomised double blind, placebo controlled, crossover study testing two doses of fish oil compared with placebo [4]. Here we evaluate the association of oily fish intake, sex, age, BMI and *APOE* genotype with fasting concentrations of EPA, DPA and DHA in plasma phosphatidylcholine (PC), non-esterified fatty acids (NEFAs), cholesteryl esters (CEs) and triacylglycerols (TGs) at baseline, prior to intervention. Habitual oily fish intake was estimated by food frequency questionnaire (FFQ), using self-reported portions completed at baseline. Oily fish was defined as salmon, herring, mackerel, fresh tuna, sardines, kippers, and trout.

Fatty acid analysis

The fatty acid composition of the plasma fractions was determined by gas chromatography. Dipentadecanoyl PC, heneicosanoic acid, cholesteryl heptadecanoate and tripentadecanoin internal standards were added to the plasma. Total plasma lipid was extracted using chloroform: methanol (2:1, v/v) containing butylated hydroxytoluene (50 mg/L) as described by Folch et al [16], and PC, NEFA, CE and TG fractions were separated and isolated by solid phase extraction on aminopropyl silica cartridges. CEs and TGs were eluted in a combined fraction with the addition of chloroform. PC was then eluted from the cartridge with the addition of chloroform: methanol (60:40 v/v). NEFAs were eluted from the cartridge with the addition of chloroform: methanol: glacial acetic acid (100:2:2 v/v). CEs and TGs were separated on a hexane primed aminopropyl silica cartridge with the addition of hexane to elute CEs, and the addition of hexane: methanol: ethyl acetate (100:5:5

v/v/v) to elute TGs. The fatty acids within the resulting lipid fractions were methylated by the addition of methanol in 2% (v/v) sulphuric acid at 50°C for 2 hours to produce fatty acid methyl esters (FAMEs) [17]. FAMEs were extracted into hexane and separated in a BPX-70 fused silica capillary column (30 m × 0·25 mm × 25 μm; SGE Analytical Science, United Kingdom) using an Agilent 6890 series gas chromatograph equipped with flame ionisation detection (Agilent Technologies, California, United States). The FAMEs were identified by comparison with retention times of 37 FAME and menhaden oil standards run alongside the samples, and quantified with the use of the internal standards using ChemStation software (Agilent Technologies, California, United States) and Microsoft Excel (Microsoft Corporation, Washington, United States). Fatty acid composition data are expressed as absolute concentrations (μg/ml plasma) and as relative concentrations (g/100 g total fatty acid (%)).

Statistics

Here we report baseline data obtained as part of the previous FINGEN trial[4]. Characteristics for participants included in the baseline analysis are detailed in **Supplemental Table 1** and **Supplemental Table 2**.

Results for the relative (%) and absolute concentrations (μg/ml) of fatty acids are reported for 303 to 306 and 292 to 306 participants in the four plasma lipid fractions. Data were checked for normality by plotting distributions of residuals obtained from general linear model (GLM) analysis of the data, and were analysed appropriately with a univariate GLM following $\log_{10}$ transformation. All variables were included in the univariate model with individual associations analysed using 'main effects' and interaction between age and BMI, age and fish intake, and sex and *APOE* analysed using 'interaction' analysis options within the model. *P* values were corrected for multiple analyses using Bonferroni post hoc analysis resulting in a significance value of $P = 0.006$ for whole group analysis and $P = 0.008$ for analyses where males and females were analysed separately. All statistical analyses were conducted using SPSS software (version 21; SPSS Inc, Chicago, IL). Statistical significance was defined as $P \leq 0.05$. Results are expressed as mean ± SEM or median (25^{th}, 75^{th} percentiles).

Results

The group (n = 306) mean age and BMI was 45.1 ± 0.7 y and 25.2 ± 0.2 kg/m^2, respectively.

Male and female participants were well matched for age, but males had a significantly higher average BMI ($P < 0.001$, **Supplemental Table 1** and **Supplemental Table 2**). There were no sex

differences in the proportion of total dietary energy consumed from fat, saturated fat (SFA), monounsaturated fat (MUFA) or polyunsaturated fat (PUFA) (data not shown). The average oily fish intake was 1.0 portion per week with no association of sex with oily fish intake.

For all three LC n-3 PUFAs, the greatest concentrations were evident in the PC fraction, with median absolute concentrations (μg/ml) of 15.1, 11.9 and 44.1 for EPA, DPA and DHA, respectively. The median values for EPA, DPA and DHA for the whole group and *P* values for the association of oily fish intake, sex, age, BMI and *APOE* with the plasma concentrations of these fatty acids in the four lipid fractions are presented in **Table 1.** The data according to oily fish intake are shown in **Supplemental Figures 1-4**, while data according to age and BMI are shown in **Table 2 and Supplemental Tables 3-5**, and those according to *APOE* genotype*sex in **Figures 1-3.**

Plasma EPA, DPA and DHA in the group as a whole

EPA: The concentration of EPA in plasma CEs and TGs was positively associated with oily fish intake ($P \leq 0.004$), with evidence for positive association in plasma PC also ($P = 0.018$) (**Table 1**). There was evidence for a positive association between EPA and age in plasma PC, CE's and TGs ($P = 0.021$, 0.019, and 0.034 respectively) and for the concentration of EPA in CEs to differ by sex ($P = 0.055$), (**Table 2**). A higher concentration of EPA in CEs was observed in males (**Table 2**), and the concentration of EPA in TGs was associated with an *APOE**sex interaction ($P = 0.044$, data not shown).

DPA: The concentration of DPA was positively associated with oily fish intake in plasma TGs ($P = 0.006$), with evidence for positive association in plasma PC also ($P = 0.022$) **(Table 1)**. DPA in TGs was positively associated with BMI ($P = 0.006$) (**Table 1**), and there was evidence for the positive association of DPA in NEFAs and CEs with age ($P = 0.031$ and 0.007 respectively, **Table 1**). The concentration of DPA significantly differed by sex with a higher concentration of DPA observed in plasma TGs in males ($P < 0.001$), with a trend in PC also (*P* 0.031) (**Table 1**). There was also a significant *APOE**sex interaction for the concentration of DPA in CEs ($P \leq 0.005$, data not shown). (**Table 1**),

DHA: The concentration of DHA in all plasma lipid fractions was positively associated with oily fish intake ($P \leq 0.001$). There was evidence for a positive association of DHA in TGs with BMI ($P = 0.020$) (**Table 1**) and with age in PC,-CEs and TGs ($P = 0.037$, 0.039, and 0.050 respectively, **Table 1**).

Overall in PC, NEFAs, CEs, and TGs, the highest oily fish consumers (2+ portions of oily fish per week) had 55%, 42%, 52% and 119% higher EPA+DHA, respectively, compared with those reporting no oily fish intake (**Supplemental Figure 4**).

Due to the significant evidence of sex and *APOE**sex interactions, subgroup analysis was performed in males and females separately.

Subgroup analysis of plasma EPA, DPA and DHA according to sex

Significance data (*P*) are reported for EPA, DPA and DHA in **Table 2** and median data are reported for EPA, DPA and DHA in **Supplemental Tables 3, 4, and 5** respectively.

EPA (**Table 2, Supplemental Table 3**)**:** The concentration of EPA in plasma TGs was positively associated with oily fish intake in both males and females ($P \leq 0.008$), while the concentration of EPA in PC was positively associated with oily fish intake in females only ($P \leq 0.004$. EPA concentration in TGs was positively associated with age and BMI in females ($P = 0.006$), while EPA in TGs differed by *APOE* genotype in males ($P = 0.002$), with evidence for this in CEs also ($P = 0.019$), (**Figure 1**). A greater concentration of EPA in TGs was observed in male *APOE*4 carriers ($P = 0.002$) with evidence for this in PC and CEs also ($P = 0.019$ and 0.053 respectively), (**Figure 1**).

DPA (**Table 2, Supplemental Table 4**): The concentration of DPA in plasma TGs was positively associated with oily fish intake in females ($P = 0.008$). There was evidence for DPA concentration in PC to differ with *APOE* genotype in males ($P \leq 0.053$, **Figure 2**) with further analysis revealing evidence for higher concentrations of DPA in PC in *APOE*4 allele carriers ($P = 0.032$, **Figure 2**).

DHA (**Table 2, Supplemental Table 5**): The concentration of DHA was positively associated with oily fish intake in plasma PC, NEFAs, and TGs in females ($P \leq 0.002$) and plasma PC in males ($P \leq 0.003$), (**Table 2**). There was evidence for DHA in plasma NEFAs to be associated with BMI in females ($P = 0.010$, **Table 2**), and for DHA in CEs to differ by *APOE* genotype in males. Further analysis revealed evidence for a higher concentration of DHA in CEs in *APOE*4 carriers ($P = 0.021$, **Figure 3**).

Discussion

EPA and DHA have been widely reported for their beneficial effects on cardiovascular and cognitive health [1-4 18] but a high level of variation in associations between intake and blood and tissue status has been observed [8]. The current analysis aimed to identify factors associated with concentrations of EPA, DPA and DHA in major lipid fractions in plasma from individuals consuming their usual diet in order to identify sources of variation in these concentrations. Identification of the contribution that oily fish intake, sex, age, BMI and *APOE* genotype make to EPA, DPA and DHA status is important for two reasons. First it will highlight the sources of the heterogeneity in status of these fatty acids, contributing to a better understanding of the use of fatty acid profiles as a measure of dietary intake amongst different population subgroups. Secondly, it may allow the development of sub-group specific recommendations for LC n-3 PUFA intake.

The current study reports associations for multiple confounding variables with the relative and absolute concentrations of EPA, DPA and DHA in different plasma lipids. The relative concentration allows investigation of LC n-3 PUFA concentrations in relation to all other fatty acids within the plasma pool (% unit changes), while the absolute concentration allows investigation of µg/ml unit changes in LC n-3 PUFAs independently of any other fatty acid within the plasma pool. Both ways of expressing the data are useful and informative and both are used in the literature in the field. The absolute concentration of a fatty acid within any plasma lipid fraction will be influenced by the total concentration of that fraction. The absolute concentration of a particular fatty acid may differ between individuals or between sub-groups while the relative concentration of that fatty acid may not be different between those individuals or sub-groups. Conversely, the relative concentration could be different but the absolute concentration may not be. Plasma lipids are involved in transport of fatty acids between tissues where they have different actions depending upon their structure. Hence, the absolute concentration of a fatty acid in a plasma lipid reflects the exposure of tissues to that fatty acid and hence is likely to be a meaningful way of reporting the fatty acid. Conversely, fatty acids often compete with one another for metabolism or for function and hence the relative concentration of each fatty acid (i.e. %) is also likely to be meaningful.

Quantitatively, PC is the main plasma LC n-3 PUFA pool and the current study reports a greater relative concentration of EPA+DHA in plasma PC (**Supplemental Figure 4**) in individuals consuming 2+ portions of oily fish a week compared to those who reported not consuming oily fish, as well as positive associations between EPA, DPA and DHA in other plasma lipid fractions and oily fish intake. Positive associations for oily fish intake and EPA and DHA are reported for plasma phospholipids [19-21] which are confirmed by data from the current analysis which shows 55% higher EPA+DHA in plasma PC in those consuming two portions of oily fish (each 150 g) per week

compared with those reporting no oily fish consumption. Two portions of oily fish supply about 4-5 g of EPA+DHA per week, equivalent to 600-700 mg per day[22 23]. Previous studies report comparable increases of 81% in plasma phospholipid EPA+DHA, and 8.8 µg/ml and 8.5 µg/ml in total plasma EPA and DHA respectively following 16 week consumption of oily fish providing 485 mg EPA+DHA per day [20] and 6 week consumption of oily fish providing 927 mg EPA+DHA per day, respectively[21]. Overall, the findings of the current analysis support existing reports that oily fish intake is associated with, and at a population level is the main determinant of, LC n-3 PUFAs in all major blood lipid pools, which may therefore be used as biomarkers of oily fish intake [4 19 24 25]. Our analysis does not clearly indicate which plasma lipid fraction would best reflect dietary intake of EPA and DHA, since, in general all four plasma lipid fractions showed dose-dependent increases in EPA and DHA concentration (both absolute and relative) with increasing frequency of oily fish consumption.

There is some evidence that age influences the concentration of EPA and DHA in various plasma fatty acid fractions, [10] which has been attributed in part to higher habitual fish intake with increasing age. Oily fish intake was controlled for in the current statistical analysis, allowing clearer attribution of any observed associations of age with EPA, DPA and DHA concentrations to altered metabolism and not to dietary differences in intakes of oily fish. Any influence of *APOE* group distribution was also ruled out as, despite a greater number of individuals aged 50-59 yr being included in the current analysis, there was no significant difference in the distribution of *APO E*2, *E*3 and *E*4 genotypes between age groups (data not shown). A 28 d stable isotope tracer study in young (mean age 27 y) vs older (mean age 77 y) adults reported a 1-2 fold greater enrichment of ^{13}C–DHA in plasma phospholipids and CEs in the older age group, suggesting a medium term age-related difference in DHA homeostasis associated with accumulation of DHA in the circulation in older people [26]. The findings of the current analysis support reports of increased plasma DHA with increasing age [11 27 28] and we further also report positive associations between age and EPA and DPA, suggesting LC n-3 PUFAs accumulate in plasma pools during ageing. However, this may in part be due to an increase in circulating cholesterol and CE with age (**Table 3**). Evidence of positive associations of plasma total cholesterol with age dates back to the late 1970s [29], and these have been reported in both males and females [30]. Increased circulating LDL (**Table 3**) may be reflected in higher absolute total PC and CE concentrations with age (P = 0.008 and 0.018, age 20-29 vs 60+ yr for PC and CE respectively, data not shown) and we observed that total cholesterol (TC) and LDL-cholesterol (LDLC) concentrations were significantly positively correlated with LC n-3 PUFA concentrations in PC (TC, P = <0.001, 0.003, 0.027; LDLC P = <0.001, <0.001, 0.003, absolute EPA, DPA and DHA respectively, data not shown), and that TC, LDLC and high density lipoprotein cholesterol (HDLC) concentrations were positively correlated with LC n-3 PUFA in

CEs (TC, P = <0.001, 0.002 absolute EPA and DHA respectively, LDL, P = <0.001, 0.046, 0.055 absolute EPA, relative DPA and DHA respectively, HDLC, P = 0.046 relative DPA, data not shown). These data suggest CE levels may play a significant role in the association of age with LC n-3 PUFAs reported in this analysis.

Insulin has a role in the regulation of genes involved in whole body lipid homeostasis including in the removal of lipids from the circulation[31]; in cases of insulin resistance, such removal can be compromised. The occurrence of insulin resistance is reported to rise with increasing age and BMl and despite individuals with diabetes or a fasting glucose concentration > 6.5 mmol/L being excluded from the current analysis, differences in fasting glucose were still evident between age and BMI groups (glucose positively correlated with age and BMI; P <0.001 both, data not shown). Thus, insulin resistance may contribute to the higher EPA and DPA concentrations in plasma lipid pools observed with increasing age and BMI.

Increasing body fatness and obesity influence many aspects of fatty acid and lipid metabolism and contribute to disease states such as hypertriglyceridemia, diabetes, and fatty liver disease [12 32]; loss of insulin sensitivity with increasing adiposity results in adipose tissue lipolysis and associated higher plasma NEFA concentrations [32-34]. In the current analysis, there was no correlation between total NEFA concentrations and BMI (data not shown); however, significant, but complex, associations between BMI and LC n-3 PUFAs were evident in plasma TGs, with an overall trend towards lower relative concentrations of EPA and DHA with increasing BMI, which is consistent with previous observations [33 35]. Increased β-oxidation of DHA associated with increased BMI may in part explain lower proportions of LC n-3 PUFAs in TGs [36] although altered TG synthesis and/or selective tissue uptake and partitioning in obesity may also be involved. We observed no association of BMI with absolute plasma concentrations of LC n-3 PUFAs and suggest the lower relative concentrations (i.e., %) of EPA and DHA are likely to be offset by increases in total TG concentrations with increasing BMI.

The proteins encoded by the *APOE* gene play a major role in the transport and metabolism of lipids via interaction with LDL receptors (LDLRs). Two common polymorphisms (rs7412 and rs429358) of the *APOE* gene in humans result in three protein isoforms, APOE2, E3 and E4. APOE2 and APOE3 are found in the circulation mainly on high density lipoproteins (HDLs) whereas APOE4 is found preferentially on very low density lipoproteins (VLDLs) with lower concentrations residing on HDLs [37]. The *APOE4* allele has been associated with reduced longevity [38], and enhanced risk of cardiovascular disease [39] and Alzheimer's disease [40]. Although centrally involved in fatty acid transport and handling in plasma and tissues (and in particular within the brain where *APOE* is almost the only apolipoprotein present), the impact of *APOE* genotype on these processes, and the contribution of dysregulated EPA and DHA metabolism to disease risk is

unknown. However, ^{13}C–DHA labelling studies provide evidence that DHA metabolism is disturbed in those who are *APOE4* carriers [41].

In the current analysis, *APOE4* carriers had significantly higher concentrations of TC and HDLC, and lower concentrations of LDLC (**Table 3**); however, sex**APOE* genotype interactions were evident and in male *APOE4* carriers we observed to have significantly higher concentrations of LDLC as well as of total CEs (data not shown). One advantage of investigating associations in individual plasma lipid classes as opposed to total lipid is that possible effects of *APOE* and lipoprotein transport and metabolism may be more easily identified. If the associations between *APOE* and LC n-3 PUFAs are seen to occur in lipid pools which are predominantly related to LDL and VLDL particles, they may reflect the dysregulation in lipoprotein handling in people with the *E*4 allele. However, if the associations between LC n-3 PUFA and *APOE* genotype are seen to occur across all lipid pools, they may be indicative of alternative mechanisms. Further subgroup analysis indicated higher EPA, DPA and DHA concentrations in CEs, EPA and DPA in PC, and EPA in TGs in male *APOE4* carriers relative to the non-carrier groups. The higher EPA and DHA may reflect higher overall CE and PC concentrations; however, the lack of association between *APOE* genotype and fatty acid concentrations in females is suggestive of a sex specific association independent of CE and PC metabolism.

Interestingly, we have previously reported *APOE* genotype mediated differences in the response of plasma EPA and DHA to a fish oil supplement given over eight weeks in males, with lower enrichment in total lipid and phospholipid EPA and DHA in *APOE4* carriers relative to the wild-type *APOE3/E3* genotype, but only in overweight participants [14]. The aetiology of these associations with LC n-3 PUFA metabolism is currently unknown. As with the association with age, higher plasma LC n-3 PUFAs in *APOE4* carriers may reflect reduced tissue uptake and DHA accumulating in the circulation. Although lower overall concentrations of *APOE* were observed in *APOE4* carriers (data not shown) no difference in plasma *APOE* concentrations were evident between sexes, which potentially could have contributed to the differential associations of *APOE* genotype with EPA, DPA and DHA concentrations. The preferential binding of VLDL by *APOE4* and possible associations of *APOE* genotype with PC and CE synthesis and cellular uptake of EPA and DHA via the LDLR family, LDLR concentrations and specific LC PUFA transporters such as the MFSD2A transporter in the brain [42] may be involved, and are worthy of future investigations. Associations between sex and the activity of these transporters and receptors would also be of interest, along with sex and *APOE* associations with FADS and ELOVL genes which encode desaturation and elongation enzymes required for the synthesis of LC n-3 PUFAs. Differential synthesis of EPA and DHA has been reported between sexes; Pawlosky *et al* report greater ability of females to convert ALA to DHA through increased conversion of DPA to DHA compared to

males when consuming a beef based diet. These results were not observed when consuming a fish based diet in which the capacity to convert DPA to DHA was equal between males and females. These findings suggest LC n-3 PUFA metabolism in females may be more sensitive to dietary alterations or may be affected by hormonal regulation[43]. Indeed there is evidence for up-regulation of the desaturase-elongase pathway via oestrogenic actions resulting in increased conversion of ALA to EPA[19 44 45] and to DHA[11 13 46] indicating significant effects of female sex hormones on the metabolism of LC n-3 PUFAs. Consistent with these observations, there is evidence for an increase in DHA in relation to EPA and DPA at baseline and in response to EPA+DHA intake in females compared to males [47 48]. The current analysis further reports lower concentrations of both DPA (-36% lower absolute concentration in TGs) and EPA (20% lower absolute concentration in TGs) in females but does not report higher concentrations of DHA in females or find a significant effect of sex on the ratio of DPA: DHA (P >0.50, data not shown). However, these results are also in contrast to other reports describing increased concentrations of EPA and DHA in females [19 44 45]. These data from the current analysis suggest investigation into associations between sex, *APOE,* and fatty acid synthesis enzymes and transporters would be of worthwhile to further understand the mechanisms by which these associations occur.

In conclusion, we report concentrations of EPA, DPA and DHA to vary across *APOE* genotype and that sex is an important factor to consider when evaluating LC n-3 PUFA concentrations in these genotypic subgroups. Our results also confirm that concentrations of EPA, DPA and DHA in plasma pools are suitable population markers of oily fish consumption and show that age and sex are important contributors to the variation in EPA, DPA and DHA concentrations in plasma lipids independent of *APOE* genotype. These variables should be considered when interpreting LC n-3 PUFA concentrations as a marker of dietary intake and when suggesting dietary LC n-3 PUFA recommendations to ensure benefits are achieved across population subgroups. Investigation into the handling of supplemental EPA and DHA in these subgroups is to be addressed in a further publication and could provide the basis for more detailed advice. However, the aetiology and physiological significance of the interaction between sex and *APOE* genotype and its association with EPA, DPA and DHA status still requires further investigation.

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Authors' responsibilities

The authors' responsibilities were as follows: GL, CKA, JCM, CJP, PCC and AMM (the study management group) were responsible for designing the original FINGEN study and supervising all aspects of the reported work; EAM, BMK, PJC and CKA recruited and screened volunteers, carried out the intervention, collected the blood samples and collected the anthropometric, questionnaire and compliance data; HLF conducted the laboratory analysis reported herein; HLF and MI conducted statistical analysis; HLF wrote the draft of the manuscript; all authors contributed to the final version of the manuscript.

Conflicts of interest

PCC is an advisor to Pronova BioPharma, Aker Biomarine, Smartfish, Sancilio, Solutex, Dutch State Mines, Cargill and Danone/Nutricia. None of the other authors has any conflict to declare.

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Table 1

Median EPA, DPA, and DHA in the plasma lipid fractions and statistical significance (*P*) of the association of oily fish intake, sex, age, and BMI on absolute and relative concentrations of these LC n-3 PUFAs[1]

					EPA			
	PC (*P*)	NEFAs (*P*)	CEs (*P*)	TGs (*P*)	PC (*P*)	NEFAs (*P*)	CEs (*P*)	TGs (*P*)
	%	%	%	%	μg/ml	μg/ml	μg/ml	μg/ml
Median	1.01	0.43	0.77	0.42	15.14	0.85	14.2	2.98
(25th, 75th percentile)	(0.65, 1.57)	(0.26, 0.66)	(0.46, 1.10)	(0.27, 0.65)	(8.74, 23.23)	(0.48, 1.47)	(8.45, 23.45)	(1.83, 4.72)
Oily Fish Intake[2]	0.055	-	0.004	<0.001	0.018	-	0.058	<0.001
Sex	-	-	0.055	-	-	-	-	-
Age[3]	0.063	-	-	-	0.021	-	0.019	0.034
BMI[4]	-	-	-	0.041	-	-	-	-
					DPA			
	PC (*P*)	NEFAs (*P*)	CEs (*P*)	TGs (*P*)	PC (*P*)	NEFAs (*P*)	CEs (*P*)	TGs (*P*)
	%	%	%	%	μg/ml	μg/ml	μg/ml	μg/ml
Median	0.78	0.31	0.07	0.33	11.87	0.62	1.34	2.32
(25th, 75th percentile)	(0.55, 0.98)	(0.22, 0.44)	(0.05, 0.12)	(0.23, 0.47)	(7.90, 15.67)	(0.42, 0.86)	(0.84, 2.51)	(1.31, 3.51)
Oily Fish Intake[2]	0.022	-	-	0.006	0.044	-	-	0.016
Sex	0.026	-	-	0.043	0.054	-	-	<0.001
Age[3]	-	0.031	-	-	-	-	0.007	-
BMI[4]	-	-	-	0.006	-	-	-	-
					DHA			
	PC (*P*)	NEFAs (*P*)	CEs (*P*)	TGs (*P*)	PC (*P*)	NEFAs (*P*)	CEs (*P*)	TGs (*P*)

	%	%	%	%	µg/ml	µg/ml	µg/ml	µg/ml
Median	2.86	1.1	0.46	0.61	44.13	2.07	9.01	4.28
(25th, 75th percentile)	(2.08, 3.93)	(0.80, 1.54)	(0.32, 0.61)	(0.39, 0.98)	(29.94, 57.68)	(1.43, 3.18)	(5.88, 12.59)	(2.38, 7.19)
Oily Fish Intake[2]	<0.001	<0.001	0.001	<0.001	<0.001	0.002	0.045	<0.001
Sex	-	-	-	-	-	-	-	-
Age[3]	0.037	-	-	-	0.043	-	0.039	0.050
BMI[4]	-	-	-	0.02	-	-	-	-

EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; PC, phosphatidylcholine; NEFAs, non-esterified fatty acids; CEs, cholesteryl esters; TGs, triacyglycerol.

[1] *P* values obtained using $\log_{10}$ data in univariate general linear model analysis. Individual associations were investigated for by the addition of all other variables as covariates, controlling for any associations between confounding variables that may influence the dependant variable. The resulting *P* values are therefore reflective of the sole association between the variable of interest and the dependant variable.

[2] Oily fish intake: 0 portions/week, 0.1-0.99/week, 1.0-1.99/week, and 2+/week. Oily fish defined as: salmon, herring, mackerel, fresh tuna, sardines, kippers, and trout.

[3] Age: 20-29y, 30-39y, 40-49y, 50-59y, 60+y.

[4] BMI: Normal weight = 18-25 (kg/m^2), Overweight = 25.1-30 (kg/m^2) and Obese = 30.1-46 (kg/m^2).

Table 2

Statistical significance (*P*) of the associations between oily fish intake, sex, age, BMI and LC n-3 PUFAs in males and females[1]

					MALES				
		PC	NEFAs	CEs	TGs	PC	NEFAs	CEs	TGs
		P[1]	*P*[1]	*P*[1]	*P*[1]	*P*[1]	*P*[1]	*P*[1]	*P*[1]
		%	%	%	%	µg/ml	µg/ml	µg/ml	µg/ml
EPA	Oily fish intake[2]	-	-	-	0.028	0.062	0.061	-	0.008
	Age[3]	-	-	-	-	-	-	0.058	0.019
	BMI[4]	-	-	-	0.014	-	-	-	-
DPA	Oily fish intake[2]	-	-	NS	-	0.066	0.026	-	-
	Age[3]	-	-	0.068	-	-	-	0.012	-
	BMI[4]	-	-	-	-	-	-	-	-
DHA	Oily fish intake[2]	0.003	0.023	0.016	-	0.002	0.014	-	-
	Age[3]	-	-	0.011	-	-	0.024	0.005	-
	BMI[4]	-	-	-	-	-	-	-	-
					FEMALES				
		PC	NEFAs	CEs	TGs	PC	NEFAs	CEs	TGs
		P[1]	*P*[1]	*P*[1]	*P*[1]	*P*[1]	*P*[1]	*P*[1]	*P*[1]
		%	%	%	%	µg/ml	µg/ml	µg/ml	µg/ml
EPA	Oily fish intake[2]	0.004	-	0.009	<0.001	0.003	-	-	<0.001
	Age[3]	0.04	-	-	-	0.039	-	-	0.006
	BMI[4]	-	-	-	-	-	0.052	-	0.006
DPA	Oily fish intake[2]	-	-	-	0.008	-	-	-	0.067
	Age[3]	-	-	-	-	-	-	-	-
	BMI[4]	-	-	-	-	-	-	-	-
DHA	Oily fish intake[2]	0.001	0.001	0.003	<0.001	<0.001	0.048	-	0.001

Age[3]	0.035	-	-	-	0.047	-	-	0.032
BMI[4]	-	-	-	-	-	0.010	-	-

EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; PC, phosphatidylcholine; NEFAs, non-esterified fatty acids; CEs, cholesteryl esters; TGs, triacyglycerol.

[1] *P* values obtained using $\log_{10}$ data in univariate general linear model analysis. Individual associations were investigated for by the addition of all other variables as covariates, controlling for any associations between confounding variables that may influence the dependant variable. The resulting *P* values are therefore reflective of the sole association between the variable of interest and the dependant variable.

[2] Oily fish intake: 0 portions/week, 0.1-0.99/week, 1.0-1.99/week, and 2+/week. Oily fish defined as: salmon, herring, mackerel, fresh tuna, sardines, kippers, and trout.

[3] Age: 20-29y, 30-39y, 40-49y, 50-59y, 60+y.

[4] BMI: Normal weight = 18-25 (kg/m^2), Overweight = 25.1-30 (kg/m^2) and Obese = 30.1-46 (kg/m^2).

TABLE 3

Blood cholesterol (mmol/l) concentration according to sex, age, BMI, *APOE* genotype and oily fish intake

	TC		HDLC		LDLC	
	Mean	SEM	Mean	SEM	Mean	SEM
Male	5.16	0.08	1.26	0.02	3.34	0.07
Female	5.16	0.08	1.61	0.03	3.17	0.07
[1]*P*	NS		<0.001		NS	
Age group						
20-29y	4.39	0.14	1.46	0.05	2.56	0.13
30-39y	4.68	0.1	1.34	0.04	2.93	0.1
40-49y	5.34	0.11	1.47	0.04	3.41	0.09
50-59y	5.57	0.1	1.45	0.05	3.62	0.09
60+y	5.59	0.13	1.49	0.06	3.52	0.1
[2]*P*	<0.001		NS		<0.001	
[3]BMI group						
Normal weight	4.91	0.08	1.57	0.03	2.98	0.07
Overweight	5.33	0.08	1.34	0.03	3.43	0.07
Obese	5.63	0.18	1.20	0.05	3.77	0.17
[2]*P*	<0.001		<0.001		<0.001	
*APOE*genotype[4]						
E2	4.71	0.09	1.54	0.04	2.76	0.08
E3	5.19	0.1	1.43	0.04	3.31	0.08
E4	5.46	0.08	1.37	0.03	3.57	0.07
[1]*P*	<0.001		0.006		<0.001	
Oily fish intake[5]						
0/wk	4.9	0.12	1.41	0.04	3.11	0.1
0.1-0.99/wk	5.21	0.09	1.44	0.03	3.25	0.07
1-1.99/wk	5.3	0.12	1.48	0.05	3.38	0.1
2+/wk	5.16	0.15	1.41	0.07	3.28	0.15
[2]*P*	NS		NS		NS	NS

TC, Total cholesterol; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol.

[1]*P* values obtained from one-way ANOVA model.

[2] *P* values obtained from Pearson's correlation model.

[3] BMI: Normal weight = 18-25 (kg/m2), Overweight = 25.1-30 (kg/m2) and Obese = 30.1-46 (kg/m2).

[4] APOE genotype: E2 (E2/E2 and E2/E3), E3 (E3/E3), and E4 (E3/E4 and E4/E4) .

[5] Oily fish defined as: salmon, herring, mackerel, fresh tuna, sardines, kippers, and trout.

FIGURE 1 Absolute concentrations (µg/ml) of eicosapentaenoic acid (EPA) in phosphatidylcholine (PC), non-esterified fatty acids (NEFAs), cholesteryl esters (CEs) and triacylglycerols (TGs) lipid fractions in male and female subjects according to *APOE* genotype. Distribution of participants in each *APOE* allele group are as follows; PC: Males: E2/E2 = 1, E2/E3 = 32, E3/E3 = 40, E3/E4 = 51, and E4/E4 = 2. Total: 126. PC: Females: E2/E2 = 3, E2/E3 = 39, E3/E3 = 44, E3/E4 = 43, and E4/E4 = 10. Total: 139. NEFAs: Males: E2/E2 = 2, E2/E3 = 29, E3/E3 = 45, E3/E4 = 50, and E4/E4 = 2. Total: 128. NEFAs: Females: E2/E2 = 3, E2/E3 = 42, E3/E3 = 44, E3/E4 = 45, and E4/E4 = 10. Total: 144. CEs: Males: E2/E2 = 2, E2/E3 = 33, E3/E3 = 50, E3/E4 = 52, and E4/E4 = 2. Total: 139. CEs: Females: E2/E2 = 3, E2/E3 = 44, E3/E3 = 48, E3/E4 = 49, and E4/E4 = 10. Total: 154. TGs: Males: E2/E2 = 2, E2/E3 = 32, E3/E3 = 50, E3/E4 = 53, and E4/E4 = 2. Total: 139. TGs: Females: E2/E2 = 3, E2/E3 = 45, E3/E3 = 49, E3/E4 = 48, and E4/E4 = 10. Total: 155. * $P < 0.050$, and ** $P > 0.050$ but < 0.060. *P* values were obtained using log-10 data in univariate general linear model (GLM) analysis controlling for covariates (age, BMI, and oily fish intake). Where there was a significant association with *APOE* genotype, significance between specific *APOE* alleles was assessed using parameter estimates obtained from the GLM results.

FIGURE 2 Absolute concentrations (µg/ml) of docosapentaenoic acid (DPA) in phosphatidylcholine (PC), non-esterified fatty acids (NEFAs), cholesteryl esters (CEs) and triacylglycerols (TGs) lipid fractions in male and female subjects according to *APOE* genotype. Distribution of participants in each *APOE* allele group are as follows; PC: Males: E2/E2 = 1, E2/E3 = 32, E3/E3 = 40, E3/E4 = 51, and E4/E4 = 2. Total: 126. PC: Females: E2/E2 = 3, E2/E3 = 39, E3/E3 = 44, E3/E4 = 43, and E4/E4 = 10. Total: 139. NEFAs: Males: E2/E2 = 2, E2/E3 = 29, E3/E3 = 45, E3/E4 = 50, and E4/E4 = 2. Total: 128. NEFAs: Females: E2/E2 = 3, E2/E3 = 42, E3/E3 = 44, E3/E4 = 45, and E4/E4 = 10. Total: 144. CEs: Males: E2/E2 = 2, E2/E3 = 33, E3/E3 = 50, E3/E4 = 52, and E4/E4 = 2. Total: 139. CEs: Females: E2/E2 = 3, E2/E3 = 44, E3/E3 = 48, E3/E4 = 49, and E4/E4 = 10. Total: 154. TGs: Males: E2/E2 = 2, E2/E3 = 32, E3/E3 = 50, E3/E4 = 53, and E4/E4 = 2. Total: 139. TGs: Females: E2/E2 = 3, E2/E3 = 45, E3/E3 = 49, E3/E4 = 48, and E4/E4 = 10. Total: 155. * $P < 0.050$. ** $P > 0.050$ but < 0.070. *P* values were obtained using log-10 data in univariate general linear model (GLM) analysis controlling for covariates (age, BMI, and oily fish intake). Where there was a significant association with *APOE* genotype, significance between specific *APOE* alleles was assessed using parameter estimates obtained from the GLM results.

FIGURE 3 Absolute concentrations (µg/ml) of docosahexaenoic acid (DHA) in phosphatidylcholine (PC), non-esterified fatty acids (NEFAs), cholesteryl esters (CEs) and triacylglycerols (TGs) lipid fractions in male and female subjects according to *APOE* genotype. Distribution of participants in each *APOE* allele group are as follows; PC: Males: E2/E2 = 1, E2/E3 = 32, E3/E3 = 40, E3/E4 = 51, and E4/E4 = 2. Total: 126. PC: Females: E2/E2 = 3, E2/E3 = 39, E3/E3 = 44, E3/E4 = 43, and E4/E4 = 10. Total: 139. NEFAs: Males: E2/E2 = 2, E2/E3 = 29, E3/E3 = 45, E3/E4 = 50, and E4/E4 = 2. Total: 128. NEFAs: Females: E2/E2 = 3, E2/E3 = 42, E3/E3 = 44, E3/E4 = 45, and E4/E4 = 10. Total: 144. CEs: Males: E2/E2 = 2, E2/E3 = 33, E3/E3 = 50, E3/E4 = 52, and E4/E4 = 2. Total: 139. CEs: Females: E2/E2 = 3, E2/E3 = 44, E3/E3 = 48, E3/E4 = 49, and E4/E4 = 10. Total: 154. TGs: Males: E2/E2 = 2, E2/E3 = 32, E3/E3 = 50, E3/E4 = 53, and E4/E4 = 2. Total: 139. TGs: Females: E2/E2 = 3, E2/E3 = 45, E3/E3 = 49, E3/E4 = 48, and E4/E4 = 10. Total: 155. * $P = 0.021$. *P* values were obtained using log-10 data in univariate general linear model (GLM) analysis controlling for covariates (age, BMI, and oily fish intake). Where there was a

significant association with *APOE* genotype, significance between specific *APOE* alleles was assessed using parameter estimates obtained from the GLM results.