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Effect of *APOE* Genotype on Plasma Docosahexaenoic Acid (DHA), Eicosapentaenoic Acid, Arachidonic Acid, and Hippocampal Volume in the Alzheimer's Disease Cooperative Study-Sponsored DHA Clinical Trial

Natalie Tomaszewski^{a,1}, Xulei He^{a,1}, Victoria Solomon^a, Mitchell Lee^a, Wendy J. Mack^b, Joseph F. Quinn^c, Meredith N. Braskie^d, Hussein N. Yassine^{a,d,*}

^aDepartment of Medicine, University of Southern California, Los Angeles, CA, USA

^bDepartment of Preventive Medicine, University of Southern California, Los Angeles, CA, USA

^cDepartment of Neurology, Oregon Health and Science University, Portland VA Medical Center

^dImaging Genetics Center, Stevens Neuroimaging and Informatics Institute, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

Abstract

Background: Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA) play key roles in several metabolic processes relevant to Alzheimer's disease (AD) pathogenesis and neuroinflammation. Carrying the *APOE* $\epsilon 4$ allele (*APOE4*) accelerates omega-3 polyunsaturated fatty acid (PUFA) oxidation. In a pre-planned subgroup analysis of the Alzheimer's Disease Cooperative Study-sponsored DHA clinical trial, *APOE4* carriers with mild probable AD had no improvements in cognitive outcomes compared to placebo, while *APOE4* non-carriers showed a benefit from DHA supplementation.

Objective: We sought to clarify the effect of *APOE* $\epsilon 4/\epsilon 4$ on both the ratio of plasma DHA and EPA to AA, and on hippocampal volumes after DHA supplementation.

Methods: Plasma fatty acids and *APOE* genotype were obtained in 275 participants randomized to 18 months of DHA supplementation or placebo. A subset of these participants completed brain MRI imaging ($n = 86$) and lumbar punctures ($n = 53$).

Results: After the intervention, DHA-treated *APOE* $\epsilon 3/\epsilon 3$ and *APOE* $\epsilon 2/\epsilon 3$ carriers demonstrated significantly greater increase in plasma DHA/AA compared to $\epsilon 4/\epsilon 4$ carriers. *APOE* $\epsilon 2/\epsilon 3$ had a greater increase in plasma EPA/AA and less decline in left and right hippocampal volumes compared to compared to $\epsilon 4/\epsilon 4$ carriers. The change in plasma and cerebrospinal fluid DHA/AA was strongly correlated. Greater baseline and increase in plasma EPA/AA was

*Correspondence to: Hussein N. Yassine, Departments of Medicine and Neurology, University of Southern California, Los Angeles, CA, USA. hyassine@usc.edu.

¹These authors contributed equally to this work.

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SUPPLEMENTARY MATERIAL

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associated with a lower decrease in the right hippocampal volume, but only in *APOE4* non-carriers.

Conclusion: The lower increase in plasma DHA/AA and EPA/AA in *APOE ε4/ε4* carriers after DHA supplementation reduces brain delivery and affects the efficacy of DHA supplementation.

Keywords

Arachidonic acid; Alzheimer's disease; apolipoprotein E; docosahexaenoic acid; eicosapentaenoic acid

INTRODUCTION

There is evidence that among highly unsaturated fatty acids (HUFAs, Fig. 1) docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA) play key roles in several metabolic processes relevant to Alzheimer's disease (AD) pathogenesis [1, 2]. The omega-3 (n-3) HUFAs DHA and EPA compete with the omega-6 (n-6) HUFA AA at the sn-2 position of phospholipids affecting their levels in tissues and in the circulation [3]. This results in a diet-determined HUFA tissue balance where increasing dietary consumption of DHA or EPA lowers AA tissue levels. This change has a biological significance. A lower ratio of DHA and EPA to AA in the brain detrimentally affects neuroinflammation, oxidative stress, and alters synaptic activity and hippocampal functions [4, 5].

Carrying the *APOE ε4* allele (*APOE4*) affects systemic and brain lipid metabolism in an allele-dose dependent manner, and is the strongest genetic risk factor for late-onset AD [6]. In contrast, carrying the *APOE ε2* allele (*APOE2*) protects against AD [7]. After lipolysis, enrichment of lipoproteins with *APOE* accelerates their clearance from plasma [8]. The preferential binding of *APOE4* compared to *APOE ε3* (*APOE3*) and *APOE2* alleles (*APOE4* > *APOE3* > *APOE2*) with larger fat-containing particles contributes to altered lipid and cholesterol metabolism [9–11]. For example, DHA and EPA are transported on chylomicrons to the liver following absorption, and *APOE4* carriers have faster DHA and EPA clearance from plasma compared to non-carriers [12–15]. *APOE4* carriers also have a lower DHA/AA in human plasma and in brains of human *APOE*-targeted replacement (*APOE-TR*) mice [16]. In *APOE-TR* mouse models, *APOE4* was associated with an increase in mitochondrial carnitine palmitoyltransferase I (CPT-1) expression, which promotes n-3 polyunsaturated fatty acid (PUFA) oxidation [17]. These studies support the notion that *APOE4* affects the ratio of DHA and EPA to AA in tissues.

One biomarker of AD severity is the volume of the hippocampus, which predicts the conversion of patients with mild cognitive impairment to AD [18]. The rate of hippocampal volume loss is greatest in patients with AD carrying *APOE4* [19]. Red blood cell and plasma DHA and EPA measurements positively associate with the volume of the hippocampus in both cross-sectional and longitudinal studies [20–22]. The lipiDiDiet trial used a multi-nutrient intervention that included high doses of DHA in patients with prodromal AD. The nutrient intervention arm was associated with significantly lower decrease in hippocampal

volumes compared to placebo over 24 months [23]. Hippocampal volume may therefore offer a surrogate biomarker of the efficacy of n-3 supplementation in the brain.

DHA supplementation has generally been ineffective in trials conducted in patients with AD [24, 25]. In the Alzheimer's Disease Cooperative Study (ADCS)-sponsored DHA clinical trial, the main outcomes—change in the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog), the Clinical Dementia Rating (CDR) sum of boxes, and the rate of brain atrophy—did not differ by treatment groups. A preplanned subgroup analysis of this trial showed a significant benefit of DHA supplementation compared to placebo on changes in ADAS-Cog and the Mini-Mental Status Examination (MMSE) when analyzed among *APOE4* non-carriers; this benefit was not evident in *APOE4* carriers [24]. We subsequently examined cerebrospinal fluid (CSF) samples obtained at baseline and at 18-month follow-up in a small subsample from this DHA trial to understand the changes in CSF DHA levels relative to randomized treatment and *APOE4* status. DHA-treated *APOE4* carriers had less increase in CSF DHA compared to DHA-treated non-carriers [24]. The mechanisms for the different clinical response to DHA supplementation noted in the trial subgroup analysis and the reduced DHA CSF delivery associated with *APOE4* are not well understood.

In the current study, we sought to clarify the effect of *APOE4* on plasma DHA/AA, EPA/AA, and hippocampal volumes in the setting of this trial. We hypothesized that *APOE4* $\epsilon 4/\epsilon 4$ would be associated with a smaller increase in plasma phospholipid DHA/AA and EPA/AA after supplementation, as well as a greater loss of hippocampal volume compared with *APOE4* non-carriers.

METHODS

ADCS-sponsored clinical trial

This trial randomized 402 participants with mild AD to two treatment arms over 18 months: DHA arm (2 g DHA per day) and placebo arm. All participants were required to take four 950 mg soft-gel capsules which contained either 510 mg DHA (treatment arm) or corn/soy oil (placebo arm) [24]. Fifty-one US centers participated in this trial after obtaining approval from their local institutional review boards. Randomization was 60% assigned to active treatment, and 40% assigned to placebo. The disproportionate randomization to active treatment was intended to enhance recruitment. Randomization was not stratified by *APOE* groups. Written informed consent was obtained from study participants, legally authorized representatives, or both.

Fatty acid analysis and omega-3 dietary intake

Fasting plasma and CSF phospholipid fatty acid levels were determined at DSM Nutritional Products using established methods [26]. The fatty-acid profiles were expressed as a percentage of the total fatty acid in micrograms (weight percent). DHA dietary intake was assessed using a validated fatty fish intake questionnaire at baseline [27].

MR imaging

Trial participants without contraindication to MRI (e.g., pacemaker) who were enrolled at trial sites that were also certified Alzheimer's Disease Neuroimaging Initiative (ADNI) sites were invited (but not required) to participate in an MRI substudy, with MRIs obtained at baseline and at end of the 18-month period. The MRI sequence, as well as methods for across-site standardization and quality control, were those used in the ADNI study. FreeSurfer software v5.3.0 (<https://surfer.nmr.mgh.harvard.edu/>) was used to automatically segment the bilateral hippocampus and calculate hippocampal volumes at baseline and 18 months. Quality control of the hippocampal segmentations was performed by a researcher who was blind to genetic and treatment data, using the recommendations of the European Alzheimer's Disease Consortium and the Alzheimer's Disease Neuroimaging Initiative (EADC – ADNI) harmonized hippocampal protocol [28, 29]. Intracranial volume (ICV) was estimated using FreeSurfer.

Power analysis

In a previous analysis of the ADCS-sponsored DHA clinical trial [26], forty-four participants completed lumbar punctures at baseline and 18 months after allocation to placebo ($n = 15$, 4 non-*APOE4* carriers, 11 *APOE4* carriers) or DHA ($n = 29$, 4 non-*APOE4* carriers, 25 *APOE4* carriers). The difference () in CSF DHA levels between placebo and treatment arms was 59% lower in *APOE4* carriers compared to *APOE4* non-carriers. With the observed SD of 1.027, this yields an effect size of 0.78, and an effect size for the interaction effect of 0.29. To detect a treatment *APOE4* interaction with 80% power and $\alpha = 0.05$ (two sided) yields a required sample size of 96 (24 per treatment/*APOE4* cell). Therefore, a sample size of 275 would be sufficient to detect an interaction of plasma DHA by genotype in this trial.

Statistical analysis

Statistical analysis was conducted using R Studio software version 3.5.

Intervention changes in fatty acid ratios were calculated by taking the difference between 18-month and baseline measures. Baseline hippocampal volumes were divided by respective ICV measures. Analysis for post-intervention hippocampal volumes were also calculated by taking the difference between the absolute values of the 18-month and baseline hippocampus volume (without dividing by ICV). At baseline, the percentage of fatty acids by the individual *APOE* genotype subgroups (*APOE* $\epsilon 2/\epsilon 3$, $\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 3$, $\epsilon 3/\epsilon 4$, and $\epsilon 4/\epsilon 4$) was compared using a univariate linear regression model. 18-months fatty acid ratios were modeled as a function of treatment group and *APOE* genotype in individual subgroups and their interaction using a multivariable linear regression model. An interaction model between *APOE* genotype subgroups and sex was modeled for plasma fatty acid ratios. The association between plasma DHA/AA or EPA/AA and hippocampal volumes was studied using a Pearson correlation coefficient and a multivariate regression model to adjust for *APOE* combined subgroups (*APOE4* and non carriers). An interaction model for *APOE* genotype and fatty acid ratios on the change in hippocampal volumes was also performed. Given the *APOE* allele-dose effect, with *APOE* $\epsilon 4/\epsilon 4$ having a stronger effect on AD risk and lipid metabolism than the other alleles, we used *APOE* $\epsilon 4/\epsilon 4$ carriers as the reference

group. A 2-sided 5.0% alpha-level was defined as significant. For this exploratory analysis, we did not control for multiple comparisons, and therefore, results with a $p < 0.05$ listed throughout the paper should be considered only nominally significant.

RESULTS

Study sample description

Fatty acid and *APOE* genotype information was available in 275 participants at baseline and 18 months following randomization to DHA supplementation or placebo. 161 were randomized to 2 grams of DHA per day and 114 were randomized to a placebo. A summary of the number of participants by *APOE* genotype is presented in Table 1. DHA dietary intake did not differ among *APOE* genotype subgroups (group comparison, $p > 0.3$).

Baseline plasma DHA, EPA, and AA within HUFAs and *APOE* genotype

Among plasma HUFAs, the percentage of AA was significantly greater in *APOE* $\epsilon 4/\epsilon 4$ compared to $\epsilon 3/\epsilon 3$ ($p = 0.027$), $\epsilon 2/\epsilon 3$ ($p = 0.049$), and $\epsilon 2/\epsilon 4$ carriers ($p = 0.0019$, Fig. 2A). In contrast, there was a trend toward a lower ratio of plasma DHA to HUFAs in *APOE* $\epsilon 4/\epsilon 4$ compared to $\epsilon 3/\epsilon 3$ ($p = 0.068$, Fig. 2B). Similarly, the ratio of ALA to HUFA trended toward a lower value in *APOE* $\epsilon 4/\epsilon 4$ compared to $\epsilon 3/\epsilon 3$ ($p = 0.061$, Fig. 2C). In addition, baseline plasma ALA/HUFA was significantly lower in *APOE* $\epsilon 4/\epsilon 4$ compared to $\epsilon 2/\epsilon 3$ ($p = 0.031$). The ratio of plasma EPA to HUFA did not differ by genotype at baseline (Fig. 2D). For plasma DHA/AA at baseline, *APOE* $\epsilon 3/\epsilon 3$ carriers exhibited a small but significantly greater ratio ($p = 0.045$) than $\epsilon 4/\epsilon 4$ carriers (Fig. 3A). Similarly, DHA/AA trended to be greater in *APOE* $\epsilon 2/\epsilon 3$ carriers compared to $\epsilon 4/\epsilon 4$ carriers ($p = 0.078$), and was significantly greater in *APOE* $\epsilon 2/\epsilon 4$ carriers ($p = 0.01$) compared to $\epsilon 4/\epsilon 4$ carriers. The plasma EPA/AA at baseline in *APOE* $\epsilon 3/\epsilon 3$ carriers was not significantly different from $\epsilon 4/\epsilon 4$ carriers (Fig. 3B). However, baseline plasma EPA/AA trended toward being greater in *APOE* $\epsilon 2/\epsilon 3$ ($p = 0.061$) and $\epsilon 2/\epsilon 4$ carriers ($p = 0.084$) compared to $\epsilon 4/\epsilon 4$ carriers. For plasma ALA/AA at baseline, *APOE* $\epsilon 2/\epsilon 3$ carriers exhibited a small but significantly greater ratio ($p = 0.01$) than $\epsilon 4/\epsilon 4$ carriers (Fig. 3C). In a similar fashion, baseline plasma ALA/AA was significantly greater in *APOE* $\epsilon 3/\epsilon 3$ carriers ($p = 0.02$) compared to $\epsilon 4/\epsilon 4$ carriers. Baseline plasma ALA/AA was also significantly greater in *APOE* $\epsilon 2/\epsilon 4$ carriers ($p = 0.003$) compared to $\epsilon 4/\epsilon 4$ carriers. However, after removing the outlier in the *APOE* $\epsilon 2/\epsilon 4$ group, the difference between $\epsilon 2/\epsilon 4$ and $\epsilon 4/\epsilon 4$ became non-significant. The ratio of all individual fatty acids of the total fatty acids in plasma by *APOE* genotype is presented in Supplementary Table 1.

Effect of sex on the association of *APOE* genotype with plasma fatty acids

Female sex appeared to influence the association of genotype with HUFAs at baseline, and this effect was stronger for EPA than DHA. We examined the interaction of *APOE* genotype and female sex on plasma fatty acid ratios. The percentage of DHA of total fatty acids (interaction p value = 0.071) and as a ratio to AA (interaction p value = 0.083) trended to differ by genotype and sex (Fig. 4A, B). For men, *APOE* $\epsilon 4$ carriers had a higher ratio than non-carriers. In contrast, in women, *APOE* $\epsilon 4$ carriers had a lower ratio than non-carriers. The interaction of sex and genotype on the ratio of DHA to HUFA was not significant. However,

after the removal of an outlier in the *APOE4* group of women, the sex-genotype interaction on fatty acid ratio became significant (p value = 0.048, Fig. 4C). EPA as a percentage of total fatty acids, as a ratio to HUFAs, and as a ratio to AA significantly differed in *APOE4* women compared to men (interaction p value < 0.05 for all) in a similar pattern to DHA (Fig. 4D–F).

Ratio of DHA and EPA of HUFAs at 18 months following supplementation

At 18 months following DHA supplementation, the ratios of plasma n-6 DPA, AA, and n-3 DPA to HUFAs were significantly decreased (p < 0.0001 for all, Fig. 5A–C). It is known that plasma EPA levels increase after DHA supplementation by retro conversion of DHA to EPA [30]. The ratios of both EPA and DHA to HUFAs increased after DHA supplementation (p < 0.0001 for all, Fig. 5D, E). These changes reflect the competition of dietary DHA with other HUFAs at the sn-2 position of phospholipids where an increase in DHA intake is able to incorporate into phospholipids replacing AA, n-6 DPA, and n-3 DPA. To understand the effect of *APOE* genotype on these fatty acid ratios after supplementation, AA/HUFA in *APOE* $\epsilon 4/\epsilon 4$ had the lowest decrease in the treatment arm (Fig. 5B), compared to all other genotypes except $\epsilon 2/\epsilon 4$. There were no differences by genotype in the change of n-3 and n-6 DPA/HUFA (Fig. 5A, C). The increase in EPA/HUFA at 18-months was greater in *APOE* $\epsilon 2/\epsilon 3$ compared with $\epsilon 4/\epsilon 4$ (p = 0.006, Fig. 5D). There was a significant interaction between *APOE* $\epsilon 4/\epsilon 4$ subgroup and treatment arm on the 18 months EPA/HUFA (interaction p value = 0.041). The increase in DHA/HUFA was least pronounced in the *APOE* $\epsilon 4/\epsilon 4$ group compared to all other genotypes except *APOE* $\epsilon 2/\epsilon 4$ (p < 0.05 for all, Fig. 5E). There was no significant difference in change in DHA/HUFA and EPA/HUFA as a function of sex. The 18-month ratio of individual fatty acids of total fatty acids by *APOE* genotype are presented in Supplementary Table 2.

Ratio of plasma DHA and EPA to AA at 18 months following supplementation

There was a significant difference between the placebo and DHA-treatment arms in plasma DHA/AA and EPA/AA after the intervention (p < 0.001, Fig. 6A, B). DHA-treated *APOE* $\epsilon 2/\epsilon 3$ carriers exhibited a 23% greater increase in plasma DHA/AA compared to DHA-treated $\epsilon 4/\epsilon 4$ carriers (p = 0.002, Fig. 6A). DHA-treated *APOE* $\epsilon 2/\epsilon 3$ carriers exhibited a 38% greater increase in plasma DHA/AA compared to DHA-treated $\epsilon 4/\epsilon 4$ carriers (p = 0.006). DHA-treated *APOE* $\epsilon 3/\epsilon 4$ carriers exhibited a small, but significantly greater increase in plasma DHA/AA compared to DHA-treated $\epsilon 4/\epsilon 4$ carriers (p = 0.031). *APOE* $\epsilon 2/\epsilon 4$ trended toward a slightly greater increase in plasma DHA/AA compared to $\epsilon 4/\epsilon 4$; however, this trend was non-significant. There was no significant sex and genotype interaction on the change of plasma DHA/AA after supplementation.

Similarly, DHA-treated *APOE* $\epsilon 3/\epsilon 3$ carriers exhibited a 23% greater increase in plasma EPA/AA compared to DHA-treated $\epsilon 4/\epsilon 4$ carriers (p = 0.036 Fig. 6B). In addition, DHA-treated *APOE* $\epsilon 2/\epsilon 3$ carriers exhibited a 57% significantly greater increase in plasma EPA/AA compared to DHA-treated $\epsilon 4/\epsilon 4$ carriers (p = 0.0001). *APOE* $\epsilon 4/\epsilon 4$ carriers trended toward slightly less change in plasma EPA/AA after supplementation compared to both $\epsilon 2/\epsilon 4$ and $\epsilon 3/\epsilon 4$ carriers. There was a significant interaction between *APOE* $\epsilon 4/\epsilon 4$ subgroup and treatment arm on the 18 months EPA/AA (interaction p value = 0.021). There

was no significant interaction between the change in DHA/AA and EPA/AA as a function of sex and genotype.

Association of plasma and CSF fatty acids by APOE genotype

There was a significant association between the ratio of DHA/AA ($r = 0.47$, $p = 0.0004$) and EPA/AA ($r = 0.32$, $p = 0.019$) measured in plasma and CSF in a subset of individuals completing both studies ($n = 53$). *APOE* genotype affected this relationship. There was a trend toward a stronger association of CSF DHA/AA to plasma DHA/AA in *APOE4* non-carriers than in *APOE4* carriers (interaction p value = 0.06, Fig. 7A). In the treatment arm ($n = 29$), there was a strong and significant association between the change in plasma with CSF DHA/AA ($r = 0.82$, $p < 0.0001$). Similarly, this association trended to be stronger in *APOE4* non-carriers compared to carriers (interaction p value = 0.09, Fig. 7B). The association of the change in CSF EPA/AA with plasma EPA/AA was non-significant ($p = 0.18$) and was not influenced by *APOE* genotype (Fig. 7C, D).

Association of APOE genotype with hippocampal volume

Hippocampal volumes provide a surrogate brain efficacy biomarker for n-3 fatty acid supplementation and are affected by *APOE* genotype. In the whole group, baseline left hippocampal volume did not differ significantly among *APOE* $\epsilon 2/\epsilon 3$ carriers, $\epsilon 2/\epsilon 4$ carriers, and $\epsilon 3/\epsilon 3$ carriers when compared to $\epsilon 4/\epsilon 4$ carriers. Similarly, the right hippocampal volume did not differ among *APOE* $\epsilon 2/\epsilon 3$ carriers, $\epsilon 2/\epsilon 4$ carriers, and $\epsilon 3/\epsilon 3$ carriers compared to $\epsilon 4/\epsilon 4$ carriers (Table 2).

In the combined sample of DHA- and placebo-treated participants, the 18-month change in left and right hippocampal volume was investigated by *APOE* genotype individual groups. *APOE* $\epsilon 2/\epsilon 3$ carriers exhibited a 10% less decline in left hippocampal volume than *APOE* $\epsilon 4/\epsilon 4$ carriers ($p < 0.001$, Fig. 8A). Additionally, *APOE* $\epsilon 2/\epsilon 3$ carriers ($p = 0.0052$) and *APOE* $\epsilon 2/\epsilon 4$ ($p = 0.007$) carriers experienced significantly less decline in the right hippocampal volume than *APOE* $\epsilon 4/\epsilon 4$ carriers ($p < 0.01$, Fig. 8B).

The association of plasma DHA/AA and EPA/AA with hippocampal volume

At baseline, plasma DHA/AA and EPA/AA ratios were not correlated with the baseline left or right hippocampal volumes. However, greater baseline plasma DHA/AA was significantly correlated with less decline in right hippocampal volume ($r = 0.38$, $p = 0.0003$, Fig. 9A). Using a multivariable linear regression model, baseline DHA/AA and *APOE* groups were both independently correlated ($p < 0.001$) with the right hippocampal volume change and there was no significant interaction between plasma DHA/AA and *APOE* genotype on the change in right hippocampal volume. Furthermore, baseline plasma DHA/AA was not significantly associated with decline in left hippocampal volume.

Higher baseline plasma EPA/AA was significantly correlated with less decline in right hippocampal volume ($r = 0.24$, $p = 0.03$, Fig. 9C). Using a multivariable linear regression model, baseline EPA/AA and *APOE* groups were independently correlated ($p < 0.01$ for both) with the right hippocampal volume change. In addition, there was no significant interaction between plasma EPA/AA and *APOE* genotype on the change of the right

hippocampal volume. Baseline plasma EPA/AA was not significantly associated with decline in left hippocampal volume.

After the intervention, changes in right and left hippocampal volumes did not significantly differ between the DHA and placebo study groups. Furthermore, in the DHA arm, there was no significant association between increasing plasma DHA/AA ratio and decline in right or left hippocampal volume after supplementation (Fig. 9B). However, there was a significant association between increasing plasma EPA/AA ratio and decline in right hippocampal volume after DHA supplementation ($r = 0.31$, $p = 0.03$, Fig. 9D). Using a multivariable linear regression model, the change of EPA/AA and *APOE* groups were independently correlated ($p < 0.01$ for both) with right hippocampal volume change. In addition, there was a significant interaction between plasma EPA/AA and *APOE* genotype on the change of right hippocampal volume ($p = 0.01$). The increase in plasma EPA/AA was correlated with the less decline in right hippocampal volume in *APOE4* non-carriers, but not in *APOE4* carriers.

DISCUSSION

In this study, patients with *APOE4* and mild probable AD had a lower increase in DHA/AA and EPA/AA after DHA supplementation compared to patients with mild AD carrying *APOE2* or *APOE3*. Plasma and CSF DHA/AA were strongly correlated but higher plasma DHA/AA did not translate as efficiently into higher CSF DHA/AA in *APOE4*. *APOE2* carriers with mild AD had the least decline in hippocampal volumes after the 18-month follow-up. The greater increase in EPA/AA in the DHA treatment arm was associated with lower decline in the right hippocampal volume, but only in *APOE4* non-carriers. These data suggest that *APOE4* reduces CSF DHA and EPA delivery by either affecting transport across the blood-brain barrier or through increased catabolism, in addition to its effects on plasma DHA and EPA.

These findings confirm earlier reports that n-3 PUFA metabolism is dysregulated in *APOE4* (illustrated in Fig. 10C). We hypothesize that the inability of *APOE4* carriers to incorporate DHA into plasma phospholipids may be due to the preferential oxidation of this fatty acid in tissues. In addition, we hypothesize that *APOE2* limits this oxidation. This hypothesis is supported by several lines of investigation. After consumption of [^{13}C]DHA, *APOE4* participants had a 77% shorter whole-body half-life of [^{13}C]DHA compared to non- $\epsilon 4$ carriers [14]. In an earlier study, we found an increase in [^{11}C]DHA brain uptake using PET scanning in younger cognitively normal *APOE4* carriers compared to non-carriers; supporting greater brain DHA loss that is compensated for by an increase in DHA transport from plasma to brain in *APOE4* [31]. The mechanisms for this DHA brain loss may include greater n-3 PUFA oxidation with *APOE4*. CPT1, the rate limiting enzyme of long-chain fatty acid oxidation, was expressed at greater levels in *APOE4*-TR mice livers than in *APOE3*-TR mice [17]. This was associated with lower n-3 PUFA concentrations in the liver, plasma, and adipose tissues of *APOE4*-TR mice models. Taken together, *APOE4* appears to accelerate n-3 PUFA's mitochondrial oxidation leading to lower levels of esterified DHA and EPA and compromising brain delivery after supplementation [17].

The ratio of n-3 HUFAs to total HUFAs or to AA represents a meaningful assessment of tissue levels [3]. The absolute concentrations of these fatty acids also serve as a useful indicator of tissue levels as there is a strong correlation between absolute and relative (% weight) plasma ratios [32]. Although the weight % value (percentage of all plasma fatty acids) has a simple rationale and is widely reported, it has no clear metabolic or biological significance. This is because the HUFA balance in tissues results from the HUFA indiscriminate action of calcium-dependent phospholipase A2 (cPLA2) or the acyl transferase that regulate HUFA recycling into and out of the sn-2 position of phospholipids. Unlike HUFAs, less saturated, unsaturated, and shorter fatty acids have less ability to recycle in and out of the sn-2 position of phospholipids. When DHA and EPA intake are increased, they replace n-6 DPA, n-3 DPA, or AA as seen in Fig. 5 and illustrated in Fig. 10.

The biological effects of these fatty acids (for example, eicosanoid release) is dependent on the activity of cPLA2 [33], which is increased in association with amyloid plaques in the human brain [34]. Increased cPLA2 activity is involved in AD pathogenesis [1] by releasing the relatively abundant AA from membrane phospholipids; this process directly contributes to changes in synaptic activity, neuroinflammation, and cognitive changes in AD mouse models [1]. At baseline, the mean ratio of plasma DHA/AA was 0.26 and the mean ratio of plasma EPA/AA was 0.07, reflecting the relatively greater consumption of n-6 s compared to n-3 s in the American diet. In the treatment arm, the mean 18-month plasma DHA/AA increased to 1.24, and the mean 18-month plasma EPA/AA increased to 0.23. This increase after supplementation was modest and was further attenuated by *APOE4*. In comparison, an EPA/AA of greater than 0.7 in a Japanese population with a high consumption of n-3 s predicted lower incidence of cardiovascular disease events [35]. It is interesting to note that the greatest increase in EPA/AA was observed in *APOE ε2/ε3* carriers (mean ratio of 0.5 after supplementation); this genotype had the least decline in hippocampal volume after 18 months, and is protected against AD.

Cellular studies demonstrate that DHA, EPA, and AA play key roles in several metabolic processes relevant to AD pathogenesis and neuroinflammation [1, 36]. High levels of AA affect hippocampal function and AD in many detrimental ways. In one process, AA increases the activity of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA receptors) [37]. These receptors cause increased stimulation of nerves by neurotransmitters due to increased mediation in the c-Jun n-terminal kinase (JNK) pathway [38], providing excitotoxic injury to the brain. AA in the brain may also cause oxidative stress [1]. In the presence of high levels of AA, neuronal activity undergoes an abnormal increase that reduces neuron survivability [4]. Additionally, acrolein, a byproduct of AA, has been shown to be detrimental to AD brains as it contributes to oxidative stress and lipid peroxidation [39]. In contrast to AA, DHA increases the functionality of the hippocampus with regards to volume and memory capacity/capability. In a rodent study, hippocampal volumes increased along with increased performance in behavior and learning tests after DHA supplementation [5]. EPA has stronger anti-inflammatory effects than DHA [40], and higher doses of EPA supplementation have recently been shown to reduce cardiovascular events in a large clinical trial [41].

Female sex affects how the *APOE* genotype influences the abundance of HUFAs. Women in the age range of 65–75 may have a higher increased risk of AD than men [42]. *APOE*-TR mice exhibit a sex-specific effect of *APOE4* on plasma fatty acids [43]. Female *APOE4*-TR mice had lower DHA/AA in the cortex at 18 months of age (equivalent to 60 years in humans) compared to *APOE3*-TR mice. These differences were less prominent in male mice. Consistent with these observations, we found a trend for lower DHA/AA and significantly lower EPA/AA in *APOE4* women compared to men at baseline. One possible explanation for this observation is the greater increase in mitochondrial PUFA oxidation in women carrying *APOE4* that is magnified by aging and the post-menopausal state.

Our findings of greater hippocampal volume loss in *APOE4* carriers and lower loss in *APOE2* carriers is consistent with prior studies. In an Alzheimer's Disease Neuroimaging Initiative (ADNI) study, 112 cognitive normal elderly individuals, 226 individuals with mild cognitive impairment, and 96 AD patients completed three MRIs over a 1-year span [44]. The presence of the *APOE4* allele was associated with higher rates of hippocampal volume loss in AD patients [44]. Expansion of this study into additional ADNI cohorts ($n = 725$) confirmed lower hippocampal volumes in both *APOE4* carriers with and without AD; the difference in volumes was more pronounced with *APOE4* homozygosity [45]. In another study, the rate of hippocampal atrophy was examined in *APOE2* elderly normal subjects [46]. With a total of 134 subjects, 27 of whom were *APOE* $\epsilon 2/\epsilon 3$ or *APOE* $\epsilon 2/\epsilon 3$ and 107 of whom were *APOE* $\epsilon 3/\epsilon 3$, the rate of hippocampal decrease in volume in *APOE2* carriers was significantly slower compared to the other groups.

Both cross-sectional and longitudinal studies have reported a positive association between plasma DHA and EPA levels with hippocampal volumes [20, 22]. In this study, we found an association between greater baseline DHA/AA and EPA/AA plasma levels and lower loss of the right but not left hippocampal volumes. The reason for this asymmetric association is not clear. In one longitudinal study of 245 participants who had been followed for up to 18 years, the volume of the right hippocampus but not left was associated with time to symptom onset in persons with mild cognitive impairment [47]. In the ADNI cohorts, there was greater atrophy of the left hippocampus than the right, and this asymmetry was more pronounced in *APOE4* homozygotes than heterozygotes [45, 48]. However, we did not find significant differences between left and right hippocampal volume loss in this study population and it was not affected by *APOE4* homozygosity.

This study has several strengths and weaknesses. The main strength of this study is the relatively large number of samples from participants with *APOE* $\epsilon 4/\epsilon 4$ ($n = 47$) allowing evaluation of contributions of E4 homozygosity on plasma DHA, EPA, and AA. Participants were carefully followed with multiple visits over 18 months in the setting of a clinical trial that included assessments of dietary n-3 intake, compliance by pill counting, imaging, and clinical outcomes. Even so, the study also has several limitations. Our findings are a *post-hoc* analysis and hypothesis generating. Notwithstanding, the aims of this analysis were internally reviewed and approved by the ADCS publication committee prior to the data analysis. In addition, we did not correct for multiple comparisons, and therefore, our results can only be considered nominally significant. These results should be validated in additional large sample studies. The placebo intervention contained soy/corn oil. However, we did not

observe significant changes in the placebo arm in plasma fatty acids (% by weight) at 18 months compared to baseline. The sample size for the *APOE2* carriers with hippocampal volumes was low, but this would be expected given the protective effect of *APOE2* on AD. In addition, our finding of a protective effect of *APOE2* on hippocampal volume loss is consistent with past literature from ADNI. Another limitation of this study was that DHA/AA and EPA/AA ratios were associated with change in right but not left hippocampal volumes; this may have been a chance finding related to normal variability in a limited sample size. In addition, the lack of significant differences in hippocampal volumes in some of the genotype groups is likely due to the small sample size of this sub-study. Finally, the AD diagnosis was clinical as there was no amyloid imaging and limited CSF exams. Therefore, the probability of making an accurate diagnosis of AD is less certain, particularly among non-*APOE4* carriers. Therefore, interpretation of the differences in plasma DHA/AA and EPA/AA may be confounded by the clinical diagnosis.

We conclude that *APOE4* carriers with mild AD may be less responsive to increases in plasma DHA/AA or EPA/AA after DHA supplementation, providing one explanation for the differential clinical response to DHA supplementation by *APOE4* status. The increase in plasma DHA/AA and EPA/AA was greatest in *APOE2* carriers with mild AD and was associated with lower decline in right hippocampal volume. Understanding how *APOE* genotype affects plasma DHA/AA and EPA/AA ratios may offer critical insights helping to reverse or slow down *APOE4*-driven AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HUFA

Highly Unsaturated Fatty Acids

with 20- and 22-carbon chains and 3 or more double bonds

Fig. 1.

The categorization of highly unsaturated fatty acid (HUFAs), defined as fatty acids containing 20 or more carbons and 3 or more double bonds.

Fig. 2. Distribution of HUFAs by *APOE* genotype at baseline. Comparison of baseline plasma ratios of (A) AA/HUFA, (B) DHA/HUFA, (C) ALA/HUFA, and (D) EPA/HUFA among *APOE* subgroups was performed using a linear regression and $p < 0.05$ was defined as significant ($n = 275$, $e2/e3$ $n = 9$, $e2/e4$ $n = 9$, $e3/e3$ $n = 88$, $e3/e4$ $n = 122$, and $e4/e4$ $n = 47$). The group comparison p values to $e4/e4$ are shown in the figures.

Fig. 3.

Distribution of DHA/AA, EPA/AA, and ALA/AA by *APOE* genotype at baseline.

Comparison of baseline plasma ratios of (A) DHA/AA, (B) EPA/AA, and (C) ALA/AA, among *APOE* subgroups was performed using a linear regression and sample sizes ($n = 275$, $\epsilon 2/\epsilon 3$ $n = 9$, $\epsilon 2/\epsilon 4$ $n = 9$, $\epsilon 3/\epsilon 3$ $n = 88$, $\epsilon 3/\epsilon 4$ $n = 122$, and $\epsilon 4/\epsilon 4$ $n = 47$). The group comparison p values to $\epsilon 4/\epsilon 4$ are shown in the figures. After exclusion of the *APOE* $\epsilon 2/\epsilon 4$ outlier in C, the difference between the baseline plasma ALA/AA levels became non-significant between *APOE* $\epsilon 2/\epsilon 4$ and $\epsilon 4/\epsilon 4$.

Fig. 4.

Interaction of sex and *APOE* genotype. The interaction between sex and *APOE4* at baseline for (A) percent plasma DHA of total fatty acids, (B) ratio of plasma DHA/AA, (C) ratio of plasma DHA/HUFA, (D) percent plasma EPA of total fatty acids, (E) ratio of plasma EPA/AA, and (F) ratio of plasma EPA/HUFA. Analysis was performed using a linear regression and sample sizes ($n = 275$, non-*APOE4* males $n = 29$, non-*APOE4* females $n = 68$, *APOE4* males $n = 64$, and *APOE4* females $n = 114$). After removing the *APOE4* female outlier, the interaction between sex and *APOE4* status became more significant in all cases, making ratio of plasma DHA/HUFA significant, however percent plasma DHA of total fatty acids and ratio of plasma DHA/AA remained non-significant. The red point indicates the mean per group.

Fig. 5.

Post-intervention difference in 18-month and baseline ratios of (A) n-6 DPA/HUFA, (B) AA/HUFA, (C) n-3 DPA/HUFA, (D) EPA/HUFA, and (E) DHA/HUFA in plasma of both treatment groups. The change in plasma fatty acids was modeled as a function of treatment arm and *APOE* subgroups using a multivariate linear regression. The group comparison *p* values to *APOE* $\epsilon 4/\epsilon 4$ are shown in the figures. Sample sizes (total $n = 275$; DHA-treated total $n = 161$, DHA-treated $\epsilon 2/\epsilon 3$ $n = 5$, DHA-treated $\epsilon 2/\epsilon 4$ $n = 6$, DHA-treated $\epsilon 3/\epsilon 3$ $n = 50$, DHA-treated $\epsilon 3/\epsilon 4$ $n = 75$, and DHA-treated $\epsilon 4/\epsilon 4$ $n = 25$; placebo-treated total $n = 114$, placebo-treated $\epsilon 2/\epsilon 3$ $n = 4$, placebo-treated $\epsilon 2/\epsilon 4$ $n = 3$, placebo-treated $\epsilon 3/\epsilon 3$ $n = 38$, placebo-treated $\epsilon 3/\epsilon 4$ $n = 47$, and placebo-treated $\epsilon 4/\epsilon 4$ $n = 22$).

Fig. 6.

Post-intervention difference in 18-month and baseline ratios of (A) DHA/AA and (B) EPA/AA in plasma of both treatment groups. The change in DHA/AA and EPA/AA were modeled as function of the treatment arm and *APOE* subgroups using a multivariate linear regression. The group comparison *p* values to *APOE* $\epsilon 4/\epsilon 4$ are shown in the figures. Sample sizes (total $n = 275$; DHA-treated total $n = 161$, DHA-treated $\epsilon 2/\epsilon 3$ $n = 5$, DHA-treated $\epsilon 2/\epsilon 4$ $n = 6$, DHA-treated $\epsilon 3/\epsilon 3$ $n = 50$, DHA-treated $\epsilon 3/\epsilon 4$ $n = 75$, and DHA-treated $\epsilon 4/\epsilon 4$ $n = 25$; placebo-treated total $n = 114$, placebo-treated $\epsilon 2/\epsilon 3$ $n = 4$, placebo-treated $\epsilon 2/\epsilon 4$ $n = 3$, placebo-treated $\epsilon 3/\epsilon 3$ $n = 38$, placebo-treated $\epsilon 3/\epsilon 4$ $n = 47$, and placebo-treated $\epsilon 4/\epsilon 4$ $n = 22$).

Fig. 7.

Correlation between (A) DHA/AA in plasma and CSF at baseline (total $n = 53$, non-*APOE4* $n = 12$, *APOE4* $n = 41$; $r = 0.47$, $p = 0.0004$), (B) change in DHA/AA in plasma and CSF post-18-month-intervention (total $n = 29$, non-*APOE4* $n = 4$, *APOE4* $n = 25$; $r = 0.82$, $p < 0.0001$), (C) EPA/AA in plasma and CSF at baseline (total $n = 53$, non-*APOE4* $n = 12$, *APOE4* $n = 41$; $r = 0.32$, $p = 0.019$), and (D) change in EPA/AA in plasma and CSF post-18-month-intervention (total $n = 29$, non-*APOE4* $n = 4$, *APOE4* $n = 25$; $r = 0.26$, $p = 0.18$) by *APOE4* status. The interaction between *APOE* genotype subgroups and among baseline and changes in plasma DHA/AA were non-significant. Analysis was done using Pearson's correlation and interaction modeling.

Fig. 8.

Post-intervention difference in 18-month and baseline A) right and B) left hippocampal volume (cm³) among *APOE* subgroups in both DHA-treated participants ($n = 86$, $\epsilon 2/\epsilon 3$ $n = 3$, $\epsilon 2/\epsilon 4$ $n = 2$, $\epsilon 3/\epsilon 3$ $n = 28$, $\epsilon 3/\epsilon 4$ $n = 41$, and $\epsilon 4/\epsilon 4$ $n = 12$). Comparison of the change in hippocampal volumes as a function of *APOE* subgroup was performed using a linear regression. The group comparison p values to $\epsilon 4/\epsilon 4$ are shown in the figures.

Fig. 9.

Post-intervention difference in 18-month and baseline right hippocampal volume (cm^3) was correlated with (A) baseline plasma DHA/AA (total $n = 86$, non-*APOE4* $n = 31$, *APOE4* $n = 55$; $r = 0.38$, $p = 0.0003$) and (B) change in plasma DHA/AA (total $n = 48$, non-*APOE4* $n = 19$, *APOE4* $n = 29$; $r = 0.25$, $p = 0.09$) in the DHA-treated arm. Post-intervention difference in 18-month and baseline right hippocampal volume (cm^3) was correlated with (C) baseline plasma EPA/AA (total $n = 86$, non-*APOE4* $n = 31$, *APOE4* $n = 55$; $r = 0.24$, $p = 0.03$) and (D) change in plasma EPA/AA (total $n = 48$, non-*APOE4* $n = 19$, *APOE4* $n = 29$; $r = 0.32$, $p = 0.03$) in the DHA-treated arm. The interaction between *APOE* genotype subgroups and the change in plasma EPA/AA on the change in right hippocampal volume in was significant ($p = 0.01$). Analysis was done using Pearson's correlation and an interaction model. Exclusion of the $\epsilon 4$ outlier made both the correlation between change in right hippocampal volume and both (C) baseline plasma EPA/AA ($r = 0.30$, $p = 0.005$), as well as (D) change in plasma EPA/AA ($r = 0.42$, $p = 0.003$) more significant. Exclusion of the outlier, however, made the interaction between *APOE* subgroups and change in plasma EPA/AA on the change in right hippocampal volume non-significant ($p = 0.06$).

Fig. 10.

A) Competition between esterified DHA and AA in triglycerides (TG) and phospholipids (PL) from circulatory lipoproteins into the sn-2 position of phospholipids of the membrane bilayer by lipases. Due to greater intake of AA in the western diet, more AA is incorporated into the membrane bilayer. B) Ingestion of dietary DHA after supplementation remodels membrane phospholipid bilayer by the activities of cPLA₂ and the acyl-coA transferase. In addition DHA is able to retroconvert into EPA. EPA also incorporated into the bilayer. C) Preferential mitochondrial oxidation of DHA by the *APOE4* genotype is associated with greater expression of mitochondrial CPT1, providing a mechanism for the lower incorporation of DHA into the membrane bilayer.

Table 1DHA dietary intake by *APOE* genotype at baseline

<i>APOE</i> genotype	Sample Size N (%)	Baseline DHA Intake (g) mean (sd)
$\epsilon 2/\epsilon 3$	9 (3)	106.55 (60.27)
$\epsilon 2/\epsilon 4$	9 (3)	81.33 (52.73)
$\epsilon 3/\epsilon 3$	88 (32)	96.40 (51.20)
$\epsilon 3/\epsilon 4$	122 (44)	87.00 (57.22)
$\epsilon 3/\epsilon 4$	47 (18)	93.77 (57.95)

Table 2

Baseline Hippocampus Volume as percent of ICV (ratio)

<i>APOE</i> genotype	Sample Size N(%)	Baseline Left Hippocampus mean (sd)	Baseline Right Hippocampus mean (sd)
$\epsilon 2/\epsilon 3$	3 (3.49)	0.27 (0.12)	0.27 (0.11)
$\epsilon 2/\epsilon 4$	2 (2.33)	0.26 (0.004)	0.27 (0.05)
$\epsilon 3/\epsilon 3$	28 (32.56)	0.26 (0.06)	0.26 (0.06)
$\epsilon 3/\epsilon 4$	41 (47.67)	0.25 (0.05)	0.25 (0.05)
$\epsilon 4/\epsilon 4$	12 (13.95)	0.23 (0.05)	0.23 (0.04)

All baseline hippocampal volume/ICV ratios were multiplied by 100 for presentation purposes. Data presented are means and SD.