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

Helena L Fisk*[1], Michael Irvine[2], Elizabeth A Miles[1], John C Mathers[3], Chris J Packard[4], Christopher K Armah[5], Bettina M Kofler[5,*] , Peter J Curtis[2], Anne M Minihane[2] and Philip C Calder[1,6]

[1]Faculty of Medicine, University of Southampton, Southampton, United Kingdom.

[2]Norwich Medical School, The Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, United Kingdom.

[3]Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom.

[4] College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom.

[5]The School of Chemistry, Food Biosciences and Pharmacy, University of Reading, Reading, United Kingdom.

[6]The National Institute for Health Research Southampton Biomedical Research Centre, University Hospital Southampton National Health Service Foundation Trust and University of Southampton, Southampton, United Kingdom.

[2]Supported by grant no: RRD7/N02/A from the UK Food Standards Agency, and by Ocean Nutrition, which donated the oil capsules used in the study.

[3]Reprints not available. Address correspondence to Helena L. Fisk, Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, IDS Building, MP887 Southampton General Hospital, Tremona Road, Southampton SO16 6YD, United Kingdom

E-mail: h.fisk@soton.ac.uk

[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

60 The FINGEN study was a multi-centre trial conducted at the Universities of Glasgow,
61 Newcastle, Reading and Southampton in the United Kingdom. Three hundred and twelve
62 participants were recruited prospectively on the basis of *APOE* genotype (87 were *APOE*2
63 homozygotes or *APOE*2/*APOE*3, 111 were homozygous for *APOE3,* and 114 were *APOE*4/APOE3
64 or APOE4 homozygotes), sex (149 male and 163 female) and age (20 to 71 years, with
65 approximately equal numbers in each of the 5 decades) [4]. Data from 306 participants were included
66 in the current analysis, with the numbers in each subgroup detailed in **Supplemental Table 1** and
67 **Supplemental Table 2**. Exclusion criteria included: diagnosed endocrine dysfunction including
68 diabetes or fasting glucose concentration > 6.5 mmol/L, myocardial infarction in the previous 2
69 years, the use of medication that may interfere with lipid metabolism, fasting total cholesterol of >
70 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
71 weight loss diet. Individuals taking n-3 fatty acid supplements were also excluded. The study was
72 approved by the research ethics committee at each of the participating centres and written informed
73 consent was obtained from all subjects prior to participation.
74

75 **Study design**

76 The FINGEN study was a randomised double blind, placebo controlled, crossover study
77 testing two doses of fish oil compared with placebo [4]. Here we evaluate the association of oily fish
78 intake, sex, age, BMI and *APOE* genotype with fasting concentrations of EPA, DPA and DHA in
79 plasma phosphatidylcholine (PC), non-esterified fatty acids (NEFAs), cholesteryl esters (CEs) and
80 triacylglycerols (TGs) at baseline, prior to intervention. Habitual oily fish intake was estimated by
81 food frequency questionnaire (FFQ), using self-reported portions completed at baseline. Oily fish
82 was defined as salmon, herring, mackerel, fresh tuna, sardines, kippers, and trout.
83

84 **Fatty acid analysis**

85 The fatty acid composition of the plasma fractions was determined by gas chromatography.
86 Dipentadecanoyl PC, heneicosanoic acid, cholesteryl heptadecanoate and tripentadecanoin internal
87 standards were added to the plasma. Total plasma lipid was extracted using chloroform: methanol
88 (2:1, v/v) containing butylated hydroxytoluene (50 mg/L) as described by Folch et al [16], and PC,
89 NEFA, CE and TG fractions were separated and isolated by solid phase extraction on aminopropyl
90 silica cartridges. CEs and TGs were eluted in a combined fraction with the addition of chloroform.
91 PC was then eluted from the cartridge with the addition of chloroform: methanol (60:40 v/v).
92 NEFAs were eluted from the cartridge with the addition of chloroform: methanol: glacial acetic acid
93 (100:2:2 v/v). CEs and TGs were separated on a hexane primed aminopropyl silica cartridge with
94 the addition of hexane to elute CEs, and the addition of hexane: methanol: ethyl acetate (100:5:5

95 v/v/v) to elute TGs. The fatty acids within the resulting lipid fractions were methylated by the
96 addition of methanol in 2% (v/v) sulphuric acid at 50°C for 2 hours to produce fatty acid methyl
97 esters (FAMEs) [17]. FAMEs were extracted into hexane and separated in a BPX-70 fused silica
98 capillary column (30 m × 0·25 mm × 25 μm; SGE Analytical Science, United Kingdom) using an
99 Agilent 6890 series gas chromatograph equipped with flame ionisation detection (Agilent
100 Technologies, California, United States). The FAMEs were identified by comparison with retention
101 times of 37 FAME and menhaden oil standards run alongside the samples, and quantified with the
102 use of the internal standards using ChemStation software (Agilent Technologies, California, United
103 States) and Microsoft Excel (Microsoft Corporation, Washington, United States). Fatty acid
104 composition data are expressed as absolute concentrations (µg/ml plasma) and as relative
105 concentrations (g/100 g total fatty acid (%)).
106

107 **Statistics**

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

111 Results for the relative (%) and absolute concentrations (µg/ml) of fatty acids are reported
112 for 303 to 306 and 292 to 306 participants in the four plasma lipid fractions. Data were checked for
113 normality by plotting distributions of residuals obtained from general linear model (GLM) analysis
114 of the data, and were analysed appropriately with a univariate GLM following $\log_{10}$ transformation.
115 All variables were included in the univariate model with individual associations analysed using
116 'main effects' and interaction between age and BMI, age and fish intake, and sex and *APOE*
117 analysed using 'interaction' analysis options within the model. *P* values were corrected for multiple
118 analyses using Bonferroni post hoc analysis resulting in a significance value of $P = 0.006$ for whole
119 group analysis and $P = 0.008$ for analyses where males and females were analysed separately. All
120 statistical analyses were conducted using SPSS software (version 21; SPSS Inc, Chicago, IL).
121 Statistical significance was defined as $P \leq 0.05$. Results are expressed as mean ± SEM or median
122 (25th, 75th percentiles).
123

124 **Results**

125 The group (n = 306) mean age and BMI was 45.1 ± 0.7 y and 25.2 ± 0.2 kg/m^2, respectively.
126

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

129 differences in the proportion of total dietary energy consumed from fat, saturated fat (SFA),
130 monounsaturated fat (MUFA) or polyunsaturated fat (PUFA) (data not shown). The average oily
131 fish intake was 1.0 portion per week with no association of sex with oily fish intake.
132
133 For all three LC n-3 PUFAs, the greatest concentrations were evident in the PC fraction, with
134 median absolute concentrations (µg/ml) of 15.1, 11.9 and 44.1 for EPA, DPA and DHA,
135 respectively. The median values for EPA, DPA and DHA for the whole group and *P* values for the
136 association of oily fish intake, sex, age, BMI and *APOE* with the plasma concentrations of these
137 fatty acids in the four lipid fractions are presented in **Table 1.** The data according to oily fish intake
138 are shown in **Supplemental Figures 1-4**, while data according to age and BMI are shown in **Table**
139 **2 and Supplemental Tables 3-5**, and those according to *APOE* genotype*sex in **Figures 1-3.**
140
141 <u>Plasma EPA, DPA and DHA in the group as a whole</u>
142
143 *EPA*: The concentration of EPA in plasma CEs and TGs was positively associated with oily fish
144 intake ($P \leq 0.004$), with evidence for positive association in plasma PC also ($P = 0.018$) (**Table 1**).
145 There was evidence for a positive association between EPA and age in plasma PC, CE's and TGs (*P*
146 = 0.021, 0.019, and 0.034 respectively) and for the concentration of EPA in CEs to differ by sex (*P*
147 = 0.055), (**Table 2**). A higher concentration of EPA in CEs was observed in males (**Table 2**), and
148 the concentration of EPA in TGs was associated with an *APOE**sex interaction ($P = 0.044$, data not
149 shown).
150
151 *DPA:* The concentration of DPA was positively associated with oily fish intake in plasma TGs (*P* =
152 0.006), with evidence for positive association in plasma PC also ($P = 0.022$) **(Table 1)**. DPA in TGs
153 was positively associated with BMI ($P = 0.006$) (**Table 1**), and there was evidence for the positive
154 association of DPA in NEFAs and CEs with age (*P* = 0.031 and 0.007 respectively, **Table 1**). The
155 concentration of DPA significantly differed by sex with a higher concentration of DPA observed in
156 plasma TGs in males ($P < 0.001$), with a trend in PC also (*P* 0.031) (**Table 1**). There was also a
157 significant *APOE**sex interaction for the concentration of DPA in CEs ($P \leq 0.005$, data not shown).
158 (**Table 1**),
159
160 *DHA:* The concentration of DHA in all plasma lipid fractions was positively associated with oily
161 fish intake ($P \leq 0.001$). There was evidence for a positive association of DHA in TGs with BMI (*P*
162 = 0.020) (**Table 1**) and with age in PC,-CEs and TGs (*P* = 0.037, 0.039, and 0.050 respectively,
163 **Table 1**).

164

165 Overall in PC, NEFAs, CEs, and TGs, the highest oily fish consumers (2+ portions of oily fish per
166 week) had 55%, 42%, 52% and 119% higher EPA+DHA, respectively, compared with those
167 reporting no oily fish intake (**Supplemental Figure 4**).
168
169 Due to the significant evidence of sex and *APOE**sex interactions, subgroup analysis was
170 performed in males and females separately.
171
172 <u>Subgroup analysis of plasma EPA, DPA and DHA according to sex</u>

173
174 Significance data (*P*) are reported for EPA, DPA and DHA in **Table 2** and median data are reported
175 for EPA, DPA and DHA in **Supplemental Tables 3, 4, and 5** respectively.
176
177 *EPA* (**Table 2, Supplemental Table 3**)**:** The concentration of EPA in plasma TGs was positively
178 associated with oily fish intake in both males and females ($P \leq 0.008$), while the concentration of
179 EPA in PC was positively associated with oily fish intake in females only ($P \leq 0.004$. EPA
180 concentration in TGs was positively associated with age and BMI in females ($P = 0.006$), while
181 EPA in TGs differed by *APOE* genotype in males ($P = 0.002$), with evidence for this in CEs also (P
182 $= 0.019$), (**Figure 1**). A greater concentration of EPA in TGs was observed in male *APOE*4 carriers
183 ($P = 0.002$) with evidence for this in PC and CEs also ($P = 0.019$ and 0.053 respectively), (**Figure**
184 **1**).
185
186 *DPA* (**Table 2, Supplemental Table 4**): The concentration of DPA in plasma TGs was positively
187 associated with oily fish intake in females ($P = 0.008$). There was evidence for DPA concentration
188 in PC to differ with *APOE* genotype in males ($P \leq 0.053$, **Figure 2**) with further analysis revealing
189 evidence for higher concentrations of DPA in PC in *APOE*4 allele carriers ($P = 0.032$, **Figure 2**).
190
191 *DHA* (**Table 2, Supplemental Table 5**): The concentration of DHA was positively associated with
192 oily fish intake in plasma PC, NEFAs, and TGs in females ($P \leq 0.002$) and plasma PC in males ($P \leq$
193 0.003), (**Table 2**). There was evidence for DHA in plasma NEFAs to be associated with BMI in
194 females ($P = 0.010$, **Table 2**), and for DHA in CEs to differ by *APOE* genotype in males. Further
195 analysis revealed evidence for a higher concentration of DHA in CEs in *APOE*4 carriers ($P = 0.021$,
196 **Figure 3**).
197

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

232 compared with those reporting no oily fish consumption. Two portions of oily fish supply about 4-5
233 g of EPA+DHA per week, equivalent to 600-700 mg per day[22 23]. Previous studies report
234 comparable increases of 81% in plasma phospholipid EPA+DHA, and 8.8 µg/ml and 8.5 µg/ml in
235 total plasma EPA and DHA respectively following 16 week consumption of oily fish providing 485
236 mg EPA+DHA per day [20] and 6 week consumption of oily fish providing 927 mg EPA+DHA per
237 day, respectively[21]. Overall, the findings of the current analysis support existing reports that oily
238 fish intake is associated with, and at a population level is the main determinant of, LC n-3 PUFAs
239 in all major blood lipid pools, which may therefore be used as biomarkers of oily fish intake [4 19 24
240 25]. Our analysis does not clearly indicate which plasma lipid fraction would best reflect dietary
241 intake of EPA and DHA, since, in general all four plasma lipid fractions showed dose-dependent
242 increases in EPA and DHA concentration (both absolute and relative) with increasing frequency of
243 oily fish consumption.

244 There is some evidence that age influences the concentration of EPA and DHA in various
245 plasma fatty acid fractions, [10] which has been attributed in part to higher habitual fish intake with
246 increasing age. Oily fish intake was controlled for in the current statistical analysis, allowing clearer
247 attribution of any observed associations of age with EPA, DPA and DHA concentrations to altered
248 metabolism and not to dietary differences in intakes of oily fish. Any influence of *APOE* group
249 distribution was also ruled out as, despite a greater number of individuals aged 50-59 yr being
250 included in the current analysis, there was no significant difference in the distribution of *APO E*2,
251 *E*3 and *E*4 genotypes between age groups (data not shown). A 28 d stable isotope tracer study in
252 young (mean age 27 y) vs older (mean age 77 y) adults reported a 1-2 fold greater enrichment of
253 ^{13}C–DHA in plasma phospholipids and CEs in the older age group, suggesting a medium term age-
254 related difference in DHA homeostasis associated with accumulation of DHA in the circulation in
255 older people [26]. The findings of the current analysis support reports of increased plasma DHA with
256 increasing age [11 27 28] and we further also report positive associations between age and EPA and
257 DPA, suggesting LC n-3 PUFAs accumulate in plasma pools during ageing. However, this may in
258 part be due to an increase in circulating cholesterol and CE with age (**Table 3**). Evidence of positive
259 associations of plasma total cholesterol with age dates back to the late 1970s [29], and these have been
260 reported in both males and females [30]. Increased circulating LDL (**Table 3**) may be reflected in
261 higher absolute total PC and CE concentrations with age (*P* = 0.008 and 0.018, age 20-29 vs 60+ yr
262 for PC and CE respectively, data not shown) and we observed that total cholesterol (TC) and LDL-
263 cholesterol (LDLC) concentrations were significantly positively correlated with LC n-3 PUFA
264 concentrations in PC (TC, *P* = <0.001, 0.003, 0.027; LDLC *P* = <0.001, <0.001, 0.003, absolute
265 EPA, DPA and DHA respectively, data not shown), and that TC, LDLC and high density
266 lipoprotein cholesterol (HDLC) concentrations were positively correlated with LC n-3 PUFA in

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

271 Insulin has a role in the regulation of genes involved in whole body lipid homeostasis
272 including in the removal of lipids from the circulation[31]; in cases of insulin resistance, such
273 removal can be compromised. The occurrence of insulin resistance is reported to rise with
274 increasing age and BMl and despite individuals with diabetes or a fasting glucose concentration >
275 6.5 mmol/L being excluded from the current analysis, differences in fasting glucose were still
276 evident between age and BMI groups (glucose positively correlated with age and BMI; *P* <0.001
277 both, data not shown). Thus, insulin resistance may contribute to the higher EPA and DPA
278 concentrations in plasma lipid pools observed with increasing age and BMI.

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

292 The proteins encoded by the *APOE* gene play a major role in the transport and metabolism
293 of lipids via interaction with LDL receptors (LDLRs). Two common polymorphisms (rs7412 and
294 rs429358) of the *APOE* gene in humans result in three protein isoforms, APOE2, E3 and E4.
295 APOE2 and APOE3 are found in the circulation mainly on high density lipoproteins (HDLs)
296 whereas APOE4 is found preferentially on very low density lipoproteins (VLDLs) with lower
297 concentrations residing on HDLs [37]. The *APOE4* allele has been associated with reduced longevity
298 [38], and enhanced risk of cardiovascular disease [39] and Alzheimer's disease [40]. Although centrally
299 involved in fatty acid transport and handling in plasma and tissues (and in particular within the
300 brain where *APOE* is almost the only apolipoprotein present), the impact of *APOE* genotype on
301 these processes, and the contribution of dysregulated EPA and DHA metabolism to disease risk is

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

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

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

337 males when consuming a beef based diet. These results were not observed when consuming a fish
338 based diet in which the capacity to convert DPA to DHA was equal between males and females.
339 These findings suggest LC n-3 PUFA metabolism in females may be more sensitive to dietary
340 alterations or may be affected by hormonal regulation[43]. Indeed there is evidence for up-regulation
341 of the desaturase-elongase pathway via oestrogenic actions resulting in increased conversion of
342 ALA to EPA[19 44 45] and to DHA[11 13 46] indicating significant effects of female sex hormones on the
343 metabolism of LC n-3 PUFAs. Consistent with these observations, there is evidence for an increase
344 in DHA in relation to EPA and DPA at baseline and in response to EPA+DHA intake in females
345 compared to males [47 48]. The current analysis further reports lower concentrations of both DPA (-
346 36% lower absolute concentration in TGs) and EPA (20% lower absolute concentration in TGs) in
347 females but does not report higher concentrations of DHA in females or find a significant effect of
348 sex on the ratio of DPA: DHA (P >0.50, data not shown). However, these results are also in
349 contrast to other reports describing increased concentrations of EPA and DHA in females [19 44 45].
350 These data from the current analysis suggest investigation into associations between sex, *APOE,*
351 and fatty acid synthesis enzymes and transporters would be of worthwhile to further understand the
352 mechanisms by which these associations occur.
353
354 In conclusion, we report concentrations of EPA, DPA and DHA to vary across *APOE*
355 genotype and that sex is an important factor to consider when evaluating LC n-3 PUFA
356 concentrations in these genotypic subgroups. Our results also confirm that concentrations of EPA,
357 DPA and DHA in plasma pools are suitable population markers of oily fish consumption and show
358 that age and sex are important contributors to the variation in EPA, DPA and DHA concentrations
359 in plasma lipids independent of *APOE* genotype. These variables should be considered when
360 interpreting LC n-3 PUFA concentrations as a marker of dietary intake and when suggesting dietary
361 LC n-3 PUFA recommendations to ensure benefits are achieved across population subgroups.
362 Investigation into the handling of supplemental EPA and DHA in these subgroups is to be
363 addressed in a further publication and could provide the basis for more detailed advice. However,
364 the aetiology and physiological significance of the interaction between sex and *APOE* genotype and
365 its association with EPA, DPA and DHA status still requires further investigation.
366
367 **Acknowledgements**
368 The authors thank Christine Williams and Muriel Caslake for study design contributions, and
369 Dorothy Bedford, Josephine Cooney, Frances L Napper, Lesley Farrell, Christine Gourlay, Jilly P
370 Grew, Elaine McDonald, Elizabeth Murray, Julie Stannard, Grace Stewart, May Stewart, Philip
371 Stewart, Elli Vastardi and Jan Luff for technical assistance.

372

373 **Authors' responsibilities**

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

381

382 **Conflicts of interest**

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

385

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504

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 (25th, 75th percentile)	1.01 (0.65, 1.57)	0.43 (0.26, 0.66)	0.77 (0.46, 1.10)	0.42 (0.27, 0.65)	15.14 (8.74, 23.23)	0.85 (0.48, 1.47)	14.2 (8.45, 23.45)	2.98 (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 (25th, 75th percentile)	0.78 (0.55, 0.98)	0.31 (0.22, 0.44)	0.07 (0.05, 0.12)	0.33 (0.23, 0.47)	11.87 (7.90, 15.67)	0.62 (0.42, 0.86)	1.34 (0.84, 2.51)	2.32 (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	NEFAs	CEs	TGs	PC	NEFAs	CEs	TGs
	(*P*)	(*P*)	(*P*)	(*P*)	(*P*)	(*P*)	(*P*)	(*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.