Interaction between BMI and APOE genotype is associated with changes in the plasma long-chain–PUFA response to a fish-oil supplement in healthy participants

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ABSTRACT:

Background: Carriers of the apolipoprotein E ε4 (APOE4) allele are lower responders to a docosahexaenoic acid (DHA) supplement than are noncarriers. This effect could be exacerbated in overweight individuals because DHA metabolism changes according to body mass index (BMI; in kg/m²).

Objectives: We evaluated the plasma fatty acid (FA) response to a DHA-rich supplement in APOE4 carriers and noncarriers consuming a high–saturated fat diet (HSF diet) and, in addition, evaluated whether being overweight changed this response.

Design: This study was part of the SATgen trial. Forty-one APOE4 carriers and 41 noncarriers were prospectively recruited and consumed an HSF diet for 8 wk followed by 8 wk of consumption of an HSF diet with the addition of DHA and eicosapentaenoic acid (EPA) (HSF + DHA diet; 3.45 g DHA/d and 0.5 g EPA/d). Fasting plasma samples were collected at the end of each intervention diet. Plasma total lipids (TLs) were separated into free FAs, neutral lipids (NLs), and phospholipids by using solid-phase extraction, and FA profiles in each lipid class were quantified by using gas chromatography.

Results: Because the plasma FA response to the HSF + DHA diet was correlated with BMI in APOE4 carriers but not in noncarriers, the following 2 groups were formed according to the BMI median: low BMI (<25.5) and high BMI (≥25.5). In response to the HSF + DHA diet, there were significant BMI × genotype interactions for changes in plasma concentrations of arachidonic acid and DHA in phospholipids and TLs and of EPA in NLs and TLs (P ≤ 0.05). APOE4 carriers were lower plasma responders to the DHA supplement than were noncarriers but only in the high-BMI group.

Conclusions: Our findings indicate that apolipoprotein E genotype and BMI may be important variables that determine the plasma long-chain PUFA response to dietary fat manipulation. APOE4 carriers with BMI ≥25.5 may need higher intakes of DHA for cardiovascular or other health benefits than do noncarriers. The SATgen trial was registered at clinicaltrials.gov as NCT01384032.

Keywords: apolipoprotein E ε4, BMI, DHA, fatty acid metabolism, lipids

INTRODUCTION

Consumption of fatty fish rich in the long-chain (LC) ω–3 PUFAs DHA (22:6n–3) and EPA (20:5n–3) is associated with lower risk of developing cognitive decline (1, 2) and cardiovascular diseases (3, 4). DHA is of particular importance to brain homeostasis because it is the most-concentrated n–3 PUFA in the central nervous system and is a key fatty acid (FA) involved in neurotransmission, membrane repair, and anti-inflammatory processes (5–7). However, carriers of at least one apolipoprotein E ε4 (APOE4) allele do not seem to gain the same cognitive (8, 9) and potentially cardiovascular benefits (10) associated with increased EPA and DHA intakes. Evidence suggested that this lack of protection could be related to disturbances in DHA kinetics (11, 12). Usually, the concentration of DHA in plasma total lipids (TLs) tends to level off when the intake of DHA and EPA is >1 g/d (13), whereas at intakes <1 g/d, the DHA plasma dose-response relation is generally linear (14, 15). However, in APOE4 carriers, this dose-response relation may not hold because the plasma response to a LC ω–3 PUFA supplement was lower than in noncarriers (12). In support of this proposition, DHA was increased by 75% in plasma triglycerides in APOE4 carriers compared with 240% in noncarriers.

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2Supplemental Tables 1–4 are available from the “Supplemental data” link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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8Abbreviations used: AA, arachidonic acid; apoE, apolipoprotein E; APOE, apolipoprotein E; APOE3/3, homozygous for the apolipoprotein E ε3 allele or APOE4 noncarriers; APOE3/4, heterozygous for the apolipoprotein E ε3 allele or APOE4 carriers; APOE4, apolipoprotein E ε4; CE, cholesterol ester; FA, fatty acid; HSF diet, high–saturated fat diet; HSF + DHA diet, high–saturated fat diet with the addition of DHA and EPA; LC, long chain; NL, neutral lipid; TL, total lipid.

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after receiving 3 g DHA + EPA/d for 6 wk (12). Recently, the kinetics of $^{13}$C-DHA showed that $^{13}$C-DHA in plasma TLs was 31% lower in APOE4 carriers, and β-oxidation was higher than in noncarriers (11). Therefore, in addition to the previously reported impact of the APOE4 genotype on postprandial lipemia (16) and the plasma lipid response to altered fat and EPA + DHA intake (10, 17), DHA homeostasis, itself, seems to be disrupted in APOE4 carriers (11, 12). This disruption could be specific to certain lipid classes because of the differential involvement of apolipoprotein E (apoE) in the synthesis and turnover of the various lipid pools (18). Also, BMI has been reported to explain 5% of the variation of $^{13}$C-DHA kinetics toward higher β-oxidation (19), and higher BMI is associated with higher plasma triglyceride concentrations (20). Because apoE is a key protein for lipid homeostasis, and variations in BMI also affects blood FA partitioning and turnover, both BMI and APOE genotype could interactively influence plasma FA status. Hence, the hypothesis is that BMI might be an important confounder with regards to the plasma PUFAs response to dietary lipid manipulation in APOE4 carriers, and this would likely vary according to the plasma lipid class. We used data and analyzed samples collected from the participants of the SATgen study (clinicaltrials.gov; NCT01384032) because this trial was designed to determine whether the dietary fat quantity, quality, and composition modified markers of cardiovascular diseases with regards to APOE4 genotype (21).

METHODS

Ninety healthy participants, aged between 35 and 70 y, were prospectively recruited by APOE genotype for the SATgen: study performed at the University of Reading, United Kingdom (21). Serum samples were available for 82 of 90 participants for the retrospective analysis of plasma lipids. Forty-one participants were heterozygous for the APOE4 allele (APOE3/4) and were identified as APOE4 carriers (22 men and 19 women), whereas 41 participants were homozygous for the apolipoprotein E ε3 allele (APOE3/3) and, thereafter, were identified as APOE4 noncarriers (20 men and 21 women). The genotype groups were matched for age, BMI, sex, and menopausal status. Exclusion criteria were as follows: anemia; hypertriglyceridemia (>4 mmol/L) or cholesterolemia (>8 mmol/L); liver or endocrine dysfunction; pregnancy or breastfeeding; and alcohol intakes ≥21 units/wk for men or >14 units/wk for women (17, 21). The study protocol was approved by the University of Reading Research Ethics Committee, and all participants gave their written informed consent to participate in this study. Moreover, the Human Ethics Research Committee of the Health and Social Sciences Center – Sherbrooke University Geriatrics Institute approved the additional analysis for plasma lipids.

Study design

As previously described (17, 21), participants in the SATgen: trial followed a sequential dietary intervention design in which 3 isoenergetic diets were consumed each for an 8-wk duration. The 3 diets were consumed in the following sequence: a low-fat diet, a high–saturated fat diet (HSF diet), and a high–saturated fat diet with the addition of DHA and EPA (HSF + DHA diet). To achieve dietary targets, all participants were advised to substitute spreads, oils, and snack foods in their diets to manipulate the overall fat composition by using a food-exchange model (21). Detailed dietary advice and diet sheets were given to participants, and their diets were monitored throughout the intervention period by nutritionists. Moreover, participants were asked to complete weighed diet diaries during weeks 4–5 of each dietary intervention to assess dietary intake. To determine compliance with the DHA supplement, capsules were returned at the end of the HSF + DHA diet and counted (21). In the current analysis, the results of 2 dietary phases (HSF and HSF + DHA diets) are included. The HSF diet was chosen as the most appropriate control group for our HSF + DHA intervention group because the dietary intake was identical except for the addition of DHA supplements. This method allowed the primary objective of establishing whether APOE4 genotype is associated with modifications in the plasma FA response to the DHA supplement to be tested. In the HSF diet, 38% of energy was derived from fat with 18% from saturated fats and 45% of energy from carbohydrates. The DHA supplement in the HSF + DHA diet provided 3.45 g DHA/d and 0.5 g EPA/d. The total dose of DHA + EPA was chosen to be within the 2–4 g/d range recommended by the American Heart Association for triglyceride lowering (22). The n−3 FA extract was highly enriched in DHA because the SATgen: trial aimed to examine the differential response according to APOE4 genotype of LDL cholesterol, triglycerides, and FAs to DHA supplementation.

Anthropometric measures and biochemical analysis

Fasted plasma samples and anthropometric measures were collected at the end of each 8-wk dietary intervention. Anthropometric measures, plasma and serum biochemistry profiles, and genotyping were all performed at the University of Reading as reported elsewhere (17). The fasting biochemistry profile included the following: glucose, insulin, HDL cholesterol, LDL cholesterol, small, dense LDL cholesterol, total cholesterol, triglycerides, and apolipoproteins B, C3, and E.

FA extraction, separation of lipid classes, and analysis

Lipids were extracted from 200 μL plasma by using the method of Folch et al. (23). The internal standard heptadecanoic acid (17:0) was added to the preparation to quantify FA in each fraction. Quantitatively, the mixture of the internal standard represented 10% of the TL extract with 25% of the internal standard in the form of triglycerides, 25% of the internal standard in the form of cholesteryl esters (CEs), 45.5% of the internal standard in the form of phospholipids, and 4.5% of the internal standard in the form of free FAs. The lipid extract that contained the internal standard mixture was evaporated under N2 and reconstituted in 200 μL chloroform. A modified version of the solid-phase extraction method described previously (24) was used to separate neutral lipids (NLs, composed of CEs and triglycerides), free FAs, and phospholipids. Briefly, the BondElut NH2 cartridge (Agilent) was washed with 3 mL hexane before the lipid-extract solution was loaded to the cartridges. NLs were eluted by using 6 mL of a mixture of chloroform: isopropanol [2:1 (volume:volume)] followed by 2 mL chloroform. Free FAs were extracted by using 2.25 mL of a mixture of diethyl ether: acetic acid [98.7:1.3 (volume:volume)], and finally, phospholipids were eluted with 2 mL methanol. The elutions were conducted under normal gravity (no vacuum). Thereafter, we
validated the purity of the fractions by using thin-layer chromatography as previously described (25). Separated lipid classes were dried under N₂, and NLs and phospholipids were saponified by using 1 mol KOH in methanol at 90°C for 1 h, thereby releasing the FAs from CE and glycerolipids. FA methyl esters were generated by adding boron trifluoride in methanol (14%; Sigma-Aldrich) to the free FAs and heated at 90°C for 30 min. FA methyl esters were analyzed as previously described (19). The FA profile of the TL class was defined as the summation of FA profiles in NLs, free FAs, and phospholipids.

Statistical analysis

This study was a secondary analysis of the SATgen trial (17). The sample size was originally calculated with the response of LDL cholesterol and total cholesterol as primary phenotypic outcomes (17). To confirm that the sample size provided sufficient power for the current study, a retrospective power calculation was conducted by using results of plasma DHA from a previous trial (12). We calculated a mean increase of DHA in triglycerides + DHA in CE (12) in APOE4 carriers and noncarriers. We estimated that the mean ± SD increase of DHA after the HSF + DHA diet would be 0.54% ± 0.53% in APOE4 carriers and 1.16% ± 0.67% in noncarriers in relative percentages of total FAs (12). With a required power set at 80% and a significance level set at 0.05, we calculated that 14 participants/group were needed.

In this study, bivariate correlations were performed between ∆ concentrations of FA in plasma lipid classes (∆FA) and BMI with SPSS Statistics 20 software (IBM Corp.). BMI × genotype interactions were tested for ∆ variables of plasma biochemistry (∆plasma biochemistry) and for ∆FA and were investigated by using a univariate general linear model. In this model, ∆plasma biochemistry or ∆FA was the dependent variable and was calculated as follows: the fasting concentration of plasma biochemistry variable or plasma FA after the HSF + DHA diet minus the fasting concentration of the plasma biochemistry variable or plasma FA after the HSF diet. In this model, BMI and genotype were fixed factors. BMI and genotype were also tested individually by using the general linear model. When the BMI × genotype interaction was significant, a subgroup analysis was performed by using independent t tests to compare ∆s between genotypes in low- or high-BMI subgroups. Data are presented as means ± SEMs unless stated otherwise. Statistical significance was set at P ≤ 0.05.

RESULTS

Anthropometric measures and fasting plasma biochemistry after the HSF and HSF + DHA diets are presented in Table 1. The mean age of participants was 52 ± 6 years for APOE4 carriers and 50 ± 1 y for noncarriers. Because APOE4 genotype and BMI interfere with DHA kinetics (11, 19) and lipid metabolism (10, 16, 17, 20), correlations were performed between the plasma FA response and BMI according to APOE4 genotype. In APOE4 carriers, ∆ arachidonic acid (∆AA or 20:4n–6), ∆EPA, and ∆DHA were inversely correlated with BMI in NLs (rAA = −0.371, P = 0.017; rEPA = −0.479, P = 0.002; rDHA = −0.318, P = 0.043), phospholipids (rAA = −0.424, P = 0.006; rEPA = −0.474, P = 0.002; rDHA = −0.465, P = 0.002) and TLs (rAA = −0.420, P = 0.011; rEPA = −0.502, P = 0.002; rDHA = −0.447, P = 0.006). However, in APOE4 noncarriers, there was no significant correlation between ∆AA, ∆EPA, or ∆DHA with BMI (data not shown). Hence, because of these APOE4-specific correlations between ∆AA, ∆EPA, or ∆DHA with BMI, participants were separated by the median BMI value as follows: low BMI (in kg/m²; <25.5) or high BMI (≥25.5). Thereafter, we tested for potential BMI × genotype interactions for ∆plasma biochemistry or ∆FA. For ∆plasma biochemistry, there was no significant BMI × genotype interaction or individual genotype effect (Table 1). However, there was a significant BMI effect for ∆glucose (Table 1).

Plasma FA concentrations

Plasma concentrations of palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n–9), α-linolenic acid (18:3n–3), EPA, docosapentaenoic acid (22:5n–3), DHA, linoleic acid (18:2n–6), and AA in lipid classes after the HSF diet and the response to the HSF + DHA diet according to BMI and APOE genotype are presented in Supplemental Tables 1–4. For ∆16:0, ∆18:0, ∆18:1n–9, ∆18:3n–3, ∆22:5n–3, and ∆18:2n–6, there was no significant BMI × genotype interaction in any lipid class. However, there were significant BMI effects for ∆16:0 and ∆18:2n–6 in NLs (P = 0.024 and P = 0.007, respectively), for ∆16:0, ∆18:0, and ∆18:2n–6 in phospholipids (P = 0.005, P = 0.047, and P = 0.043, respectively) and for ∆16:0 and ∆18:2n–6 in TLs (P = 0.022 and P = 0.025, respectively). Also, there were significant genotype effects for ∆22:5n–3 in NLs, phospholipids, and TLs (P = 0.049, P = 0.022, and P = 0.011, respectively).

Changes in AA

There were significant BMI × genotype interactions for ∆AA in NLs, phospholipids, and TLs (Figure 1). In the high-BMI group, the subgroup analysis reported a trend for a genotype effect in NLs and a significant genotype effect in phospholipids and TLs; the ∆AA in plasma NLs, phospholipids, and TLs was −11.0 ± 5.6, −30.4 ± 7.1, and −44.9 ± 14.0 mg/mL, respectively, in APOE4 carriers compared with +7.4 ± 7.5, −5.0 ± 8.6, and +7.9 ± 17.2 mg/mL, respectively, in noncarriers (between-genotype analysis: P = 0.055 for NLs, P = 0.029 for phospholipids, and P = 0.023 for TLs; Figure 1). In contrast, in the low-BMI group, there was no significant genotype effect; the ∆AA in plasma NLs, phospholipids, and TLs was +13.7 ± 5.2, −13.5 ± 5.3, and +14.1 ± 11.1 mg/mL, respectively, in APOE4 carriers compared with +7.4 ± 4.4, −7.4 ± 6.1, and +4.5 ± 9.0 mg/mL, respectively, in noncarriers (between-genotype analysis: P = 0.356 for NLs, P = 0.458 for phospholipids, and P = 0.507 for TLs; Figure 1).

Changes in EPA

There were significant BMI × genotype interactions for ∆EPA in NLs and TLs (Figure 2). In the low-BMI group, the subgroup analysis reported a significant genotype effect in NLs and a trend for a genotype effect in TLs; ∆EPA in plasma NLs and TLs was +26.7 ± 4.1 and +50.4 ± 7.8 mg/mL, respectively, in APOE4 carriers compared with +15.2 ± 3.1 and +32.0 ± 6.3 mg/mL, respectively, in noncarriers (between-genotype analysis: P = 0.029 for NLs and P = 0.073 for TLs; Figure 2). In contrast, in the high-BMI group, there was no significant genotype effect,
TABLE 1
Anthropometric measures and fasting plasma biochemistry of APOE4 allele noncarriers (APOE3/3; n = 41) and carriers (APOE3/4; n = 41) after the HSF diet and response to the HSF + DHA diet

<table>
<thead>
<tr>
<th>Variable and genotype</th>
<th>Low-BMI</th>
<th>High-BMI</th>
<th>P-interaction</th>
<th>P-BMI</th>
<th>P-genotype</th>
</tr>
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<tr>
<td></td>
<td>HSF</td>
<td>Δ</td>
<td>HSF</td>
<td>Δ</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>52 ± 2</td>
<td>—</td>
<td>47 ± 2</td>
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<tr>
<td>APOE3/3</td>
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</tr>
<tr>
<td>APOE3/4</td>
<td>52 ± 2</td>
<td>—</td>
<td>52 ± 2</td>
<td>—</td>
<td>—</td>
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<td>Sex, M/F, n</td>
<td>6/15</td>
<td>—</td>
<td>14/6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>APOE3/3</td>
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<td>—</td>
<td>10/11</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.2 ± 0.4</td>
<td>0.3 ± 0.1</td>
<td>27.8 ± 0.4</td>
<td>0.1 ± 0.1</td>
<td>— 0.161</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.41 ± 0.12</td>
<td>0.16 ± 0.07</td>
<td>5.57 ± 0.11</td>
<td>−0.08 ± 0.1</td>
<td>0.727 0.024 0.748</td>
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<td>5.47 ± 0.11</td>
<td>0.10 ± 0.08</td>
<td>5.72 ± 0.11</td>
<td>−0.08 ± 0.1</td>
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<td>Insulin, pmol/L</td>
<td></td>
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<td></td>
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<tr>
<td>APOE3/3</td>
<td>27.5 ± 2.5</td>
<td>4.0 ± 2.5</td>
<td>37.9 ± 3.6</td>
<td>−0.7 ± 3.5</td>
<td>0.919 0.184 0.752</td>
</tr>
<tr>
<td>APOE3/4</td>
<td>30.0 ± 3.5</td>
<td>2.7 ± 4.3</td>
<td>47.8 ± 6.0</td>
<td>−1.4 ± 2.7</td>
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<td>HDL cholesterol, mmol/L</td>
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<td>APOE3/3</td>
<td>1.63 ± 0.09</td>
<td>0.08 ± 0.05</td>
<td>1.37 ± 0.08</td>
<td>0.04 ± 0.02</td>
<td>0.671 0.195 0.953</td>
</tr>
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<td>APOE3/4</td>
<td>1.62 ± 0.09</td>
<td>0.1 ± 0.05</td>
<td>1.45 ± 0.08</td>
<td>0.02 ± 0.04</td>
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<tr>
<td>LDL cholesterol, mmol/L</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>APOE3/3</td>
<td>3.53 ± 0.13</td>
<td>0.06 ± 0.06</td>
<td>4.14 ± 0.19</td>
<td>0.11 ± 0.09</td>
<td>0.249 0.526 0.069</td>
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<tr>
<td>APOE3/4</td>
<td>3.79 ± 0.17</td>
<td>0.01 ± 0.1</td>
<td>4.14 ± 0.15</td>
<td>−0.14 ± 0.07</td>
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<td>sLDL cholesterol, mmol/L</td>
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<tr>
<td>APOE3/3</td>
<td>0.56 ± 0.04</td>
<td>0.03 ± 0.03</td>
<td>0.87 ± 0.09</td>
<td>−0.05 ± 0.05</td>
<td>0.822 0.070 0.099</td>
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<td>0.91 ± 0.08</td>
<td>−0.11 ± 0.04</td>
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<tr>
<td>TC, mmol/L</td>
<td>5.41 ± 0.16</td>
<td>0.14 ± 0.09</td>
<td>6.02 ± 0.19</td>
<td>−0.04 ± 0.1</td>
<td>0.959 0.095 0.158</td>
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<td>APOE3/3</td>
<td>5.67 ± 0.22</td>
<td>−0.01 ± 0.13</td>
<td>5.98 ± 0.15</td>
<td>−0.19 ± 0.09</td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>1.01 ± 0.09</td>
<td>−0.15 ± 0.09</td>
<td>1.64 ± 0.23</td>
<td>−0.4 ± 0.13</td>
<td>0.134 0.744 0.569</td>
</tr>
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<td>APOE3/4</td>
<td>1.42 ± 0.25</td>
<td>−0.43 ± 0.21</td>
<td>1.51 ± 0.13</td>
<td>−0.27 ± 0.1</td>
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<tr>
<td>apoB, mg/L</td>
<td>840 ± 29</td>
<td>24 ± 21</td>
<td>1023 ± 44</td>
<td>9 ± 18</td>
<td>0.614 0.219 0.238</td>
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<td>APOE3/3</td>
<td>849 ± 64</td>
<td>10 ± 24</td>
<td>988 ± 39</td>
<td>−26 ± 19</td>
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<tr>
<td>APOE3/4</td>
<td>105 ± 6</td>
<td>−5 ± 5</td>
<td>115 ± 9</td>
<td>−13 ± 5</td>
<td>0.594 0.463 0.800</td>
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<tr>
<td>apoC3, mg/L</td>
<td>110 ± 10</td>
<td>−6 ± 9</td>
<td>123 ± 7</td>
<td>−8 ± 5</td>
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<tr>
<td>APOE3/3</td>
<td>35.9 ± 2.8</td>
<td>6.5 ± 1.9</td>
<td>45.3 ± 2.3</td>
<td>0.9 ± 1.7</td>
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<td>APOE3/4</td>
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<td>37.5 ± 5.2</td>
<td>−3.7 ± 4.9</td>
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</table>

1All values are means ± SEMs. Data are for Low-BMI (APOE3/3: n = 21; APOE3/4: n = 20) and High-BMI (APOE3/3: n = 20, APOE3/4: n = 21). Δ denotes the fasting plasma concentration of biochemistry variables after the HSF + DHA diet (which included 3.45 g DHA/d and 0.5 g EPA/d) minus the fasting plasma concentration of biochemistry variables after the HSF diet. Data were analyzed by using a univariate general linear model with SPSS statistics 20 software (IBM Corp.) with Δ as the dependent variable and BMI and genotype as fixed factors. P-interaction = P-BMI × genotype for Δ. There was no significant (P < 0.05) BMI × genotype interaction for any Δ. However, there was a significant BMI effect for Δglucose. apoB, apolipoprotein B; apoC3, apolipoprotein C3; apoE, apolipoprotein E; APOE3/3, homozygous for the apolipoprotein E 3 allele; APOE4/3, heterozygous for the apolipoprotein E 3 allele; APOE4, apolipoprotein E 4 allele; High-BMI, participants with BMI ≥25.5; HSF diet, high–saturated fat diet; HSF + DHA diet, high–saturated fat diet with the addition of DHA and EPA. Low-BMI, participants with BMI <25.5; sLDL cholesterol, small, dense LDL; TC, total cholesterol.

but there was a trend for a genotype effect in NLs and TLs; ∆EPA in plasma NLs and TLs was +0.5 ± 1.9 and +18.6 ± 3.9 mg/L, respectively, in APOE4 carriers compared with +15.7 ± 2.4 and +30.9 ± 5.4 mg/L, respectively, in noncarriers (between-genotype analysis: P = 0.100 for NLs and P = 0.074 for TLs; Figure 2). There was also a significant BMI effect for ∆EPA in phospholipids (P = 0.018, Figure 2). ∆EPA was 63% higher in low- compared with high-BMI participants, and this was independent of APOE genotype.

Changes in DHA
There were significant BMI × genotype interactions for ∆DHA in phospholipids and TLs (Figure 3), which provided support that APOE4 carriers were lower plasma responders to the HSF + DHA diet than were noncarriers but only in the high-BMI group. ∆DHA in plasma phospholipids and TLs was +26.8 ± 6.0 and +48.7 ± 10.1 mg/L, respectively, in high-BMI APOE4 carriers compared with +49.2 ± 7.5 and +86.4 ± 15.1 mg/L, respectively, in high-BMI noncarriers (between-genotype analysis:
PLASMA FA RESPONSE TO DHA IN APOE4 CARRIERS

**FIGURE 1** Mean (±SEM) ∆AA of participants according to BMI (in kg/m²) and APOE genotype. Participants consumed an HSF diet for 8 wk followed by an HSF + DHA diet for another 8 wk. ∆AA equals the plasma AA concentration after the HSF + DHA diet minus the plasma AA concentration after the HSF diet. Data are presented for ∆AA in plasma free fatty acids, neutral lipids, phospholipids, and total lipids. In each graph, data on the left are for Low-BMI (APOE3/3: n = 19–21; APOE3/4: n = 18–20), and data on the right are for High-BMI (APOE3/3: n = 18–20; APOE3/4: n = 18–21). The HSF + DHA diet included 3.45 g DHA/d and 0.5 g EPA/d. Data were analyzed by using a univariate general linear model with SPSS Statistics 20 software (IBM Corp.). When there was a significant BMI × genotype interaction, subgroup analyses were performed by using independent t tests to compare ∆AA values between genotypes. P-interaction = P-BMI × genotype for ∆AA. There was a significant BMI × genotype interaction for ∆AA in neutral lipids, phospholipids, and total lipids. *P = 0.029 between High-BMI APOE4 carriers and noncarriers for ∆AA in phospholipids, and P = 0.023 between High-BMI APOE4 carriers and noncarriers for ∆AA in total lipids. P = 0.055 between High-BMI APOE4 carriers and noncarriers for ∆AA in neutral lipids. AA, arachidonic acid; APOE, apolipoprotein E; APOE3/3, homozygous for the apolipoprotein E ε3 allele or APOE4 noncarriers; APOE3/4, heterozygous for the apolipoprotein E ε4 allele or APOE4 carriers; APOE4, apolipoprotein E ε4; High-BMI, participants with BMI ≥25.5; HSF diet, high–saturated fat diet; HSF + DHA diet, high–saturated fat diet with the addition of DHA and EPA; Low-BMI, participants with BMI <25.5; ∆AA, ∆arachidonic acid.

\[ P = 0.024 \text{ for phospholipids and } P = 0.046 \text{ for TLs; Figure 3}. \]

In contrast, in the low-BMI group, the subgroup analysis reported no significant genotype effect; ∆DHA in plasma phospholipids and TLs was +51.7 ± 6.6 and +94.8 ± 13.9 mg/L, respectively, in APOE4 carriers compared with +46.0 ± 6.7 and +75.6 ± 12.7 mg/L, respectively, in noncarriers (between-genotype analysis: P = 0.543 for phospholipids and P = 0.313 for TLs; Figure 3).

**DISCUSSION**

This study reported that there was an interaction between BMI and APOE genotype on AA, EPA, and DHA plasma responses to a DHA supplement. In high-BMI participants, the DHA plasma response in phospholipids and TLs was lower in APOE4 carriers than in noncarriers whereas there was no difference between genotypes in low-BMI participants. High-BMI APOE4 carriers also had a more-pronounced lowering of AA concentrations in plasma phospholipids after the DHA supplement than did high-BMI noncarriers. In low-BMI participants, both genotypes had similar AA plasma response to the HSF + DHA diet.

One hypothesis as to why high-BMI APOE4 carriers were lower plasma responders to the DHA supplement is that there was greater hepatic uptake and β-oxidation of DHA in this subgroup than in high-BMI noncarriers. In line with this hypothesis, transgenic mice knock-in for the human APOE4 had higher FA transport proteins in the liver together with higher hepatic carnitine palmitoyl transferase 1 than did APOE3 mice (26). Carnitine palmitoyl transferase 1 is the rate-limiting enzyme for mitochondrial β-oxidation. Furthermore, we previously showed that \(^{13}\)C-DHA was more β-oxidized in human APOE4 carriers than in noncarriers (11) because there was a higher recovery of \(^{13}\)C-CO₂ in the breath of APOE4 participants after receiving a single oral dose of 40 mg \(^{13}\)C-DHA. Consistent with this finding, the \(^{13}\)C-DHA whole-body half-life was 77% lower in APOE4 carriers than in noncarriers. The slope of the linear regression between \(^{13}\)C-DHA and \(^{13}\)C-CO₂ was 117% steeper in APOE4 carriers, which indicated that, for a given plasma concentration of \(^{13}\)C-DHA, \(^{13}\)C-CO₂ was higher in APOE4 carriers than in noncarriers, which supported a more rapid β-oxidation of DHA (11). With regards to EPA and AA, there is currently no data, to
our knowledge, on their hepatic uptake and β-oxidation according to APOE genotype, in part because of the lack of commercially available 13C-labeled isotope tracers for these FAs.

ApoE is a key protein centrally involved in lipid metabolism (27) including VLDL synthesis in the liver and in lipoprotein clearance from the circulation via its affinity for the LDL-receptor family (28). APOE4 carriers have lower plasma concentrations of apoE, which is in part attributed to lower hepatic apoE recycling, and apoE4 is preferentially associated with VLDL than with HDL, which is opposite to that of the apoE3 protein (29). Hence, although the precise molecular cause is currently unknown, these differences in overall protein concentrations and lipoprotein partitioning in APOE4 carriers likely contribute to the disconnection between BMI and plasma lipids in this population. This effect, in turn, may contribute to the deregulated PUFA metabolism reported in overweight APOE4 carriers compared with noncarriers. One potential explanation for this deregulation is that, compared with normal-weight participants, subjects who were overweight displayed higher concentrations of apolipoprotein B and small, dense LDL cholesterol in the plasma, which may be associated with higher amounts of small, dense LDL particles. This effect could have been exacerbated in APOE4 carriers and contributed to a shift toward more CEs in the blood because small, dense LDLs have a higher proportion of CEs and less triglycerides than do large LDLs. Because 13C-DHA seems to be preferentially incorporated into triglycerides than in CEs (14, 25), this differential incorporation could have contributed to explain why overweight APOE4 carriers were lower responders to the DHA supplement. This hypothesis could have also played a role in the BMI genotype interactions reported for DAA and D EPA in plasma, but it is, at this point, premature to make the same assumption as for DHA because there has been no study, to our knowledge, on the kinetics of AA or EPA by using a 13C tracer. In this study, BMI genotype interactions for D DHA were reported in phospholipids and TLs but not in NLs, which contrasted with what was previously reported (12). However, we did not separate triglycerides from the NL fraction. Therefore, potential BMI × genotype interactions for D EPA of participants according to BMI (in kg/m²) and APOE genotype. Participants consumed an HSF diet for 8 wk followed by an HSF + DHA diet for another 8 wk. D EPA equals the plasma EPA concentration after the HSF + DHA diet minus the plasma EPA concentration after the HSF diet. Data are presented for D EPA in plasma free fatty acids, neutral lipids, phospholipids, and total lipids. In each graph, data on the left are for Low-BMI (APOE3/3: n = 19–21; APOE3/4: n = 18–20), and data on the right are for High-BMI (APOE3/3: n = 18–20; APOE3/4: n = 18–21). The HSF + DHA diet included 3.45 g DHA/d and 0.5 g EPA/d. Data were analyzed by using a univariate general linear model with SPSS Statistics 20 software (IBM Corp.). When there was a significant BMI × genotype interaction, subgroup analyses were performed by using independent t tests to compare D EPA values between genotypes. P interaction = P-BMI × genotype for D EPA. There was a significant BMI × genotype interaction for D EPA in neutral lipids and total lipids. *P = 0.029 between Low-BMI APOE4 carriers and noncarriers for D EPA in neutral lipids. P = 0.073 between Low-BMI APOE4 carriers and noncarriers for D EPA in total lipids, P = 0.100 between High-BMI APOE4 carriers and noncarriers for D EPA in neutral lipids, and P = 0.074 between High-BMI APOE4 carriers and noncarriers for D EPA in total lipids. APOE, apolipoprotein E; APOE3/3, homozygous for the apolipoprotein E e3 allele or APOE4 noncarriers; APOE3/4, heterozygous for the apolipoprotein E e4 allele or APOE4 carriers; APOE4, apolipoprotein E e4; High-BMI, participants with BMI ≥25.5; HSF diet, high–saturated fat diet; HSF + DHA diet, high–saturated fat diet with the addition of DHA and EPA; Low-BMI, participants with BMI <25.5.
interactions for ΔDHA in the triglyceride fraction may have been masked by the presence of CEs in the NL faction.

In participants who consumed a high-fat diet, BMI could have modified the plasma lipid response to the DHA supplement in several ways. This contribution could have been mediated through dysfunction in insulin metabolism in overweight participants because BMI is inversely correlated with insulin sensitivity in healthy individuals (30). Moreover, high BMI is associated with reduced insulin sensitivity and higher insulin secretion in humans (31). Because insulin is closely related to lipids and particularly FA homeostasis, a disrupted insulin metabolism could lead to ectopic fat accumulation and adipose tissue dysfunction (32), which may result in higher triglycerides and VLDL synthesis but lower HDL synthesis by the liver. In the current study, high-BMI participants had overall higher plasma concentrations of insulin, HDL cholesterol, LDL cholesterol, and triglycerides than those of low-BMI participants, and these differences were independent of APOE genotype. Hence, this deregulated plasma lipid profile reported in participants with high BMI suggested that BMI could also affect the plasma lipoprotein response to dietary interventions involving modifications of dietary fat. A study reported that, after consuming a 6-wk diet that provided an additional 31 g total fat/d and 650 mg cholesterol/d than did a baseline low-fat diet, the rise in HDL cholesterol was 2-fold higher in men with BMI $\geq 25$ than in men with BMI $<25$, whereas the rise in LDL cholesterol was similar between the 2 BMI groups (33).

Considering the importance of DHA for brain function (5–7) and cardiovascular health (34–37), BMI × genotype interactions for ΔDHA reported in the current study could help explain why APOE4 carriers are at higher risk of cardiovascular diseases and cognitive decline. Also, it was reported that central obesity is associated with lower cognitive scores in dementia-free humans but only in APOE4 carriers (38). Hence, disturbances in DHA homeostasis could play a role in this APOE4-specific association. Preliminary data indicated that, when the dosage is adequate (approximately 3 g EPA + DHA/d), DHA homeostasis could be rebalanced in APOE4 carriers (39).

This study has strengths and limitations. First, in contrast with many previous studies that relied on retrospective genotyping and
low numbers in the rarer APOE4-carrier group (11, 12), this study was powered to examine APOE genotype by phenotype interactions. Furthermore, in addition to BMI, genotype groups were matched for age, sex, and menopausal status, all of which have been reported to modify PUFA metabolism (39–42). However, the separation of results according to the BMI median resulted in heterogeneity in the ratio of men to women between carriers and noncarriers of APOE4. However, no effect of sex on ΔAA, ΔEPA, and ΔDHA was evident (data not shown), and thus, it is unlikely that sex disparities between groups influenced BMI × genotype interactions reported in the current study. Last, the study design, namely the sequential intervention approach with no washout between intervention arms, may be considered a limitation. However, this design was specifically chosen to assess an optimal approach because of the inherent difficulties in returning participants to their habitual diet during the washout period in studies that involved extensive fat manipulation. It is anticipated that, after 8 wk exposure to a particular FA dietary exposure, the FA profile will be fully adopted and reflective of dietary intake. Such an approach has been used in previous studies (43).

In conclusion, the current study suggests that APOE4 allele and BMI may be important variables that determine the plasma response of AA, EPA, and DHA to a DHA supplement. Hence, these variables should be considered when designing future studies examining how dietary lipids influence AA, EPA, or DHA homeostasis in humans. Compared with noncarriers, APOE4 carriers with BMI ≥25.5 may need higher intakes of DHA for cardiovascular or other health benefits.

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