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APOE genotype, eicosapentaenoic acid (EPA) supplementation and *n*-3 highly unsaturated fatty acid (HUFA) levels in patients with multiple colorectal polyps: A secondary analysis of the seAFOod polyp prevention trial

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ABSTRACT

Introduction: We examined the relationship between *Apolipoprotein E* (*APOE*) genotype and *n*-3 highly unsaturated fatty acid (HUFA) levels in participants of the seAFOod trial, who were undergoing colonoscopy surveillance after removal of colorectal polyps.

Methods: Baseline and on-treatment (eicosapentaenoic acid [EPA] 2 g daily or placebo for 6 months) levels of *n*-3 HUFAs, and plasma 18-hydroxyeicosapentaenoic acid (HEPE), were analysed according to *APOE* genotype (based on polymorphisms rs429358 and rs7412) in 584 participants.

Results: Before treatment, *APOE2/2* individuals had lower levels, and *APOE4/4* participants had higher levels, of *n*-3 HUFAs, including EPA, than *APOE3/3* counterparts ($P < 0.01$ for the *APOE2/2 versus APOE4/4* comparison). After EPA supplementation, *n*-3 HUFA levels were not significantly different when stratified by *APOE* genotype, although *APOE4* carriers displayed lower plasma 18-HEPE levels than individuals without an *APOE4* allele ($P = 0.002$).

Conclusions: APOE genotype is associated with differential *n*-3 HUFA and 18-HEPE levels in individuals with multiple colorectal polyps.

1. Introduction

Apolipoprotein E (APOE) is an important regulator of the transport and metabolism of lipids, including omega (*n*)-3 highly unsaturated fatty acids (HUFAs) [1]. Functional genetic variants of the *APOE* gene have been implicated in risk of several non-communicable diseases particularly Alzheimer's disease [1,2]. There are three *APOE* alleles ε2, ε3 and ε4, which give rise to three APOE protein isoforms based on the combination of two functional, non-synonymous, single nucleotide polymorphisms (SNPs) (rs429358 [Cys112Arg] and rs7412 [Cys158Arg]), with *APOE3* (Cys112/Arg158) being the common, wild-type variant [1]. The relationship between *APOE* genotype and plasma levels of the two main bioactive *n*-3 HUFAs C20:5*n*-3 eicosapentaenoic acid (EPA) and C22:6*n*-3 docosahexaenoic acid (DHA), as well as *n*-3 HUFA-derived

Abbreviations: APOE, Apolipoprotein E; BCSP, Bowel Cancer Screening Programme; BMI, body mass index; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; EPIC, European Prospective Investigation into Cancer and Nutrition; HEPE, hydroxyeicosapentaenoic acid; HUFA, highly unsaturated fatty acid; RBC, red blood cell; SNP, single nucleotide polymorphism.

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oxylipin metabolites, before and after mixed *n*-3 HUFA supplementation has been restricted to studies in healthy human participants and patients with Alzheimer's disease, with variable findings [3–6]. Carriers of at least one *APOE4* allele have been reported to display a reduced plasma *n*-3 HUFA response to mixed EPA/DHA and DHA-predominant *n*-3 HUFA interventions in some [3,4,6], but not all [5], studies. However, the relationship between *APOE* genotype and *n*-3 HUFA levels in response to pure EPA supplementation has not been addressed previously.

The seAFOod trial was a randomised, placebo-controlled, double-blind, 2 × 2 factorial colorectal polyp prevention trial (ISRCTN05926847), which tested the efficacy of pure EPA 2 g free fatty acid daily and aspirin 300 mg daily, taken for 12 months in individuals aged 55–73 years with 'high risk' colorectal polyp findings (≥3 polyps, if one polyp was ≥10 mm; or ≥5 polyps of any size) from a national Bowel Cancer Screening Programme (BCSP) colonoscopy, at which all visible colorectal polyps were removed [7]. The seAFOod trial population is predominantly White British and male, consistent with the demographic of individuals undergoing English BCSP screening colonoscopy [7]. The primary outcome of the trial (the number of individuals with one or more colorectal polyps at surveillance colonoscopy one year later) was null for both interventions. However, in a secondary analysis, although EPA use did not reduce total (adenomatous and serrated) colorectal polyp number per participant, allocation to active EPA was associated with a statistically significant reduction in risk of left-sided (distal to the splenic flexure) adenomatous polyps [7]. By contrast, aspirin use was associated with a significant reduction in total colorectal polyp number compared with placebo [7]. A secondary genetic analysis of the seAFOod trial has already been conducted using microfluidic PCR technology to genotype 78 SNPs, relevant to oxylipin synthesis, fatty acid metabolism and colorectal cancer risk [8]. An investigation of plasma levels of *n*-3 HUFA-derived oxylipin metabolites including 18-hydroxyeicosapentaenoic acid (HEPE) has also been reported in seAFOod trial participants [9].

Herein, we report the relationship between *APOE* genotype and levels of *n*-3 HUFAs in red blood cells (RBCs) at baseline and after EPA treatment for six months in individuals, who had undergone clearance colonoscopy after a finding of multiple colorectal polyps in the seAFOod polyp prevention trial. We also investigated the relationship between *APOE* genotype and plasma concentration of 18-HEPE as a major monohydroxy metabolite of EPA, levels of which have been reported to be linked to *APOE* genotype in healthy adult humans [5].

2. Methods

This secondary analysis of the seAFOod polyp prevention trial is part of a wider programme of investigations using the seAFOod trial biobank called STOP-ADENOMA (ISRCTN05926847). Ethical approval for this study was granted by the London and Surrey Borders Research Ethics Committee (19/LO/1655).

2.1. Genotype analysis

DNA was extracted from buffy coat samples from seAFOod trial participants [8]. Fluidigm microfluidic SNP genotyping, which included *APOE* SNPs rs429358 and rs7412, was performed, as described [8].

Single nucleotide polymorphism rs7412 did not satisfy Hardy-Weinberg equilibrium ($P < 0.05$ with Benjamini-Hochberg correction for multiple testing) [8]. However, on inspection of the scatter plot for individual genotypes, "true calls" did not overlap with "fails" suggesting that this was not caused by sampling error. Conceivably, rs7412 genotype could be causally related to colorectal polyp development (and thus seAFOod trial recruitment), thereby explaining absence of Hardy–Weinberg equilibrium, so rs7412 was retained for analysis.

Cases which were genotyped as rs429358 C/T and rs7412 C/T were assigned as *APOE2/4* (CT/CT) on the basis that only a few cases of APOE3r (APOE1), which is the other possible allele combination, have ever been recorded worldwide [10].

2.2. Blood sample collection and laboratory analysis

Blood sampling during seAFOod trial participation is described in detail in the Trial protocol [11]. Baseline samples were obtained prior to starting trial interventions. Blood samples were also obtained during (6 months), and at the end of (12 months), the intervention period. The majority of seAFOod trial participants provided an end of intervention blood sample immediately after the trial exit colonoscopy at 12 months [11]. Previous secondary analysis of plasma oxylipin and urine biomarker levels in seAFOod trial participants has suggested that the proximity of the sample collection to colonoscopy bowel preparation, and/or the procedure itself, may alter HUFA metabolism and confound the analysis of the relationship between genotype and plasma oxylipin concentration [9,12]. Therefore, we restricted the current study to values obtained from 6-month on-treatment blood samples.

Levels of *n*-3 HUFAs (presented as % total fatty acids for EPA, *n*-3 docosapentaenoic acid [DPA] and DHA) in RBC membranes, and the plasma concentration of 18-hydroxyeicosapentaenoic acid (HEPE; a combination of both *S*- and *R*- enantiomers measured as ng/mL), were measured by liquid chromatography-tandem mass spectrometry, as reported [7,9]. In brief, total PUFAs (including esterified and free fatty acids) were extracted from RBC membranes by acid hydrolysis and chloroform extraction before quantification of a panel of nine fatty acids using authentic fatty acid standards and an internal extraction standard (deuterated alpha-linolenic acid-d14) [13].

2.3. Clinical data on seAFOod trial participants

Linked clinical data on participant sex and body mass index (BMI) were available from the seAFOod trial database. Daily dietary intake of EPA and DHA (g/day) at baseline was calculated from data on fish intake over the previous 6 months collected with the European Prospective Investigation into Cancer and Nutrition (EPIC) short-form food frequency questionnaire, which was completed at trial entry [7]. Dietary fish intake was converted into EPA and DHA intake per day using the EPIC-Norfolk fatty acid nutrient database [14].

2.4. Statistical analysis

Analysis of baseline *n*-3 HUFA levels in seAFOod participants utilised the whole seAFOod trial cohort that had available *n*-3 HUFA and *APOE* genotype data. For investigation of a genotype x treatment interaction for *n*-3 HUFA levels, we restricted analysis to participants that were randomised to active EPA, regardless of their simultaneous allocation to placebo or active aspirin, according to the 2 × 2 factorial trial design and primary trial analysis [7]. By contrast, although we did not observe a statistically significant difference in plasma 18-HEPE levels in seAFOod trial participants randomised to aspirin compared with placebo in an earlier secondary analysis [9], it is clear that aspirin could affect COX-dependent 18-HEPE synthesis [15]. Therefore, the plasma 18-HEPE analysis was restricted to trial arms that received placebos only or EPA alone.

Data on *n*-3 HUFA and oxylipin levels are presented using a logarithmic axis for ease of visualisation of a wide range of individual data points. The impact of *APOE* genotype on RBC *n*-3 HUFA and plasma 18-HEPE levels was assessed using the Kruskal-Wallis test or one-way analysis of variance (ANOVA), depending on the normality of the data distribution. Data on *n*-3 HUFA levels after EPA supplementation for 6 months were logarithmically transformed to generate a normal distribution for ANOVA testing. Post-hoc analysis was conducted if the Kruskal-Wallis test or one-way ANOVA yielded a P value ≤0.1. Factors potentially influencing EPA, DPA, and DHA levels were explored using multivariate linear regression using *APOE3/3* as the reference group.

Covariates were sex, dietary EPA+DHA intake, and body mass index (BMI). Statistical analysis was carried out using R (version 2021.09.0).

3. Results

3.1. The relationship between APOE genotype and RBC n-3 HUFA levels at baseline

Five hundred and eighty-four seAFOod trial participants (male n=463, female n=121) had an available *APOE* genotype and data on RBC HUFA levels at baseline before trial intervention (Fig. 1). The distribution of *APOE* genotypes was *APOE2/2* (n = 9; 1.5 %), *APOE2/3* (n = 72; 12.3 %), *APOE2/4* (n = 16; 2.7 %), *APOE3/3* (n = 323; 55.3 %), *APOE3/4* (n = 152; 26.0 %), and *APOE4/4* (n = 12; 2.1 %).

The level of EPA, DPA and DHA in RBCs varied in individuals with different APOE genotypes, with *APOE2/2* individuals demonstrating lower levels of EPA (median % EPA 0.35), DPA and DHA compared to *APOE3/3* counterparts (median % EPA 0.47), before EPA intervention (Fig. 2A). Conversely, *APOE4/4* participants had higher *n*-3 HUFA levels (median % EPA 0.66) than *APOE3/3* individuals (Fig. 2A). Inter-group differences were found to be borderline statistically significant by the Kruskal-Wallis test (Fig. 2A), although *post hoc* testing revealed that the difference in *n*-3 HUFA levels between *APOE2/2* and *APOE4/4* individuals was statistically significant (EPA and DHA, $P < 0.01$; DPA, $P < 0.001$; Fig. 2A). Similar results were obtained when the three major *n*-3 HUFAs EPA, DPA and DHA were pooled together as total *n*-3 HUFAs (Supplementary Figure 1).

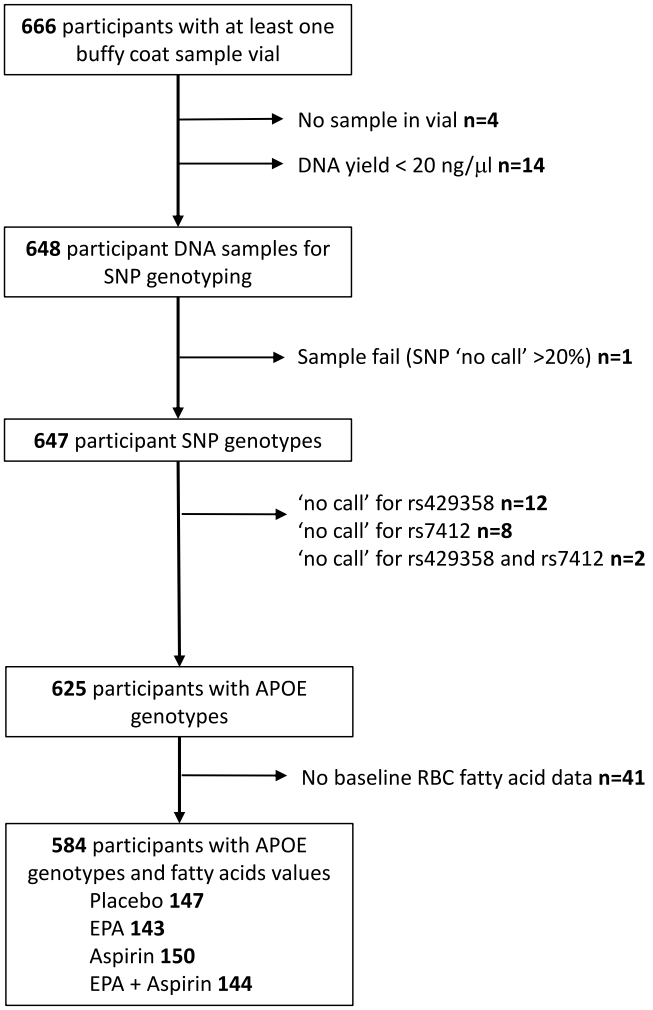

Fig. 1. seAFOod trial participant DNA and APOE genotype data. EPA, eicosapentaenoic acid; RBC, red blood cell; SNP. Single nucleotide polymorphism.

The seAFOod trial cohort displayed a preponderance of men, which reflects the sex balance of individuals undergoing BCSP colonoscopy in England, as well as a high prevalence of excess body weight (defined as BMI $\geq$ 25 Kg/m^2; n = 472 [81 %]) [7]. In the seAFOod trial population with an available APOE genotype, there was no sex difference in *n*-3 HUFA levels, although females displayed a higher level of EPA in RBCs compared to males, which just failed to reach the pre-specified statistical significance level of $\alpha = 0.05$ ($p = 0.068$, Fig. 2B). However, individuals with excess body weight exhibited statistically significantly lower EPA ($P = 0.021$), DPA ($P = 0.012$), and DHA ($P = 0.025$) levels in RBCs than those with a BMI less than 25 kg/m^2 (Fig. 2B). Age did not significantly influence the levels of EPA, DPA, or DHA in RBCs at baseline in the restricted age range displayed by seAFOod trial participants, who were aged between 55 and 73 years (data not shown).

Multivariate analysis incorporating sex, dietary EPA+DHA intake, BMI and *APOE* genotype as variables confirmed that dietary EPA+DHA intake was strongly predictive of the respective % level of *n*-3 HUFAs and that excess body weight was associated with lower EPA, DPA, and DHA levels (Table 1 and Supplementary Table 1). The *APOE2/2* variant was associated with lower DPA (beta-coefficient and 95 % CI: -0.57 [-1.16 to -0.01]) and DHA levels (beta-coefficient and 95 % CI: -0.60 [-1.16 to -0.03]) compared with *APOE3/3* individuals (Table 1).

3.2. The relationship between APOE genotype and changes in RBC n-3 HUFA levels after EPA supplementation for 6 months

The levels of the three main *n*-3 HUFAs EPA, DPA and DHA in RBC membranes after EPA supplementation for 6 months in seAFOod trial participants have been reported previously [7,16]. The increase in EPA and DPA in RBCs after EPA supplementation for 6 months is apparent in Fig. 3A compared with Fig. 2A. However, individual *n*-3 HUFA levels in RBCs after EPA supplementation for 6 months were not significantly different when stratified by *APOE* genotype (Fig. 3A). Respective median EPA values for *APOE3/3* and *APOE3/4* participants were 1.73 % and 1.86 %. Similar results were obtained when the *n*-3 HUFAs were pooled as total *n*-3 HUFAs (Supplementary Figure 2).

3.3. The relationship between APOE genotype and plasma 18-HEPE concentration after EPA supplementation for 6 months

We have previously reported that EPA treatment led to an increase in plasma concentration of the mono-hydroxy EPA metabolite 18-HEPE [9]. Therefore, we investigated the relationship between *APOE* genotype and total 18-HEPE concentration in plasma from participants who received EPA alone, excluding participants that received concurrent aspirin. Plasma 18-HEPE concentrations were comparable across different genotypes at baseline (Supplementary Figure 3). There was no significant difference in plasma 18-HEPE concentration among participants, who received placebos only for 6 months, according to *APOE* genotype ($P = 0.11$; Fig. 3B), although *APOE2/2, APOE2/4* or *APOE4/4* genotypes were not evaluable due to insufficient cases (Fig. 3B). Respective median plasma 18-HEPE levels in *APOE3/3* and *APOE3/4* individuals were 0.17 ng/ml and 0.10 ng/ml. However, the *APOE* genotype influenced 18-HEPE levels after EPA supplementation for 6 months (Fig. 3B, $P = 0.002$). *Post hoc* analysis with Bonferroni adjustment indicated that *APOE3/4* participants displayed lower plasma 18-HEPE levels (median 0.67 ng/ml) than those with *APOE2/3* (median 1.79 ng/ml; $P = 0.019$) and *APOE3/3* genotypes (median 1.92 ng/ml; $P = 0.003$, Fig. 3B).

4. Discussion

We report the relationship between *APOE* genotype and *n*-3 HUFA levels before and during supplementation with a formulation of pure

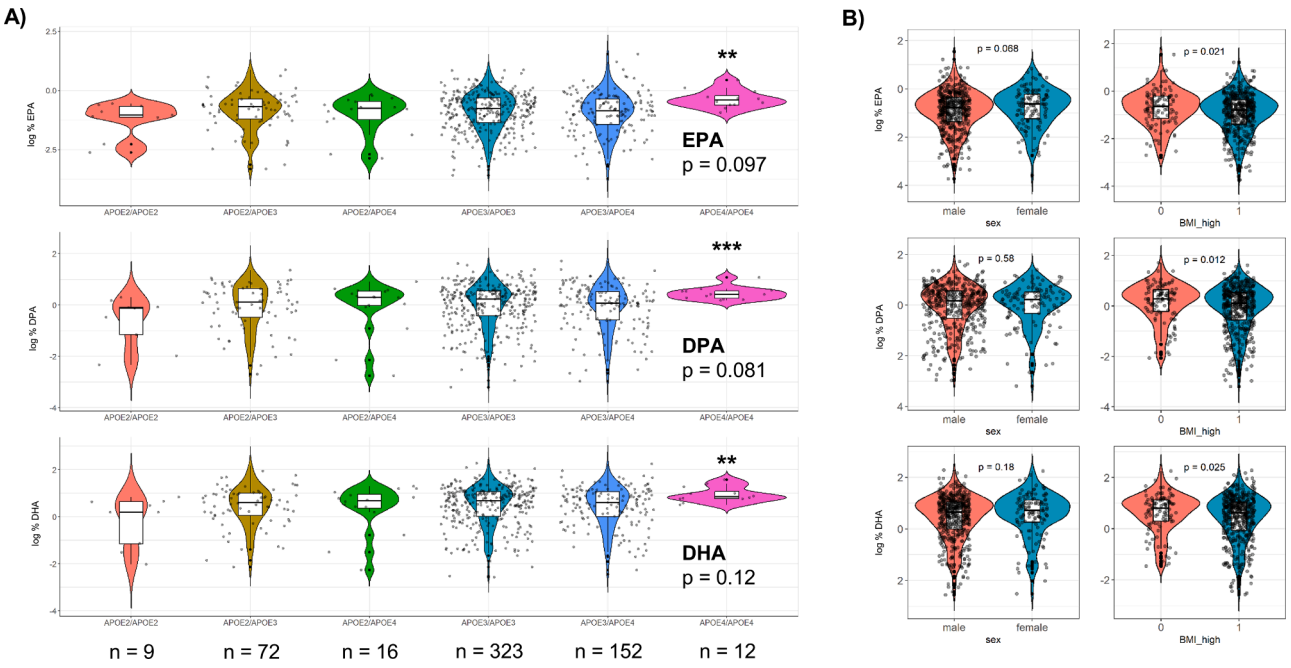

Fig. 2. *n*-3 HUFA levels in red blood cells in seAFOod trial participants at baseline. A) Relationship between *APOE* genotype and percentage *n*-3 HUFA level in RBC membranes. B) Relationship between sex and body mass index (BMI) with the percentage *n*-3 HUFA level in RBC membranes. BMI_high was defined as a BMI $\geq$25 Kg/m^2.
In both panels, data are shown as a violin plot with an embedded box and whisker plot representing the median, quartile and 95th centile values, with overlaid individual data values.
** $P < 0.01$; *** $P < 0.001$ for *post hoc* comparison between *APOE2/2* and *APOE4/4* individuals.

Table 1
Multivariate analysis of factors associated with baseline RBC *n*-3 HUFA levels in seAFOod trial participants.

	EPA			DPA			DHA		
Variables	Coefficient	95% CI	P	Coefficient	95% CI	P	Coefficient	95% CI	P
Sex									
Female	Ref			Ref			Ref		
Male	-0.06	-0.23 to 0.10	0.47	-0.02	-0.20 to 0.16	0.85	-0.04	-0.22 to 0.14	0.65
Dietary EPA+DHA intake	0.66	0.44 to 0.88	**<0.001**	0.30	0.06 to 0.54	**0.01**	0.59	0.36 to 0.83	**<0.001**
BMI $\geq$25kg/m^2	-0.19	-0.36 to -0.02	**0.03**	-0.20	-0.39 to -0.02	**0.03**	-0.18	-0.34 to -0.005	0.06
Genotype									
APOE3/APOE3	Ref			Ref			Ref		
APOE2/APOE2	-0.27	-0.80 to 0.26	0.31	-0.57	-1.16 to -0.01	**0.05**	-0.60	-1.16 to -0.03	**0.04**
APOE2/APOE3	0.002	-0.21 to 0.21	0.99	-0.07	-0.30 to 0.16	0.57	-0.06	-0.28 to 0.16	0.60
APOE2/APOE4	-0.09	-0.49 to 0.31	0.67	-0.01	-0.46 to 0.43	0.95	-0.08	-0.51 to 0.35	0.70
APOE3/APOE4	-0.07	-0.23 to 0.09	0.38	-0.06	-0.23 to 0.11	0.52	-0.05	-0.21 to 0.12	0.56
APOE4/APOE4	0.34	-0.12 to 0.80	0.15	0.38	-0.13 to 0.89	0.14	0.32	-0.18 to 0.81	0.21

EPA, DPA, and DHA data were log-transformed before inclusion in the multivariate model. Bold text represents a *P* value $\leq$0.05.

EPA in a human cohort at increased risk of colorectal cancer that was participating in a randomised clinical trial. Our data add to the literature that supports the relationship between the presence of at least one *APOE4* allele and higher levels of *n*-3 HUFAs in the absence of *n*-3 HUFA supplementation [4]. Unlike other similar (but smaller) studies [4–6], the seAFOod trial cohort contained a small number of individuals who were homozygous for *APOE2* and who displayed low *n*-3 HUFA levels in RBCs compared with the other APOE variants. The difference between *APOE2/2* individuals and other genotypes reached statistical significance for the difference from *APOE4/4* homozygotes by univariate testing, as well as in comparison with 'normal' *APOE3/3* individuals in the multivariate model. Our data in a predominantly male study cohort are consistent with the *APOE4* x sex interaction restricted to males previously observed in healthy adults [17].

Tomaszewski et al have reported that *APOE2/3* individuals had a higher increase in individual *n*-3 HUFAs (as a % of total HUFAs) in plasma in comparison to *APOE4/4* homozygotes in response to a DHA supplement in a randomised trial in patients with mild Alzheimer's disease [6]. Differences in the relationship between *APOE* genotype and *n*-3 HUFA levels in different blood fractions (plasma *versus* RBC membranes) may underlie differential findings in independent studies, in addition to other variables including the nature and formulation of the *n*-3 HUFA intervention.

Our study cohort was sufficiently large to contain a small number of cases (2.7 % of the study population) who had an *APOE2/4* genotype. *APOE2/4* individuals did not display any difference in baseline or post-treatment *n*-3 HUFA levels compared with *APOE3/3* trial participants. Comparison with other studies of *n*-3 HUFA supplementation or oily fish intake is challenging as other studies have not distinguished *APOE2/4* individuals from other *APOE4* carriers [3,4], or have not reported any *APOE2/4* cases [17].

We also took the opportunity to study the relationship between *APOE* genotype and EPA metabolism using the plasma 18-HEPE concentration as a readout of EPA metabolism. Plasma 18-HEPE concentrations were

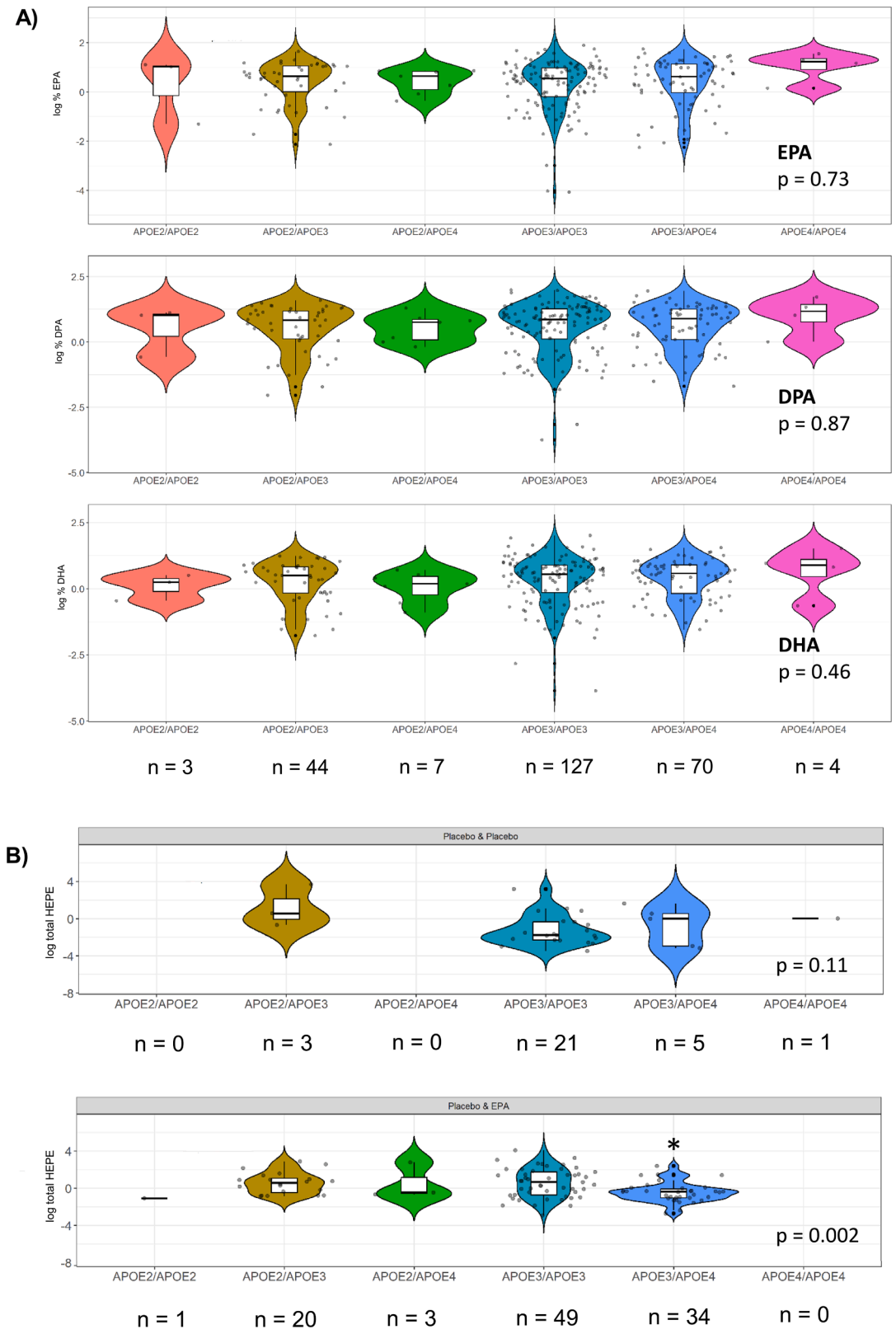

Fig. 3. *n*-3 HUFA levels in red blood cells and plasma total 18-HEPE concentration in seAFOod trial participants after treatment with EPA for 6 months. A) Relationship between *APOE* genotype and percentage *n*-3 HUFA levels in RBC membranes in seAFOod trial participants randomised to active EPA (with and without active aspirin). Statistical comparisons between *APOE* genotypes were made on log-transformed data. B) Relationship between *APOE* genotype and plasma total 18-HEPE concentration at 6 months in seAFOod trial participants randomised to either placebos alone or EPA only. Note that only a proportion of participants randomised to placebos alone had plasma 18-HEPE levels measured as part of previously published work [9]. $P < 0.05$ for the comparison with *APOE2/3* and *APOE3/3* individuals. Statistical comparisons between *APOE* genotypes were made using one-way ANOVA on log-transformed data, excluding the single *APOE4/4* individual data and single *APOE2/2* individual data from respective placebo and EPA group analyses. In both panels, data are represented as a violin plot with an embedded box and whisker plot representing the median, quartile and 95th centile values, with overlaid individual data values.

lower in *APOE3/4* individuals (no samples from *APOE4/4* individuals were available for analysis during the trial intervention phase) following EPA supplementation suggesting that *APOE* genotype could affect oxylipin synthesis from the precursor *n*-3 HUFA, at least in the context of EPA supplementation. Lower plasma 18-HEPE levels in *APOE3/4* individuals are consistent with our previous pre-clinical work which observed lower brain 18-HEPE content in *ApoE4* mice relative to *ApoE3* animals [18], but contrast with our observation in humans that a greater increase in plasma HEPE concentration was evident in *APOE4* carriers following *n*-3 HUFA supplementation for 12 months [5]. A different dose (up to 1.89 g daily), a mixed *n*-3 HUFA formulation (EPA:DHA ratio 1:1.2), and a different study group demographic (healthy subjects that were 52 % female) is likely to underpin the apparent inconsistency with our seAFOod trial data.

It is important that future APOE genotype-phenotype studies analyse blood and tissue HUFA content, in parallel with measures of HUFA metabolism, in order to understand the role of APOE in control of circulating HUFA concentration, tissue bioavailability and metabolism into bioactive oxylipins.

A strength of our study was the relatively large trial cohort size, compared with previous studies of the relationship between *APOE* genotype and *n*-3 HUFA levels, which included a small number of rare *APOE2/2, APOE2/4* and *APOE4/4* individuals. Despite this, our study could be affected by type 2 statistical error. Limitations of our study include the use of % HUFA content (of total measured HUFAs) in RBCs, rather than absolute HUFA concentrations, which does not allow direct comparison with studies that measured plasma HUFA levels. The seAFOod trial cohort that we studied was predominantly male and lacked ethnic diversity, thereby reducing applicability to other populations.

In conclusion, we report that *APOE* genotype is related to baseline RBC *n*-3 HUFA content, with *APOE2/2* individuals displaying low relative HUFA levels, and *APOE4/4* individuals displaying high relative *n*-3 HUFA levels, in comparison with wild-type *APOE3/3* individuals, in the context of a randomised trial in patients at elevated risk of colorectal cancer, who were undergoing colonoscopic surveillance. Although we found no evidence that *APOE* genotype influences EPA incorporation into RBC membranes during EPA supplementation in seAFOod trial participants, we observed a relationship between *APOE* genotype and plasma concentration of the EPA metabolite 18-HEPE during EPA supplementation suggesting that APOE influences EPA metabolism, which may have relevance to the putative anti-neoplastic activity of EPA [19, 20].

Data sharing statement

De-identified seAFOod trial meta-data, including individual fatty acid levels, are available upon request to the Chief Investigator and the trial Sponsor (University of Leeds).

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CRediT authorship contribution statement

Ge Sun: Writing – original draft, Visualization, Formal analysis. **John R. Davies:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Tracey Mell:** Writing – review & editing, Data curation. **Mark Harland:** Writing – review & editing, Formal analysis, Data curation. **Rasha M.H. Saleh:** Writing – review & editing, Investigation, Conceptualization. **Amanda D. Race:** Writing – review & editing, Data curation. **Paul M. Loadman:** Writing – review & editing, Funding acquisition, Data curation. **Elizabeth A. Williams:** Writing – review & editing, Funding acquisition, Conceptualization. **Anne Marie Minihane:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Mark A. Hull:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The Authors declare no potential Conflicts of Interest

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.plefa.2024.102623.

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