Title

Altered SPMs and age-associated decrease in brain DHA in APOE4 female mice

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Nonstandard Abbreviations

DHA: docosahexaenoic acid

EPA: eicosapentaenoic acid

APOE4: apolipoprotein E ε4 allele

AD: Alzheimer's disease

SPMs: specialised proresolving mediators

(±) 18-HEPE: 18R/S-hydroxy-5Z,8Z,11Z,14Z,16E-eicosapentaenoic acid

17S-HDHA: 17S-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid

14S-HDHA: 14S-hydroxy-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid

RvD1: 7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid (resolvin D1),

RvD3: 4S,11R,17S-trihydroxy-5Z,7E,9E,13Z,15E,19Z-docosahexaenoic acid (resolvin D3)

RvD5: 7S,17S-dihydroxy-5Z,8E,10Z,13Z,15E,19Z-docosahexaenoic acid (resolvin D5)

17R-RvD1: 7S,8R,17R-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid

MaR1: 7R,14S-dihydroxy-4Z,8E,10E,12Z,16Z,19Z-docosahexaenoic acid

10S,17S-diHDHA: 10S,17S-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid

PD1: 10R,17S-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid

RvE1: 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid (resolvin E1)

RvE2: 5S,18R-dihydroxy-6E,8Z,11Z,14Z,16E-eicosapentaenoic acid (resolvin E2)

RvE3: 17R,18R-dihydroxy-5Z,8Z,11Z,13E,15E-eicosapentanoic acid (resolvin E3)

FAME: Fatty acid methyl esters

PE: phosphatidylethanolamine

PC: phosphatidylcholine

PS: phosphatidylserine

PL: phospholipid

LTB4-d4: Leukotriene-d4

IS: internal standard

SPE: solid phase extraction

LCMS: liquid chromatography-mass spectrometry

SFA: saturated fatty acid

MUFA: mono-unsaturated fatty acid

PUFA: polyunsaturated fatty acid

DMA: dimethyl acetal fatty acid

AA: arachidonic acid

IL-6: interleukin 6

TNFα: tumor necrosis factor α

Abstract

An Apolipoprotein E4 (*APOE4*) genotype is the most important, common genetic determinant for Alzheimer's disease (AD), and female *APOE4* carriers present with an increased risk compared to males.

The study quantified cortical and hippocampal fatty acid and phospholipid profiles along with select eicosapentaenoic acid (EPA)- and docosahexaenoic acid (DHA)-derived specialised proresolving mediators (SPMs) in 2-, 9- and 18-month-old *APOE3* and *APOE4* male and female mice.

A 10% lower cortical DHA was evident in *APOE4* females at 18 months compared to 2 months, with no significant decrease in *APOE3* or *APOE4* males. This decrease was associated with a reduction in DHA-phosphatidylethanolamine. Older *APOE4* females had a 15% higher oleic acid content compared to young mice. Although no sex**APOE* genotype interactions were observed for SPMs expressed as a ratio of their parent compound, higher cortical (±) 18 HEPE, resolvin D3, protectin D1, 10S,17S-diHDHA, maresin 1, 17S-HDHA and 14S-HDHA were evident in females, and lower cortical 17R-resolvin D1, 10S,17S-diHDHA and (±) 18 HEPE in *APOE4*.

Our findings show a strong association between age, female sex and an *APOE4* genotype, with decreased cortical DHA and a number of SPMs, that together may contribute to the development of cognitive decline and AD pathology.

Keywords

Alzheimer's disease, fatty acids, specialised proresolving mediators, neuroinflammation, cortex

Introduction

The *E4* isoform of the apolipoprotein E (*APOE*) genotype is the strongest prevalent genetic determinant for late-onset Alzheimer's disease (AD), the most common form of dementia. Caucasians heterozygous or homozygous for *APOE4* are at 3-4 or 8-12-fold increased risk of developing AD respectively (1) and have an earlier age of onset relative to the wild-type *APOE3/3* genotype (2, 3). Besides, there is substantial evidence of a greater cognitive impact of the *APOE4* genotype in females (4-6). In a recent meta-analysis of the Global Alzheimer's Association Interactive Network (27 studies with nearly 58,000 individuals), females between ages 65 and 75 years with *APOE3/4* had a higher risk of developing AD compared with males, and a higher risk of mild cognitive impairment (MCI) between 55 and 70 years (6). In addition to a global five years longer life expectancy (75 versus 70 years), a greater penetrance of *APOE4* on neuropathological progression is likely to underlie the fact that about two thirds of AD patients are females (6). But the physiological basis for such stronger associations in females is currently unknown.

Within the central nervous system, the APOE protein performs numerous physiological roles including synaptic plasticity maintenance, cell signaling, neuroinflammation modulation and lipid transport and metabolism, (7). Brain tissue is particularly enriched in the n-3 fatty acid, docosahexaenoic acid (DHA), constituting about 15% of total lipids, and up to 40% in select synaptic phospholipids, relative to 2-5% in most other tissues such as the heart and liver (8). A low DHA status has been consistently associated with increased cognitive decline, progression of MCI to AD, brain atrophy and overall AD risk (9-11). Interestingly there is evidence of reduced benefits of a higher DHA intake and status in *APOE4* carriers (12). In a recent secondary analysis of the Alzheimer's Disease Cooperative Study trial, Yassine and colleagues reported lower cerebrospinal fluid:blood DHA levels in *APOE4* carriers following 18 months of DHA supplementation (13), which is suggestive of a differential DHA metabolism relative to *APOE3* (14).

Specialised proresolving mediators (SPMs) derived from n-3 fatty acids, have emerged as important contributors to the resolution of neuroinflammation associated with injury and dementia progression (15, 16). Inappropriate conversion of n-3 fatty acids to SPMs or alterations in SPMs receptors could be in part responsible for the lack of benefits of n-3 fatty acid supplementation in *APOE4* carriers (17). But the etiology for potential altered associations between *APOE4* and SPMs has not been determined.

Furthermore, the impact of sex on *APOE4-DHA* associations is unknown. In the current study, we investigated brain (cortical and hippocampal) fatty acid profiles according to *APOE* genotype and sex in an age-dependent manner in an *APOE*-targeted replacement transgenic model. We hypothesised that in females, an *APOE4* genotype will accentuate the lower brain DHA status relative to younger animals. Furthermore, we report on the impact of age, sex and *APOE* genotype on a number of specialised EPA- and DHA-derived SPMs.

Materials and methods

Study approval

All experimental procedures and protocols used in this study were reviewed and approved by the Animal Welfare and Ethical Review Body (AWERB) and were conducted within the provisions of the Home Office Animals (Scientific Procedures) Act 1986.

Animals

120 Male and female humanised *APOE3* (B6.129P2-Apoe$^{tm2(APOE*3)Mae}$ N8) and *APOE4* (B6.129P2-Apoe$^{tm2(APOE*4)Mae}$ N8) targeted replacement mice homozygous for the human *APOE3* or *APOE4* gene (Taconic, Germantown, NY, US) were used in these experiments. The model was created by targeting the murine *APOE* gene for replacement with the human *APOE3* and *APOE4* allele in E14TG2a ES cells and injecting the targeted cells into blastocysts (18). Resultant chimeras were backcrossed to C57BL/6 for eight generations (N8). 10 mice were allocated per group for a total of 12 groups (*APOE3*/female/2months, *APOE3*/male/2months, *APOE4*/female/2months, *APOE4*/male/2months, *APOE3*/female/9months, *APOE3*/male/9months, *APOE4*/female/9months, *APOE4*/male/9months, *APOE3*/female/18months, *APOE3*/male/18months, *APOE4*/female/18months and *APOE4*/male/18months). Mice were maintained in a controlled environment (21 ± 2°C; 12-h light–dark cycle; light from 07:00 hours) and fed *ad libitum* on a standard chow diet (RM3-P, Special Diet Services, Essex, UK) for the duration of the experiments. Composition and fatty acid profile of the chow diet are provided in supplementary data (Suppl. 1). Mice were anaesthetised with a mixture of isoflurane (1.5%), nitrous oxide (70%), and oxygen (30%) and transcardially perfused with an ice-cold saline solution containing heparin (10 units/mL). Body weight was recorded prior to sacrifice. Brains were rapidly removed, dissected into cortices and hippocampi, snap-frozen and stored at -80°C until further analysis. Left cortex and left hippocampus were processed for fatty acid

profile (n=7 per group) and neuroinflammatory markers (n=3 per group) and right cortices were pooled for select EPA- and DHA-derived SPMs analysis (n=5 per group).

Lipid extraction and fatty acid analysis

Total lipids were extracted according to the method of Folch *et al.* (19) and non-lipid impurities were removed by washing with 0.88% (w/v) potassium chloride. The weight of lipids was determined gravimetrically after evaporation of solvent and overnight desiccation under vacuum. Fatty acid methyl esters (FAME) were prepared by acid-catalysed transesterification of total lipids according to the method of Christie *et al.* (20). Extraction and purification of FAME was performed as described by Ghioni et al. (21) before being separated by gas-liquid chromatography using a ThermoFisher Trace GC 2000 (ThermoFisher, Hemel Hempstead, UK) equipped with a fused silica capillary column (ZBWax, 60m x 0.32 x 0.25 mm i.d.; Phenomenex, Macclesfield, UK) with hydrogen as carrier gas and using on-column injection. The temperature gradient was from 50 to 150°C at 40°C/min and then to 195°C at 1.5°C/min and finally to 220°C at 2°C/min. Individual methyl esters were identified by comparison to known standards (Supelco 37-FAME mix; Sigma-Aldrich Ltd., Poole, UK) and by reference to published data (22). Data were collected and processed using the Chromcard for Windows (version 2.00) computer package (Thermoquest Italia, Milan, Italy). Fatty acid content per g of tissue was calculated using heptadecanoic acid (17:0) as internal standard.

Phospholipid profiling

To assess the incorporation of fatty acids into cellular phospholipids, cortical and hippocampal phospholipid (PL) class compositions were determined by single-dimension double-development high-performance thin-layer chromatography (HPTLC) using methyl acetate/propan-2-ol/chloroform/methanol/0.25% aqueous KC1 (25:25:25:10:9, by vol.) and hexane/diethyl ether/acetic acid (85:15; 1.5, by vol.) as first and second development solvents, respectively (23). PL classes, including phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylserine (PS), were quantified by charring followed by calibrated scanning densitometry using a Camag 3 Scanner (Camag, Muttenz, Switzerland). Identities of individual classes were confirmed by comparison with reference to retention (Rf) values of authentic standards run alongside samples.

Specialised proresolving mediators analysis

Specialised proresolving mediators (SPMs) were extracted from tissue samples using a method described by Massey *et al.* with some modifications (24). Briefly, mice brain tissue, cortex and hippocampus, were weighed and thawed on ice. Samples were pooled in duplicate from similar treatment groups to achieve an average weight of 20-40 mg of hippocampus and 60-80 mg of cortex tissue. The samples were homogenised in Eppendorf tubes using a Branson Sonifier 150 with a slim probe in short bursts in 15% methanol kept on ice to prevent heating of samples. Homogenates were transferred to glass tubes containing 1 ng LTB4-d4 (Leukotriene-d4) as the internal standard (IS). Homogenates were incubated on ice for 30 min and centrifuged at 4000 rpm for 15 min at 4°C to remove any precipitated proteins. The supernatants were transferred to clean tubes and acidified to pH ~3.0 with 0.125 M HCl before applying to SPE cartridges (Bond Elut C18 500mg, Agilent Technologies, VIC, Australia) that were pre-equilibrated with methanol and water (25). Loaded samples were washed with 4 ml 15% methanol, 4 ml Milli-Q water followed by 4 ml hexane. SPMs were eluted with 6 ml ethyl acetate, dried under nitrogen and then reconstituted in 120 µL of 5 mM ammonium acetate (pH 8.9) and methanol (50:50; v/v), for analysis by LCMS/MS on a Thermo Scientific TSQ Quantum^{Ultra} Triple Quadrupole LCMS System equipped with an electrospray ionisation source operated in a negative ion mode as described previously (25). Quantitative analysis was performed using calibration curves of standard solutions of SPMs in mice brain tissues and LTB4-d4 as IS. Mice brain homogenates spiked with varying concentrations (0-1000 pg/mL) of standard solutions and 1 ng IS were extracted in a similar manner as described above. Linear regression analysis of ratio of SPMs to IS as a function of concentration of SPMs that typically gave $R^2 > 0.95$-0.90, were used to calculate concentration of SPMs in pg/mL which were then converted to pg/mg of brain tissue. The detection limit for 18-HEPE, 17-HDHA and 14-HDHA was 10pg/mL and for other SPMs was 20pg/mL. The following SPMs were quantified: 18R/S-hydroxy-5Z,8Z,11Z,14Z,16E-eicosapentaenoic acid ((±) 18-HEPE), 17S-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid (17S-HDHA), 14S-hydroxy-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid (14S-HDHA), 7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid (RvD1, resolvin D1), RvD3 (4S,11R,17S-trihydroxy-docosa-5Z,7E,9E,13Z,15E,19Z-hexaenoic acid, resolvin D3), RvD5 (7S,17S-dihydroxy-5Z,8E,10Z,13Z,15E,19Z-docosahexaenoic acid, resolvin D5), 7S,8R,17R-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid (17R-RvD1), 7R,14S-dihydroxy-4Z,8E,10E,12Z,16Z,19Z-

docosahexaenoic acid (MaR1, 7R-Maresin), 10S,17S-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid (10S,17S-diHDHA) and 10R,17S-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid (PD1, protectin D1). The level of other SPMs was under the detection limit of the assay. A schematic illustration of EPA- and DHA-derived SPMs pathways adapted from Serhan (16) can be found in supplementary material (Suppl. 2).

Preparation of protein homogenates for pro-inflammatory cytokines measurement

Half cortices were homogenised in 1:10 volume of CelLytic™ MT Lysis reagent (Sigma-Aldrich, Poole, UK) containing phosphatase and protease inhibitors (Sigma-Aldrich, Poole, UK) using a Precellys® 24 tissue homogeniser (VWR, Lutterworth, UK). Homogenates were centrifuged at 12,000 rpm for 10 min at 4°C and the supernatants were collected for biochemical analyses. Total protein concentration was determined using the Pierce™ BCA protein assay kit (Fisher Scientific, Loughborough, UK). Pro-inflammatory cytokines (IL-6 and TNFα) in the cortex homogenates were quantified using commercial IL-6 (Bio-Techne, Abindgon, UK) and TNFα (Fisher Scientific, Loughborough, UK) cytokine ELISA kits following the manufacturer's instructions.

Statistical analysis

Tissue FA and SPMs are presented as means ± SEMs. Data analysis was performed by using ANOVAs (1 way and 2 ways). Standard diagnostic tests (e.g. normality of residuals assessed using q-q plots and Shapiro-Wilk tests, outlier tests, high leverage/influence data points tested using Cook's distance) were used to verify that the data was appropriate for that technique. Where necessary, transformations were applied to the response variance to ensure that the data complied with ANOVA assumptions. Non-parametric equivalent tests (e.g. Kruskal-Wallis) were used where the data were not compliant with ANOVA requirements. Post-hoc tests were carried out using Tukey Honest Significant Differences. As we were interested in the association with age, sex and genotype on each of the profiled fatty acids, no multiple test correction was performed due to the exploratory, hypothesis driven nature of the analysis (26-28). Statistical analysis was performed using R statistical software version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Fatty acid profile

To determine whether the anticipated impact of age on DHA concentrations in the brain is influenced by *APOE* genotype and sex, we analysed fatty acid profiles in the parieto-temporal cortex and hippocampus of female and male *APOE3* and *APOE4* mice at 2-, 9- and 18-month of age (Table 1 and Appendix 3). Ageing was associated with a decreased DHA in the cortex ($p<0.001$), which was more pronounced in *APOE4* compared to *APOE3* (age*genotype, $p=0.024$) mice. A significant decrease in cortical DHA (22:6 n-3, % of total fatty acids) was only evident in female *APOE4* mice, with 10% lower levels at 18 months compared to 2 months (Fig. 1A). This effect was also evident in female *APOE4* mice when the data was expressed as a concentration (μg/mg of total fatty acids), with DHA in the cortex decreasing from 78.54 ± 3.05μg/mg to 58.21 ± 7.09μg/mg (n=7, $p=0.050$, Appendix 4). Ageing also affected the cortical profile of other classes of fatty acids (Table 1 and Appendix 1). Whilst unchanged in *APOE4* mice, the level of cortex arachidonic acid (AA, 20:4 n-6, % of total fatty acids) decreased in female *APOE3* (Appendix 3). Interestingly, the level of cortex oleic acid (18:1 n-9, % of total fatty acids) increased with age in both male and female of both genotypes, with 15% higher levels in female *APOE4* mice at 18 months (Fig. 1B). There were no significant differences in DHA, AA or oleic acid levels with increasing age in the hippocampus of female or male mice of either genotype (Fig. 1 C and D).

Figure 2 shows DHA levels in cortex phospholipid fractions according to age (Fig 2A, D, G), genotype (Fig. 2B, E, H) and age, sex and genotype (Fig 2C, F, I). Ageing was associated with a decrease in PE-DHA (Fig. 2A, $p=0.001$), in particular in female *APOE4* (Fig. 2C, $p<0.001$) mice. Ageing was also associated with a decrease in PS-DHA (Fig. 2D, $p<0.001$) and PE- and PC-AA ($p<0.01$ for both), whilst PS-AA increased ($p=0.000$, Appendix 5). Conversely, the oleic acid-containing fractions were all significantly increased at 18-month of age compared to young animals in *APOE4* females ($p=0.000$), as follows: from 19.4 ± 0.1% to 20.5 ± 0.1% for PC, from 8.0 ± 0.2% to 9.8 ± 0.2% for PE, and from 10.5 ± 0.2% to 14.3± 0.4% for PS (Appendix 5). No significant age*genotype interactions were observed in oleic acid-containing fractions.

As a lower DHA to arachidonic acid (DHA:AA) ratio has been proposed as a biomarker of AD risk, we looked at total and individual phospholipid species ratios. In older animals a lower total (Fig. 3A, $p=0.010$) and PS- (Fig. 3G, $p<0.001$) DHA:AA ratio was evident, with the effect of age only observed in *APOE4* animals (both male and female) for total DHA:AA (Fig. 3B, $p<0.001$). On the other hand, PC-DHA:AA significantly increased with age (Fig. 3J, $p=0.003$) and was more pronounced in *APOE3* (Fig. 3K, $p=0.037$) than in *APOE4*.

EPA- and DHA-derived specialised pro-resolving mediators

SPMs were assessed in the parieto-temporal cortex and hippocampus samples to determine whether there was an association with *APOE* genotype, age and sex. The SPMs profile differed between the two brain regions with the cortex exhibiting the most significant inter-group differences (Table 2, Appendix 6).

As shown on figure 4, relative to their parent compound EPA or DHA, females had higher levels of cortical RvD3 ($p=0.008$), 10S,17S-diHDHA ($p=0.026$), PD1 ($p=0.001$), MaR1 ($p<0.001$), 17S-HDHA ($p=0.002$), 14S-HDHA ($p=0.005$) and (±) 18-HEPE ($p<0.001$). (±) 18-HEPE, 10S,17S-diHDHA and 17R-RvD1 levels expressed relative to their parent compound, EPA or DHA, were lower in mice with an *APOE4* genotype (Fig. 5). There was a trend for a reduced ratio for MaR1 between the two genotypes (Fig. 5D, $p=0.083$). Other SPMs did not reach significance. There was an age*genotype interaction for 17S-HDHA/DHA ($p=0.011$), 14S-HDHA/DHA ($p=0.023$) and MaR1/DHA ($p=0.083$) largely driven by differences in the *APOE3* genotype in females at 2 months of age (Appendix 6). Similar results in females compared to males were observed in unadjusted data but only for PD1, 7R-Maresin, 17S-HDHA and 14S-HDHA (Table 2), and in *APOE4* compared to *APOE3* for (±) 18-HEPE, 10S,17S-diHDHA and 17R-RvD1 (Table 2).

Neuroinflammatory status in the cortex

In order to provide insight into the SPM responses, IL-6 and TNFα expressions were determined (as biomarkers of neuroinflammatory status) in the cortex of female and male *APOE3* and *APOE4* mice at 2-, 9- and 18-month of age. No independent or interactive effect of age, sex or *APOE* genotype effects were evident (Fig. 7).

Discussion

The current study presents for the first time the independent and interactive effect of age, sex and *APOE* genotype on the brain fatty acid profile and the concentration of EPA- and DHA-derived SPMs. A 10% lower total and 11% lower PE-DHA were evident in female mice carrying an *APOE4* genotype at 18 months relative to 2 months of age suggesting that a lower DHA status in *APOE4* carriers may contribute to the higher AD incidence in females. A number of SPMs were significantly higher in females and lower in *APOE4* mice.

Lower cortical DHA content in old female *APOE4* mice

Ageing is associated with brain atrophy, and a loss of total tissue and fatty acid concentration per unit volume. As recently highlighted by Lacombe *et al.* (29), it is important to consider brain fatty acid content as a percentage of total fatty acids and relative to the weight of total lipids when interpreting the physiological meaning of altered status. Here we observed a lower percentage and concentration of cortex DHA in older female *APOE4* mice compared to their *APOE3* or male counterparts. Previous studies in rodents have demonstrated the impact of ageing on brain DHA (29) and identified DHA transporters across the blood-brain barrier using transgenic animal models (for review (30)). Vandal *et al.* (31) described the impact of *APOE4* genotype on DHA uptake (31). However, unlike their study (31) here we show an age*genotype interaction with total DHA in older mice different according to *APOE* genotype. This lack of consistency may be the result of different experimental conditions including the age at which the animals were sacrificed (13-month-old versus 18 months in the present study) therefore suggesting a more deleterious effect of *APOE4* later in life. Considering the average lifespan of laboratory mice, 18-months of age would correspond to about 60 human years (32). Our results are in line with observations reported in the Global Alzheimer's Association Interactive Network meta-analysis, that showed the influence of sex on cognitive decline emerged as age-dependent (6). Women carrying one or two copies of the *APOE4* allele were at a higher and earlier risk of MCI (at ages 55-70 years) or AD (at ages 65-75 years) compared to men, with sexual dimorphism ceasing after 75 years (5, 6) (for review (30)).

Interestingly, we only observed a trend for a lower concentration of DHA in the hippocampus, which had been previously reported by Chouinard-Watkins *et al.* (33) in 12-month of age *APOE4* mice regardless of sex. Knowing that regions with a high degree of plasticity such the fronto-temporal cortex are particularly vulnerable to normal ageing-

associated detrimental effects (34), it is possible that fatty acid changes in the hippocampus, which is a more conserved region and central to AD development, could appear at an older age.

Lower cortical DHA associated with lower PE-DHA

Brain fatty acids are almost exclusively present in four major phospholipid classes: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) (35). APOE plays a crucial role in phospholipid transport and metabolism within the central nervous system. DHA-containing phospholipids play a key structural role in neuronal membranes and supporting transmembrane receptor function and subsequent signaling pathways. Changes in APOE conformation resulting from an *APOE4* genotype and changes in DHA bioavailability may impair these pathways (35). PS is the major anionic phospholipid class in neuronal membranes and is crucial for protein translocation, protein-lipid interactions and cell survival with potential therapeutic impact suggested in AD (36). PS in neuronal membranes is tightly associated with DHA and is particularly sensitive to DHA deprivation unlike in other organs such as liver and adrenal gland where PS is enriched in AA and where EPA may compensate for the lack of DHA (35, 36). PE is a precursor to PS (36).

We observed a significant decrease in PE-DHA in old female *APOE4* mice. An age-associated decline in PE-DHA compared to the other phospholipid species was also described by Sharman *et al.* (37) for total PE in 1-year old male *APOE4*. Conversely, while they reported an increase with age of total brain PS level, we have shown a decrease in cortical PS-DHA independent of sex and genotype. Kariv-Inbal *et al.* (38) also observed a decrease in PS-DHA which was restored by a DHA-enriched diet in *APOE4* mice. The lack of genotype effect in our study is unlikely to be related to the age difference between the mice in our study and that reported by Kariv-Inbal *et al.* (5-month versus 2- and 18-month of age, respectively) but could perhaps be explained by the fatty acid composition of the control diet. The chow diet in our study contained 57% PUFAs with a n-6/n-3 ratio of 5, while Kariv-Inbal *et al.* (38) used a diet composed of 46% PUFAs and a n-6/n-3 ratio of 20, suggesting that the difference in n-3 PUFA content in the chow diets might have offset the genotype difference in DHA-associated PS species.

Oleic acid to counterbalance the reduction in DHA content in brain tissue

Palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n-9), AA and DHA were the predominant fatty acids in rodent brains in our study, consistent with previous observations (39). In our study the level of MUFAs and more specifically oleic acid (18:1n-9) increased in all mice. Gimenez da Silva-Santi *et al.* (39) reported similar increases in oleic acid in rats fed either a high fat or high carbohydrate diet. Oleic acid may serve as a brain energy source when glucose availability is reduced (39). It is known that DHA deficiency in the cerebral cortex leads to reduced glucose transporter expression and impairs glucose transport (40). Knowing that there is an association between AD and DHA deprivation, it is therefore not surprising to observe higher concentrations of oleic acid in AD as reported by Fraser *et al.* (41) and Astarita *et al.* (42). In human brain samples, Astarita *et al.* (42) observed an increased mRNA expression of stearoyl-CoA desaturase, the rate-limiting enzyme in MUFAs biosynthesis, and subsequent higher concentration in oleic acid in brains of AD patients compared to controls that were correlated with cognitive impairment. In contrast to the findings of Astarita *et al.* (42), Fraser *et al.* (41) surprisingly did not observe any reduction in DHA content between AD and control brains or any correlation with *APOE* genotype and gender. The diverse age range could be a reason of this discrepancy, where AD subjects ranged from 47 to 99 years in the report of Fraser *et al.* (41), while Astarita *et al.* used AD brains from subjects aged between 63 and 95 years. The interactive effects of age, sex and *APOE* genotype in human brain tissue is currently unknown. In a recent nontargeted metabolomic study, comparing brain regions in controls, asymptomatic cognitively normal individuals with some brain pathology (ASYMAD) and AD patients, surprisingly higher DHA was observed in AD brains (43). Lower DHA was observed in the cerebellum of the ASYMAD group. Their analysis included a relatively small sample size (n=14 per cognitive group) with no subgroup analysis (e.g. by sex or APOE genotype) conducted (43).

Significant age*APOE genotype interaction in cortical DHA:AA ratio

The role of brain AA:DHA ratio in the diagnosis of MCI/AD has recently been highlighted (44), but there is little information on how it is influenced by sex, age and *APOE4* genotype. Our results showed that in the cortex, DHA:AA ratio decreased with age and this decrease was more pronounced in animals with an *APOE4* genotype independent of sex, with the reduced level of DHA mainly responsible for the decreased ratio. In contrast, Chouinard-Watkins *et al.* reported higher hippocampal AA in *APOE4* mice at 12-months of age compared to *APOE3*, with no change in DHA (33). A similar finding has been reported in

AD-like transgenic mice (45). These findings agree with the observed decreased DHA:AA ratio from the present study.

Neuroinflammation in *APOE4* carriers

Previous studies have shown that an inflammatory stimulus in an *APOE4* genotype increased cytokine secretion leading to amplified glial response and neuroinflammation (18). However, under basal conditions *APOE4* genotype does not exhibit signs of glial activation as observed by Zhu *et al.* (18) who did not observe differences in IL-1β, IL-6 or TNFα in the hippocampus of *APOE4* mice compared to *APOE3* or *APOE2* at 4 months of age. The present findings are consistent with the findings reported by Zhu *et al*.

Influence of sex and genotype on SPMs

SPMs are endogenous lipid mediators derived from DHA, EPA, and AA, and function as potent local-resolution agonists with actions ranging from nanomolar to picomolar concentrations in a variety of cell types (46). Our study has shown higher levels of cortical SPMs in females when expressed relative to their parent fatty acid (DHA or EPA). Inter-group differences were only observed using data adjusted for parent fatty acid and could be related to the fall in DHA. However, the lack of change in EPA along with a lower 18-HEPE provides support for a direct effect of sex on SPMs production. The higher ratio of SPM to parent fatty acid in females could be related to several factors including: (i) reduced fatty acid substrate in cortical tissue and/or (ii) activation of the enzymes involved in SPM synthesis by estrogen. In regard to the latter, there is evidence that estrogen increases 15-lipoxygenase activity (47). Our data is also supported by findings from a human study that showed women on a skin challenge had increased levels of SPMs associated with accelerated resolution of inflammation (16, 48).

Neuroinflammation plays a key role in neuronal function and AD pathology (49). However, the impact of sex, age and *APOE* genotype on the resolution of inflammation in the brain and in particular on SPMs profiles is unknown. Growing evidence suggests that resolution mechanisms are impaired in the development of AD pathology (17), possibly related to a lower n-3 fatty acid status (50), genetic polymorphisms in the enzymes responsible for the biosynthesis of SPMs or dysfunctional SPM-receptor signalling pathways (16). We report for the first time, a significant decrease in protectins (10S, 17S-diHDHA), resolvins (17R-RvD1) and the EPA-derived 18-HEPE in *APOE4* compared to *APOE3* genotype. In addition,

significant age*genotype interactions were observed for the DHA-derived 17S-HDHA and 14S-HDHA.

SPMs display unique biological functions and their actions are stereochemically selective with respect to their routes of biosynthesis (46). RvD3 exhibits potent pro-resolving and anti-inflammatory actions within the inflammatory secretion and is involved in the late resolution phase of the acute inflammatory response (51). MaR1 is produced in the later stages of the resolution of inflammation and promotes tissue regeneration, protects against motor dysfunction and displays analgesic actions (48). In contrast, PD1 is involved in the initial phase of inflammation and is reduced in the early stages of AD (15). In our study, PD1 levels were not affected by genotype but significantly decreased with age in the hippocampus, consistent with reduced levels detected in the CA1 region of the hippocampus from AD patients (52).

Given their neuroprotective effects, SPMs such as for PD1, 17R-RvD1 and E-resolvins, could have a novel therapeutic role in AD (For review, (17)). For instance, acute treatment with 17R-RvD1 prevented cognitive decline by protecting the brain from neuroinflammation and synaptic dysfunction in a mouse model (53). A better understanding of resolution mechanisms and fatty acid metabolism in the brain is therefore warranted.

Strengths and Limitations

The strength of our study is its comprehensive nature, comparing in a single trial the individual and interactive impact of age, sex and *APOE* genotype on fatty acid and SPM status in two brain regions. One important limitation is that we did not apply stringent control for multiple testing, which may lead to a potential overstatement of our findings. Thus, larger scale focused studies are highly encouraged to provide further confirmation.

Conclusion

Our results show a strong association between age, the female sex and an *APOE4* genotype. A novel finding is that SPMs in brain cortex were substantially lower in mice with an *APOE4* genotype. Together these data suggest that decreased cortical DHA, in particular PE-DHA, and SPMs, may contribute to the development of cognitive decline and AD pathology. Corroboration of these findings in human are needed, along with an investigation of the impact of menopause and hormone replacement therapy on DHA status and metabolism.

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Author Contributions

AM. Minihane, D. Vauzour, N. Tejera and A. Martinsen designed the research;

D. Vauzour provided the Home Office Animal Licence;

A. Martinsen, N. Tejera, G. Harden, performed the animal research and subsequent sample processing and inflammatory cytokine analysis;

J. Dick performed the fatty acid analysis;

S. Shinde, A Barden and T.A designed and performed the SPMs analysis;

A. Martinsen and N. Tejera analysed the data;

A. Martinsen, AM. Minihane, D. Vauzour, J. Dick, S. Shinde, A. Barden and T.A. Mori contributed to the writing of the paper and all authors approved the final manuscript.

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Figures legend

Fig. 1: DHA (22:6 n-3) and oleic acid (18:1 n-9) as a % of total fatty acids in the cortex (A and B) and hippocampus (C and D) of female and male *APOE3* and *APOE4* mice at 2-, 9- and 18-month of age fed on a chow diet. Values are means ± SEM, n=6-7 per group. **p<0.01, ***p<0.001.

Fig. 2: DHA-containing phospholipid fractions in cortex of female and male *APOE3* and *APOE4* mice at 2- and 18-month of age fed on a chow diet. PE, phosphatidylethanolamine, PS phosphatidylserine and PC, phosphatidylcholine. The age*genotype interaction was only significant for PE-DHA (B, p=0.005). Figures C, F and I show DHA-containing PE, PS and PC respectively at a group level where PE-DHA content was significantly decreased in female *APOE4* at 18 months (p<0.001). Values are means ± SEM, n=15-16 per group for age effect (A, D, G), n=7-8 per group for age*genotype interaction (B, E, H), n=3-4 per group for group effect (C, F, I). ***p<0.001. Inclusion of arrows indicates significant interaction.

Fig. 3: Ratios of DHA to arachidonic acid (AA), in total lipid and individual phospholipid species, in the cortex of female and male *APOE3* and *APOE4* mice at 2- and 18-month of age fed on a chow diet. The age*genotype interaction was significant for total DHA:AA ratio (B, p=0.01), PE-DHA:AA (E, p=0.00) and PC-DHA:AA (K, p=0.04). Values are means ± SEM, n=15-27 per group for age effect (A, D, G, J), n=7-14 per group for age*genotype interaction (B, E, H, K), n=4-7 per group for group effect (C, F, I, L). *p=0.01, **p<0.01, ***p<0.001. Inclusion of arrows indicates significant interaction.

Fig. 4: Gender differences in the ratios of SPMs to their parent compound, EPA or DHA, in cortex of female and male *APOE3* and *APOE4* mice at 2-, 9- and 18-month of age fed on a chow diet. Values are means ± SEM, n=20 per group, * p≤0.05, **p<0.01. Figures of SPMs and their precursors were expressed in pg/mg of tissue to calculate the ratios.

Fig. 5: Genotype differences in the ratios of SPMs to their parent compound, EPA or DHA, in cortex of female and male *APOE3* and *APOE4* mice at 2-, 9- and 18-month of age fed on a chow diet. Values are means ± SEM, n=20 per group, **p<0.01. Figures of SPMs and their precursors were expressed in pg/mg of tissue to calculate the ratios.

Fig. 6: Expression level of two pro-inflammatory cytokines, IL-6 and TNFα, in the cortex of female and male *APOE3* and *APOE4* mice at 2-, 9- and 18-month of age fed on a chow diet. Values are means ± SEM, n=11-12 per group (A, D) and n=17-18 per group for gender (B, E) or genotype effect (C, F).

Tables legend

Table 1: Fatty acid composition in % of total lipid in hippocampus and cortex of female and male 2-, 9- and 18-month of age *APOE3* and *APOE4* mice fed on chow diet. Means ± SEM (n). NS, non-significant. Mo, month, Gd, gender, Gt, genotype, SFA, saturated fatty acid, MUFA, monounsaturated fatty acid, PUFA, polyunsaturated fatty acid, DHA, docosahexaenoic acid, AA, arachidonic acid and DMA, dimethyl acetal fatty acid. P-values are shown for age, gender, genotype, two-way interactions and at a group level (comparing each group with each other).

Table 2: Specialised proresolving mediators measured in hippocampus and cortex of female and male 2-, 9- and 18-month of age *APOE3* and *APOE4* mice fed on chow diet. The levels of (±) 18 HEPE, 17S-HDHA, 14S-HDHA, 17R-resolving D1 (17R-RvD1), resolvin D1, D3 and D5 (RvD1, D3, D5), 7R-Maresin (MaR1), 10S,17S-diHDHA and Protectin D1 (PD1) were detected and analysed by LCMS/MS. Means ± SEM (n) in pg/mg of tissue. NS, non-significant. P-values are shown for age, gender, genotype, two-way interactions and at a group level (comparing each group with each other).

Figure 1

Fig. 1

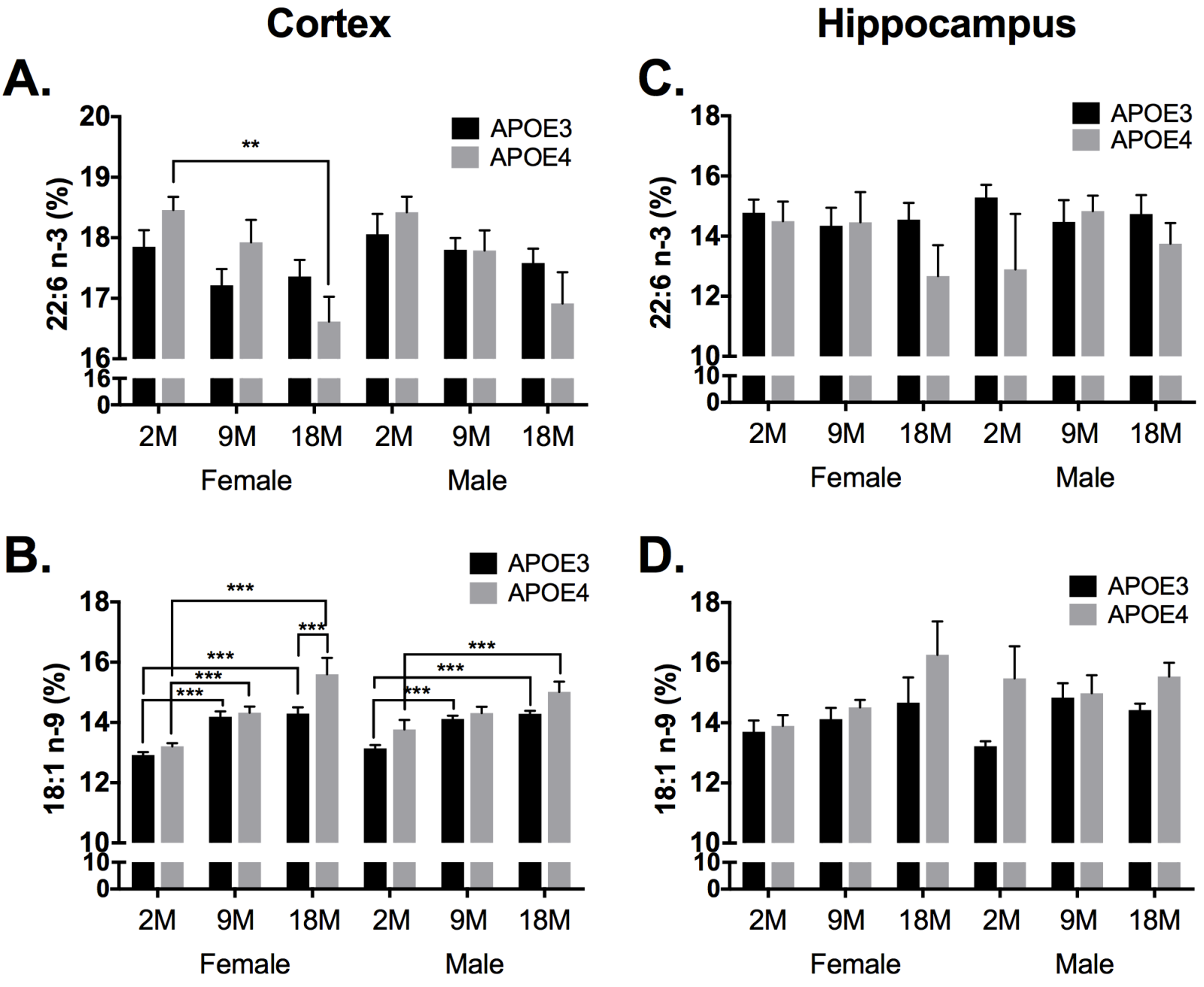


Figure 2

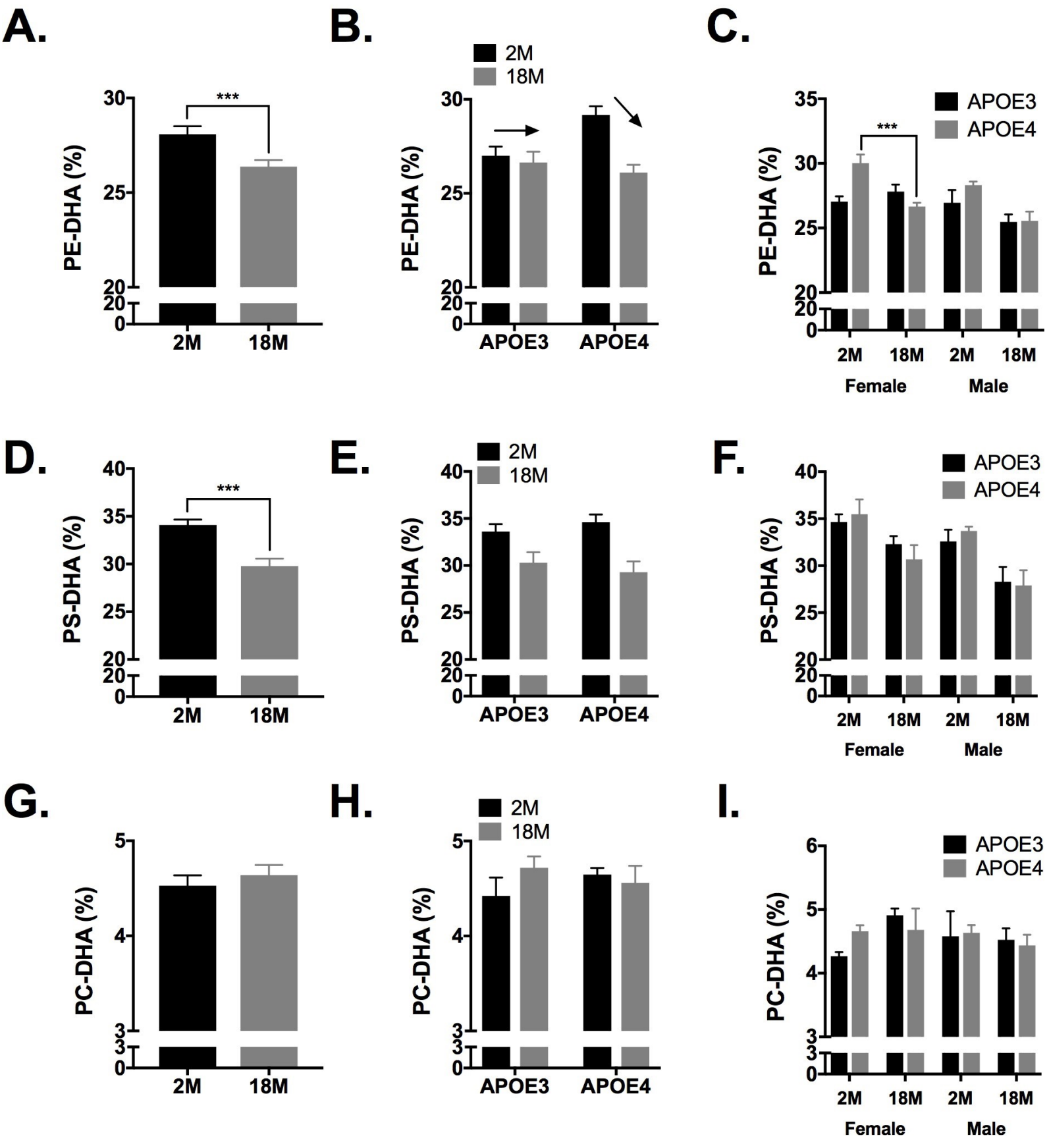


Fig. 2
A.
B.
C.
D.
E.
F.
G.
H.
I.
PE-DHA (%)
PS-DHA (%)
PC-DHA (%)
2M
18M
APOE3
APOE4
Female
Male
***

Fig. 3

Figure 3

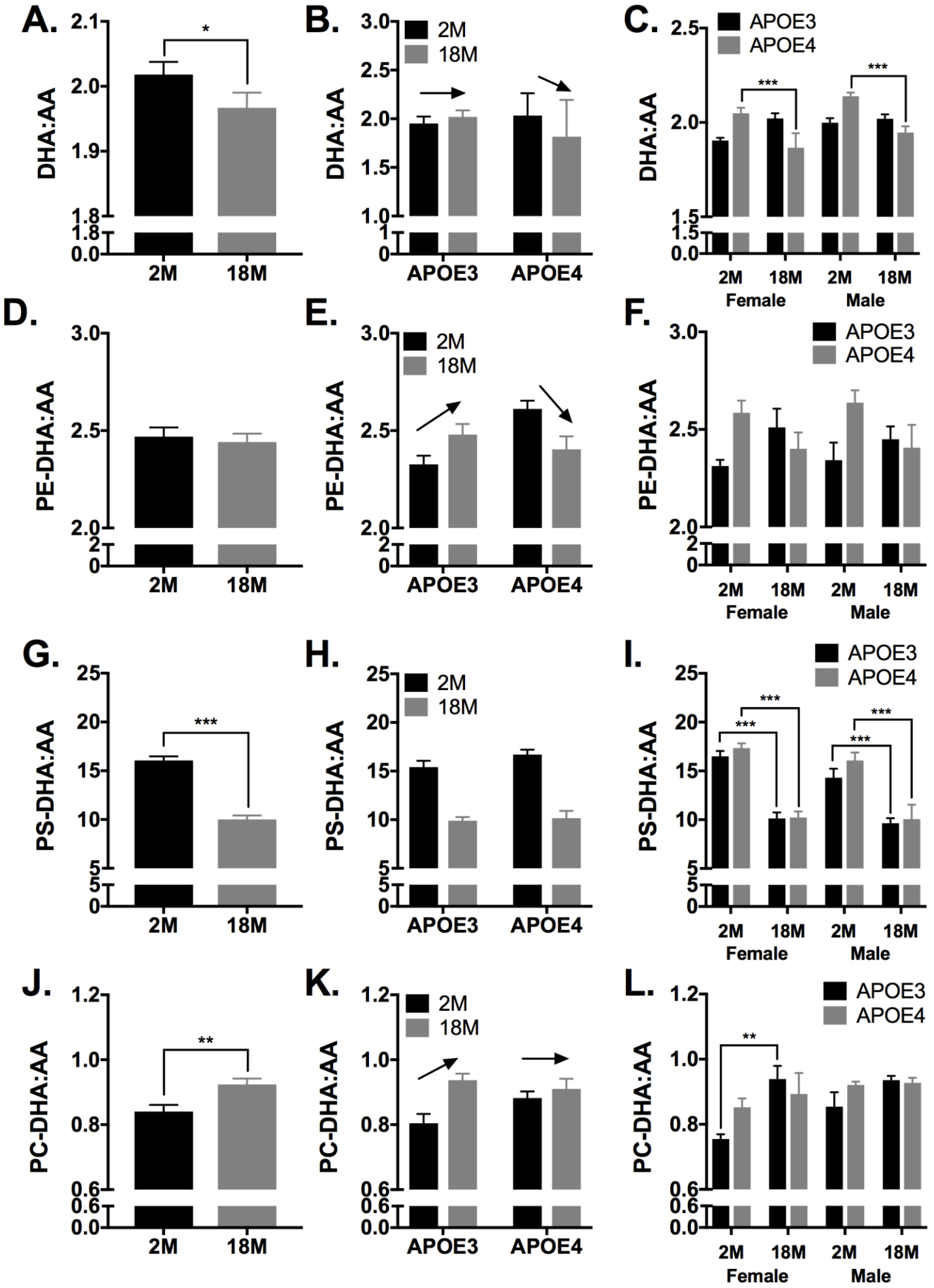


Figure 4

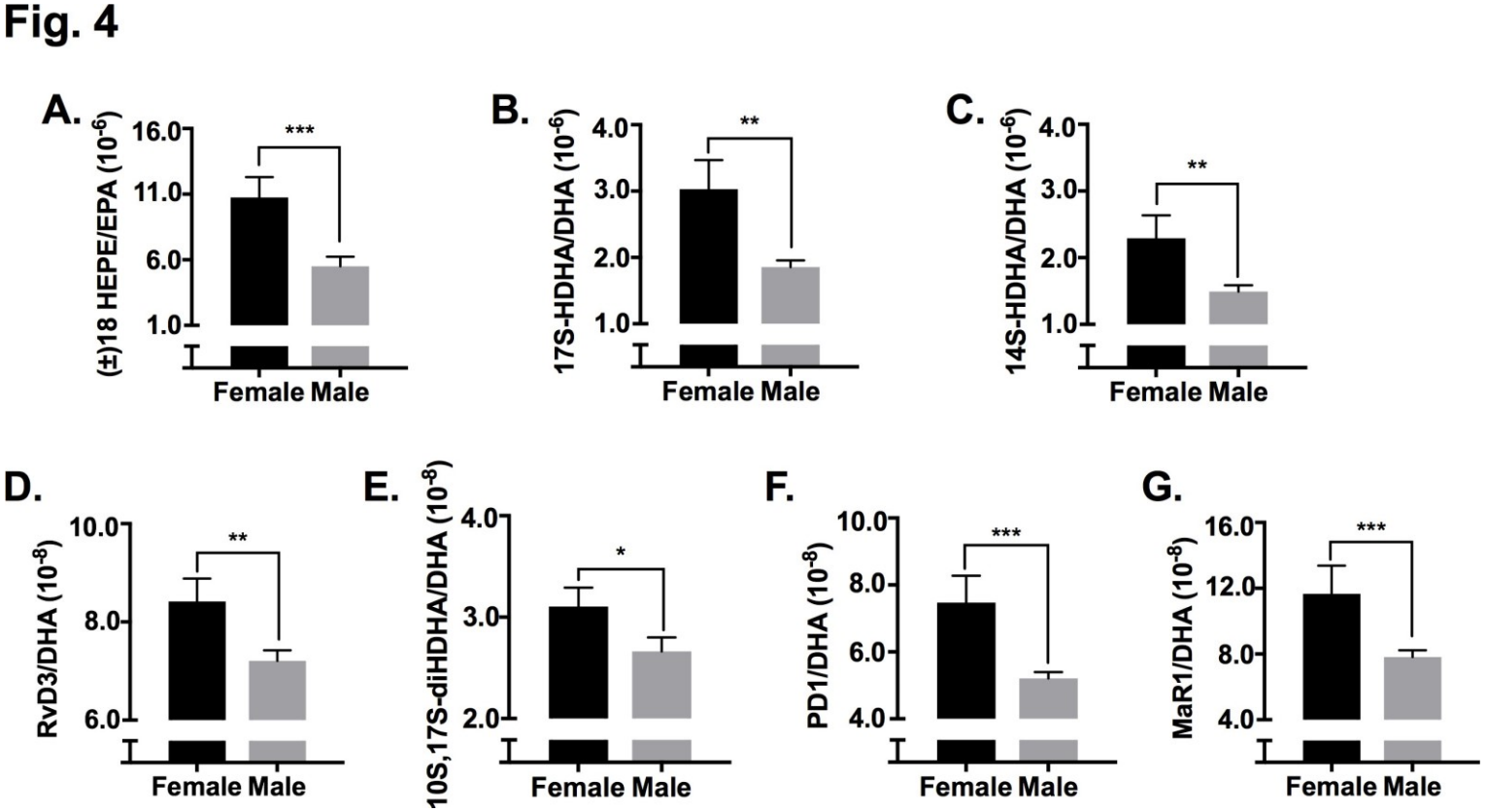


Fig. 4
A.
(±)18 HEPE/EPA (10^{-6})
16.0
11.0
6.0
1.0
***
Female Male
B.
17S-HDHA/DHA (10^{-6})
4.0
3.0
2.0
1.0
**
Female Male
C.
14S-HDHA/DHA (10^{-6})
4.0
3.0
2.0
1.0
**
Female Male
D.
RvD3/DHA (10^{-8})
10.0
8.0
6.0
**
Female Male
E.
10S,17S-diHDHA/DHA (10^{-8})
4.0
3.0
2.0
*
Female Male
F.
PD1/DHA (10^{-8})
10.0
8.0
6.0
4.0
***
Female Male
G.
MaR1/DHA (10^{-8})
16.0
12.0
8.0
4.0
***
Female Male

Figure 5

Fig. 5

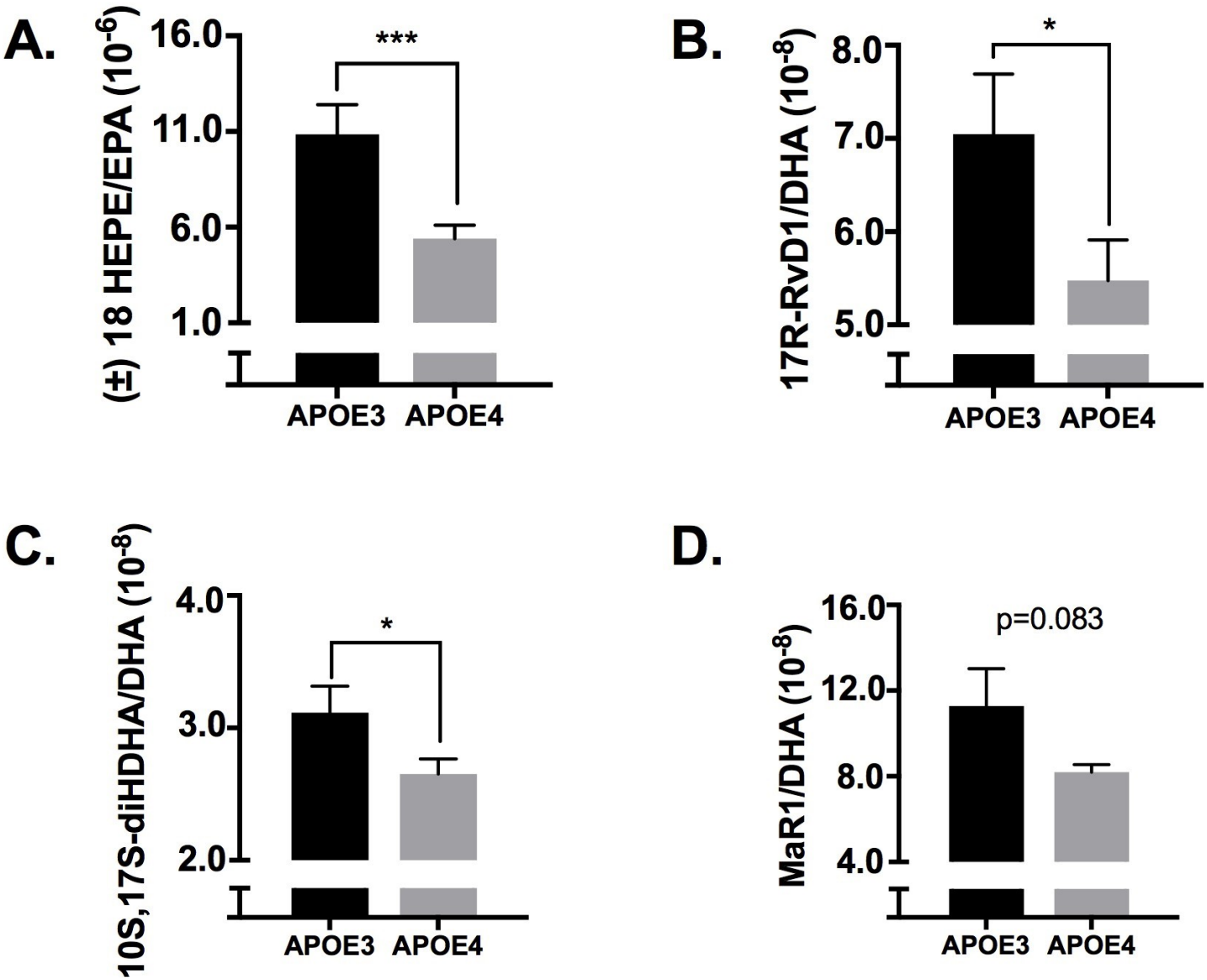
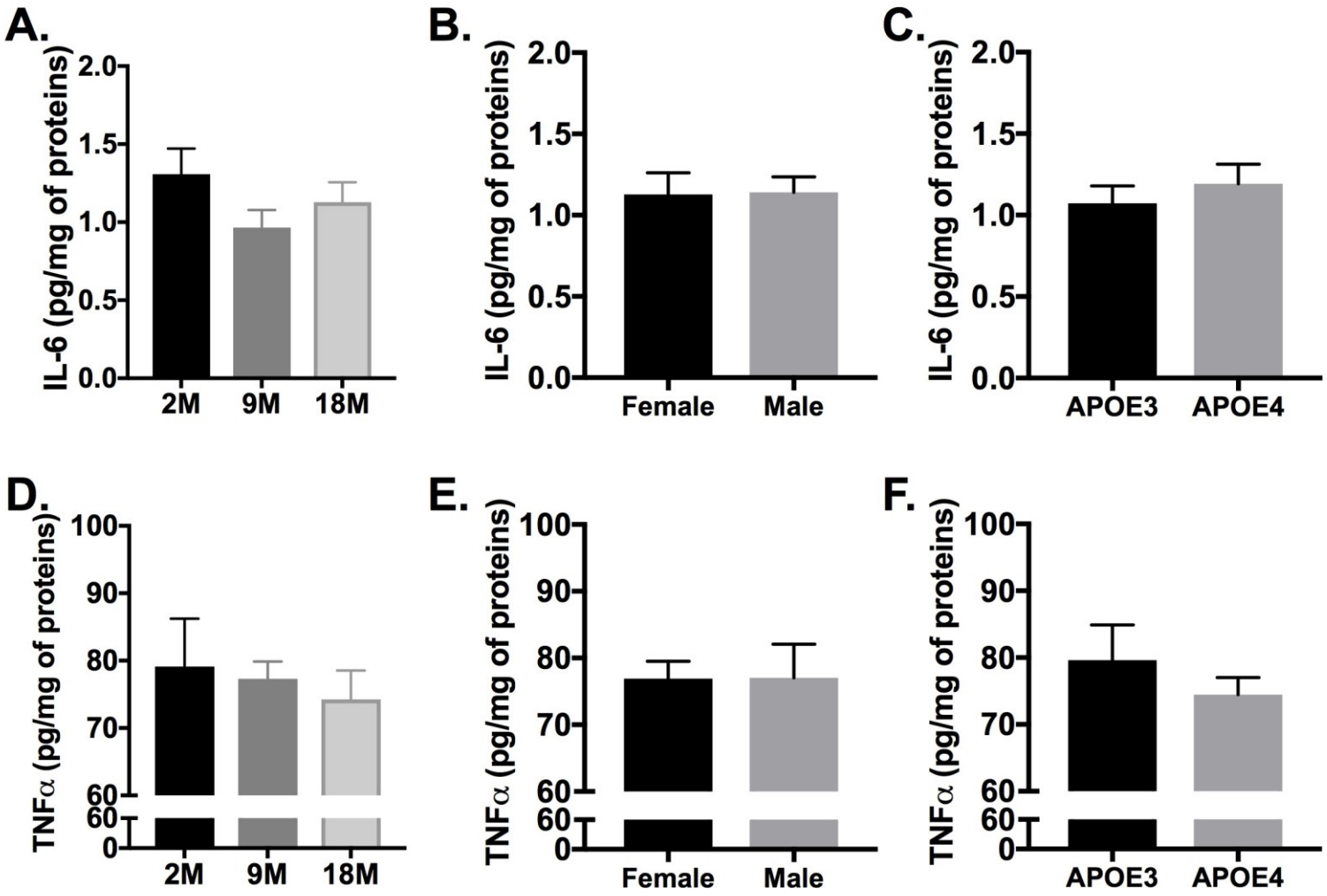


Figure 6

Fig. 6



Supplemental data 1: Fatty acid composition of RM3-P chow diet.

Oil content (% Oil)	3.54
Moisture content (% Moisture)	11.55

Fatty acid	Trivial name	%	mg/100g
14:00	Myristic	0.71	18.36
15:00	Pentadecylic	0.13	3.48
16:00	Palmitic	13.87	358.41
18:00	Stearic	2.75	71.02
20:00	Arachidic	0.27	7.01
22:00	Behenic	0.38	9.7
24:00	Lignoceric	0.22	5.66
Total SFA		18.32	473.64
16:1n-9		0.13	3.33
16:1n-7	Palmitoleic	0.81	20.89
18:1n-9	Oleic	19.17	495.54
18:1n-7	Vaccenic	1.56	40.43
20:1n-11		0.15	3.77
20:1n-9	Gondoic	1.4	36.22
20:1n-7		0	0
22:1n-11		1.38	35.63
22:1n-9	Erucic	0.27	6.95
24:1n-9	Nervonic	0.25	6.39
Total MUFA		25.11	649.17
18:2n-6	Linoleic	47.59	1229.99
18:3n-6	Gamma-linolenic	0	0
20:2n-6	Eicosadienoic	0.15	3.95
20:3n-6	Dihomo-gamma-linolenic	0	0
20:4n-6	Arachidonic	0.1	2.62
22:4n-6	Adrenic	0	0
22:5n-6	Osbond	0	0
Total n-6 PUFA		47.84	1236.56
18:3n-3	Alpha-linolenic	5.64	145.79
18:4n-3	Stearidonic	0.32	8.28
20:3n-3	Eicosatrienoic	0	0
20:4n-3	Eicosatetraenoic	0.11	2.77
20:5n-3	Eicosapentaenoic (EPA)	0.98	25.23
21:5n-3	Heneicosapentaenoic	0.1	2.53
22:5n-3	Docosapentaenoic	0.16	4.1
22:6n-3	Docosahexaenoic (DHA)	1.42	36.75
Total n-3 PUFA		8.72	225.45
Total PUFA		56.56	1462.01

Abbreviations: Total SFA: saturated fatty acids (14:0, 15:0, 16:0, 18:0, 20:0, 22:0 and 24:0). Total MUFA: monounsaturated fatty acids (16:1, 18:1, 20:1, 22:1 and 24:1). Total n-6 PUFA: n-6 polyunsaturated fatty acids (18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6 and 22:5n-6). Total n-3 PUFA: n-3 polyunsaturated fatty acids (18:3n-3, 18:4n-3, 20:3n-3, 20:5n-3, 21:5n-3, 22:5n-3 and 22:6n-3).

Supplementary data to:

Altered SPMs and age-associated decrease in brain DHA in APOE4 female mice

Anneloes Martinsen[1], Noemi Tejera[1], David Vauzour[1], Glenn Harden[1], James Dick[2], Sujata Shinde[3], Anne Barden[3], Trevor A Mori[3], Anne Marie Minihane[1]

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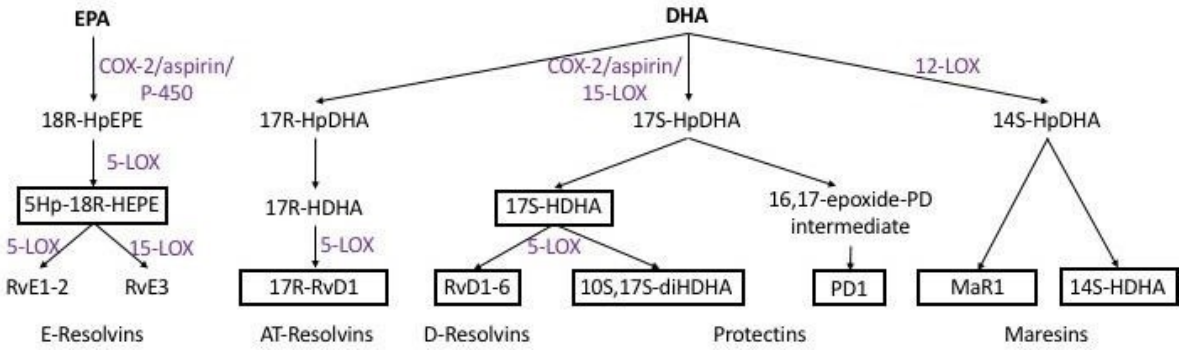
Supplemental data 2: Schematic illustration of EPA- and DHA-derived specialised proresolving mediators pathways.



The following SPMs were quantified: 18R/S-hydroxy-5Z,8Z,11Z,14Z,16E-eicosapentaenoic acid ((±) 18-HEPE), 17S-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid (17S-HDHA), 14S-hydroxy-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid (14S-HDHA), 7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid (RvD1, resolvin D1), RvD3 (4S,11R,17S-trihydroxy-docosa-5Z,7E,9E,13Z,15E,19Z-hexaenoic acid, resolvin D3), RvD5 (7S,17S-dihydroxy-5Z,8E,10Z,13Z,15E,19Z-docosahexaenoic acid, resolvin D5), 7S,8R,17R-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid (17R-RvD1), 7R,14S-dihydroxy-4Z,8E,10E,12Z,16Z,19Z-docosahexaenoic acid (MaR1, 7R-Maresin), 10S,17S-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid (10S,17S-diHDHA) and 10R,17S-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid (PD1, protectin D1). The level of other SPMs was under the detection limit of the assay. The figure has been adapted from Serhan, C. N. (2017) Treating inflammation and infection in the 21st century: new hints from decoding resolution mediators and mechanisms. *FASEB J* **31**, 1273-1288.

Supplementary data to:
Altered SPMs and age-associated decrease in brain DHA in APOE4 female mice

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Supplemental data 3: Detailed fatty acid composition in % of total lipid in cortex and hippocampus of female and male 2-, 9- and 18-month of age *APOE3* and *APOE4* mice fed on chow diet.

Cortex	APOE3						APOE4																		
	Female			Male			Female			Male															
Compound (%TL)	APOE3 F 2 Mo	APOE3 F 9 Mo	APOE3 F 18 Mo	APOE3 M 2 Mo	APOE3 M 9 Mo	APOE3 M 18 Mo	APOE4 F 2 Mo	APOE4 F 9 Mo	APOE4 F 18 Mo	APOE4 M 2 Mo	APOE4 M 9 Mo	APOE4 M 18 Mo	P-group	P-age	P-sex	P-apoe	P-age:sex	P-age:apoe	P-sex:apoe
14:0	0.59 ± 0.26	0.39 ± 0.13	0.62 ± 0.32	0.80 ± 0.50	0.29 ± 0.06	0.25 ± 0.03	0.33 ± 0.04	0.26 ± 0.06	0.33 ± 0.06	0.42 ± 0.07	0.33 ± 0.06	0.71 ± 0.40	NS	NS	NS	NS	NS	NS	NS
16:0	21.80 ± 0.27	20.73 ± 0.20	20.86 ± 0.35	22.05 ± 0.31	20.86 ± 0.33	20.66 ± 0.26	21.77 ± 0.16	21.33 ± 0.72	21.23 ± 0.85	22.56 ± 0.69	21.60 ± 0.40	21.22 ± 0.47	**0.047**	**0.001**	NS	NS	NS	NS	NS
16:1n-7	0.51 ± 0.04	0.52 ± 0.01	0.56 ± 0.03	0.55 ± 0.05	0.54 ± 0.02	0.56 ± 0.01	0.50 ± 0.01	0.53 ± 0.02	0.66 ± 0.04	0.57 ± 0.02	0.61 ± 0.02	0.64 ± 0.02	**0.000**	**0.000**	**0.001**	**0.001**	NS	NS	NS
16:1n-9	0.67 ± 0.18	0.62 ± 0.16	0.68 ± 0.19	0.59 ± 0.13	0.59 ± 0.13	0.49 ± 0.09	0.73 ± 0.17	0.53 ± 0.12	0.70 ± 0.14	0.72 ± 0.18	0.65 ± 0.14	0.69 ± 0.16	NS	NS	NS	NS	NS	NS	NS
16:1	1.18 ± 0.19	1.14 ± 0.16	1.24 ± 0.21	1.15 ± 0.17	1.13 ± 0.13	1.06 ± 0.09	1.23 ± 0.17	0.91 ± 0.19	1.36 ± 0.17	1.29 ± 0.19	1.26 ± 0.14	1.33 ± 0.17	NS	NS	NS	NS	NS	NS	NS
18:0	20.09 ± 0.13	19.85 ± 0.13	19.64 ± 0.15	19.67 ± 0.33	19.74 ± 0.14	19.71 ± 0.13	19.65 ± 0.15	19.83 ± 0.16	19.41 ± 0.22	19.45 ± 0.17	19.30 ± 0.19	18.97 ± 0.13	**0.005**	0.051	**0.011**	**0.001**	NS	NS	NS
18:1n-7	3.12 ± 0.04	3.15 ± 0.02	3.20 ± 0.02	3.25 ± 0.04	3.18 ± 0.04	3.28 ± 0.04	3.14 ± 0.05	3.19 ± 0.05	3.37 ± 0.04	3.12 ± 0.03	3.15 ± 0.02	3.25 ± 0.04	**0.000**	**0.000**	NS	NS	NS	0.081	**0.003**
18:1n-9	12.92 ± 0.10	14.19 ± 0.17	14.29 ± 0.21	13.14 ± 0.11	14.12 ± 0.11	14.29 ± 0.10	13.21 ± 0.10	14.33 ± 0.20	15.60 ± 0.55	13.77 ± 0.32	14.31 ± 0.20	15.02 ± 0.34	**0.000**	**0.000**	NS	**0.000**	0.071	NS	NS
18:1	16.04 ± 0.09	17.34 ± 0.18	17.49 ± 0.21	16.39 ± 0.14	17.30 ± 0.13	17.57 ± 0.12	16.34 ± 0.13	15.01 ± 2.51	18.97 ± 0.57	16.89 ± 0.31	17.47 ± 0.20	18.26 ± 0.32	**0.000**	**0.000**	NS	**0.000**	**0.032**	NS	NS
18:2n-6	0.65 ± 0.02	0.57 ± 0.01	0.61 ± 0.04	0.71 ± 0.06	0.67 ± 0.03	0.62 ± 0.02	0.73 ± 0.03	0.70 ± 0.11	0.81 ± 0.10	0.99 ± 0.12	0.77 ± 0.08	0.84 ± 0.10	**0.001**	**0.023**	**0.005**	**0.000**	NS	NS	NS
20:1n-9	0.79 ± 0.03	1.15 ± 0.03	1.11 ± 0.05	0.80 ± 0.01	1.08 ± 0.05	1.10 ± 0.02	0.75 ± 0.01	1.05 ± 0.05	1.10 ± 0.05	0.80 ± 0.01	0.95 ± 0.03	1.00 ± 0.07	**0.000**	**0.000**	NS	**0.005**	**0.040**	NS	NS
20:2n-6	0.14 ± 0.00	0.09 ± 0.00	0.10 ± 0.00	0.14 ± 0.00	0.12 ± 0.01	0.10 ± 0.00	0.12 ± 0.02	0.11 ± 0.01	0.10 ± 0.01	0.14 ± 0.01	0.11 ± 0.01	0.09 ± 0.02	**0.000**	**0.000**	0.052	NS	NS	NS	NS
20:4n-6	9.38 ± 0.15	8.88 ± 0.14	8.60 ± 0.15	9.04 ± 0.13	8.64 ± 0.11	8.71 ± 0.07	9.02 ± 0.09	8.58 ± 0.15	8.90 ± 0.19	8.57 ± 0.12	8.29 ± 0.15	8.52 ± 0.19	**0.000**	**0.000**	**0.001**	**0.006**	NS	**0.039**	NS
22:4n-6	1.99 ± 0.03	2.26 ± 0.04	2.08 ± 0.07	1.83 ± 0.07	2.11 ± 0.04	2.16 ± 0.03	1.85 ± 0.02	2.11 ± 0.07	2.08 ± 0.04	1.77 ± 0.03	1.94 ± 0.03	2.00 ± 0.06	**0.000**	**0.000**	**0.001**	**0.000**	**0.048**	NS	NS
24:1n-9	0.56 ± 0.09	0.86 ± 0.12	0.83 ± 0.11	0.42 ± 0.05	0.72 ± 0.11	0.84 ± 0.12	0.51 ± 0.07	0.92 ± 0.09	1.02 ± 0.19	0.59 ± 0.09	0.90 ± 0.17	1.04 ± 0.21	**0.000**	**0.000**	NS	0.095	NS	NS	NS
22:6n-3	17.85 ± 0.27	17.22 ± 0.27	17.36 ± 0.27	18.06 ± 0.34	17.80 ± 0.20	17.58 ± 0.24	18.46 ± 0.22	17.93 ± 0.37	16.62 ± 0.41	18.42 ± 0.25	17.79 ± 0.33	16.92 ± 0.52	**0.002**	**0.000**	NS	NS	NS	**0.024**	NS
16:0DMA	1.92 ± 0.05	1.68 ± 0.03	1.65 ± 0.06	1.93 ± 0.06	1.65 ± 0.03	1.63 ± 0.04	1.97 ± 0.04	1.69 ± 0.06	1.67 ± 0.07	1.96 ± 0.07	1.69 ± 0.03	1.63 ± 0.06	**0.000**	**0.000**	NS	NS	NS	NS	NS
18:0DMA	3.40 ± 0.17	3.51 ± 0.14	3.37 ± 0.13	3.28 ± 0.19	3.50 ± 0.12	3.38 ± 0.13	3.51 ± 0.11	3.47 ± 0.15	3.22 ± 0.15	3.42 ± 0.17	3.34 ± 0.14	3.05 ± 0.17	NS	0.085	NS	NS	NS	NS	NS
18:1n-9DMA	0.81 ± 0.04	1.01 ± 0.05	1.01 ± 0.05	0.82 ± 0.05	1.01 ± 0.05	1.05 ± 0.04	0.86 ± 0.02	1.02 ± 0.07	1.04 ± 0.08	0.82 ± 0.05	0.99 ± 0.04	1.10 ± 0.08	**0.000**	**0.000**	NS	NS	NS	NS	NS
18:1n-7DMA	0.74 ± 0.04	1.42 ± 0.08	1.58 ± 0.07	0.75 ± 0.04	1.39 ± 0.07	1.64 ± 0.07	0.77 ± 0.02	1.35 ± 0.10	1.65 ± 0.15	0.74 ± 0.05	1.32 ± 0.07	1.60 ± 0.13	**0.000**	**0.000**	NS	NS	NS	NS	NS
Total SFA	43.58 ± 0.47	41.98 ± 0.34	42.14 ± 0.62	43.63 ± 0.53	41.86 ± 0.35	41.57 ± 0.36	42.91 ± 0.29	42.37 ± 0.95	41.02 ± 0.41	43.65 ± 0.89	42.23 ± 0.36	41.98 ± 0.74	NS	**0.000**	NS	NS	NS	NS	NS
Total MUFA	18.67 ± 0.24	20.61 ± 0.28	20.78 ± 0.38	18.87 ± 0.23	20.36 ± 0.22	20.69 ± 0.19	18.95 ± 0.25	20.67 ± 0.35	22.57 ± 0.85	19.67 ± 0.49	20.68 ± 0.31	21.75 ± 0.42	**0.000**	**0.000**	NS	**0.002**	NS	NS	NS
Total n-6 PUFA	12.83 ± 0.17	12.35 ± 0.18	11.90 ± 0.20	12.42 ± 0.16	12.13 ± 0.14	12.13 ± 0.10	12.36 ± 0.11	12.04 ± 0.20	12.45 ± 0.21	12.16 ± 0.14	11.68 ± 0.19	12.01 ± 0.21	**0.001**	**0.004**	**0.017**	0.078	NS	**0.018**	NS
Total n-3 PUFA	18.04 ± 0.27	17.43 ± 0.27	17.58 ± 0.26	18.30 ± 0.31	18.10 ± 0.23	17.90 ± 0.29	18.67 ± 0.23	18.21 ± 0.40	16.93 ± 0.41	18.72 ± 0.26	18.06 ± 0.36	17.23 ± 0.54	**0.006**	**0.000**	NS	NS	NS	**0.033**	NS
n3/n6	1.41 ± 0.01	1.41 ± 0.02	1.48 ± 0.02	1.47 ± 0.02	1.49 ± 0.01	1.48 ± 0.02	1.51 ± 0.02	1.50 ± 0.01	1.36 ± 0.04	1.54 ± 0.01	1.55 ± 0.01	1.42 ± 0.03	**0.000**	**0.005**	**0.000**	**0.026**	NS	**0.000**	NS
DHA/AA	1.90 ± 0.01	1.94 ± 0.02	2.02 ± 0.03	2.00 ± 0.02	2.06 ± 0.01	2.02 ± 0.02	2.05 ± 0.03	1.99 ± 0.07	1.87 ± 0.08	2.14 ± 0.02	2.15 ± 0.02	1.95 ± 0.03	**0.000**	**0.010**	**0.000**	**0.034**	0.081	**0.000**	NS
Total DMA	6.88 ± 0.29	7.62 ± 0.27	7.60 ± 0.28	6.78 ± 0.32	7.55 ± 0.26	7.71 ± 0.25	7.10 ± 0.18	7.52 ± 0.35	7.59 ± 0.39	6.95 ± 0.31	7.34 ± 0.26	7.38 ± 0.42	NS	**0.002**	NS	NS	NS	NS	NS

Hippocampus	APOE3						APOE4																	
	Female			Male			Female			Male														
Compound (%TL)	APOE3F 2Mo	APOE3F 9Mo	APOE3F 18Mo	APOE3M 2Mo	APOE3M 9Mo	APOE3M 18Mo	APOE4F 2Mo	APOE4F 9Mo	APOE4F 18Mo	APOE4M 2Mo	APOE4 M 9 Mo	APOE4M 18Mo	P-group	P-age	P-sex	P-apoe	P-age:sex	P-age:apoe	P-sex:apoe
14:0	0.72 ± 0.21	1.38 ± 0.89	1.79 ± 1.30	0.93 ± 0.50	0.79 ± 0.31	0.57 ± 0.20	0.76 ± 0.22	0.67 ± 0.27	0.60 ± 0.10	1.04 ± 0.40	0.68 ± 0.16	0.71 ± 0.18	NS	NS	NS	NS	NS	NS	NS
15:0	0.51 ± 0.18	0.42 ± 0.12	0.36 ± 0.11	0.28 ± 0.08	0.53 ± 0.21	0.46 ± 0.24	0.62 ± 0.23	0.50 ± 0.19	0.45 ± 0.13	0.70 ± 0.32	0.48 ± 0.15	0.47 ± 0.16	NS	NS	NS	NS	NS	NS	NS
16:0	21.67 ± 0.52	21.38 ± 0.62	21.04 ± 0.58	21.60 ± 0.34	21.25 ± 0.44	21.04 ± 0.50	21.95 ± 0.27	21.50 ± 1.05	21.61 ± 0.75	23.11 ± 1.65	21.46 ± 0.67	21.79 ± 0.49	NS	NS	NS	NS	NS	NS	NS
16:1n-7	0.55 ± 0.04	0.51 ± 0.02	0.56 ± 0.04	0.50 ± 0.03	0.62 ± 0.09	0.54 ± 0.03	0.62 ± 0.10	0.52 ± 0.02	0.64 ± 0.05	0.58 ± 0.06	0.57 ± 0.03	0.69 ± 0.05	0.063	0.080	NS	**0.016**	NS	NS	NS
16:1n-9	1.67 ± 0.59	1.30 ± 0.40	1.19 ± 0.42	0.97 ± 0.29	1.72 ± 0.76	1.53 ± 0.81	2.04 ± 0.91	1.62 ± 0.61	1.58 ± 0.54	2.35 ± 1.13	1.55 ± 0.54	1.56 ± 0.58	NS	NS	NS	NS	NS	NS	NS
16:1	2.23 ± 0.62	1.81 ± 0.42	1.75 ± 0.45	1.46 ± 0.31	2.34 ± 0.85	2.07 ± 0.83	2.66 ± 0.91	2.14 ± 0.62	2.22 ± 0.56	2.93 ± 1.18	2.11 ± 0.56	2.25 ± 0.61	NS	NS	NS	NS	NS	NS	NS
18:0	19.50 ± 0.40	19.44 ± 0.40	19.04 ± 0.57	19.74 ± 0.28	19.04 ± 0.55	19.54 ± 0.56	19.00 ± 0.59	19.29 ± 0.50	18.61 ± 0.53	18.29 ± 1.02	18.90 ± 0.47	18.47 ± 0.39	NS	NS	NS	**0.046**	NS	NS	NS
18:1n-7	2.81 ± 0.07	2.76 ± 0.07	2.79 ± 0.08	2.96 ± 0.03	2.83 ± 0.03	2.85 ± 0.09	2.84 ± 0.13	2.73 ± 0.09	2.98 ± 0.07	2.66 ± 0.15	2.73 ± 0.05	2.91 ± 0.05	NS	0.070	NS	NS	NS	NS	**0.049**
18:1n-9	13.70 ± 0.37	14.12 ± 0.38	14.67 ± 0.84	13.22 ± 0.16	14.84 ± 0.48	14.43 ± 0.21	13.90 ± 0.36	14.51 ± 0.25	16.26 ± 1.11	15.48 ± 1.07	14.98 ± 0.60	15.54 ± 0.46	**0.005**	**0.003**	NS	**0.002**	NS	NS	NS
18:1	16.51 ± 0.38	16.87 ± 0.41	17.46 ± 0.84	16.18 ± 0.15	17.67 ± 0.45	17.28 ± 0.21	16.74 ± 0.32	17.24 ± 0.22	19.24 ± 1.09	18.14 ± 0.93	17.71 ± 0.57	18.44 ± 0.43	**0.006**	**0.004**	NS	**0.002**	NS	NS	NS
18:2n-6	0.94 ± 0.11	0.63 ± 0.04	0.86 ± 0.28	0.78 ± 0.05	0.79 ± 0.06	0.65 ± 0.05	0.87 ± 0.11	0.90 ± 0.14	0.93 ± 0.12	1.36 ± 0.29	1.01 ± 0.14	0.95 ± 0.12	**0.017**	0.052	NS	**0.002**	NS	NS	NS
20:1n-9	0.74 ± 0.08	0.95 ± 0.06	0.91 ± 0.06	0.70 ± 0.04	0.94 ± 0.07	0.88 ± 0.06	0.77 ± 0.07	0.83 ± 0.07	0.93 ± 0.08	0.76 ± 0.03	0.83 ± 0.03	0.90 ± 0.04	**0.032**	**0.000**	NS	NS	NS	NS	NS
20:3n-6	0.47 ± 0.02	0.39 ± 0.02	0.36 ± 0.02	0.52 ± 0.01	0.44 ± 0.02	0.40 ± 0.02	0.42 ± 0.02	0.38 ± 0.03	0.45 ± 0.04	0.47 ± 0.04	0.45 ± 0.02	0.48 ± 0.02	**0.000**	**0.002**	**0.001**	NS	NS	**0.000**	NS
20:4n-6	10.40 ± 0.34	10.03 ± 0.32	9.79 ± 0.43	10.73 ± 0.24	9.77 ± 0.50	10.18 ± 0.41	9.98 ± 0.42	9.94 ± 0.51	9.79 ± 0.39	9.10 ± 0.82	9.58 ± 0.39	9.84 ± 0.37	NS	NS	NS	NS	NS	NS	NS
22:0	0.44 ± 0.04	0.47 ± 0.04	0.43 ± 0.02	0.45 ± 0.04	0.44 ± 0.02	0.49 ± 0.05	0.47 ± 0.03	0.40 ± 0.02	0.50 ± 0.04	0.50 ± 0.07	0.41 ± 0.03	0.44 ± 0.03	NS	NS	NS	NS	NS	NS	NS
22:4n-6	2.26 ± 0.09	2.52 ± 0.08	2.39 ± 0.14	2.27 ± 0.06	2.32 ± 0.13	2.44 ± 0.10	2.20 ± 0.08	2.44 ± 0.14	2.35 ± 0.11	2.03 ± 0.19	2.28 ± 0.10	2.28 ± 0.09	NS	**0.017**	NS	NS	NS	NS	NS
24:0	0.47 ± 0.05	0.35 ± 0.04	0.33 ± 0.03	0.39 ± 0.02	0.38 ± 0.05	0.38 ± 0.07	0.51 ± 0.07	0.37 ± 0.05	0.44 ± 0.07	0.54 ± 0.10	0.38 ± 0.04	0.38 ± 0.05	NS	**0.003**	NS	NS	NS	NS	NS
24:1n-9	0.67 ± 0.12	0.85 ± 0.17	0.91 ± 0.13	0.51 ± 0.07	0.71 ± 0.12	0.74 ± 0.13	0.53 ± 0.09	0.89 ± 0.23	1.05 ± 0.20	0.69 ± 0.12	0.86 ± 0.13	0.76 ± 0.11	NS	**0.003**	NS	NS	NS	NS	NS
22:6n-3	14.78 ± 0.44	14.34 ± 0.60	14.55 ± 0.55	15.28 ± 0.42	14.47 ± 0.73	14.73 ± 0.63	14.50 ± 0.65	14.46 ± 1.00	12.67 ± 1.03	12.90 ± 1.84	14.83 ± 0.51	13.75 ± 0.69	NS	NS	NS	NS	NS	NS	NS
16:0DMA	1.75 ± 0.09	1.64 ± 0.09	1.61 ± 0.11	1.89 ± 0.08	1.59 ± 0.11	1.60 ± 0.07	1.84 ± 0.11	1.63 ± 0.09	1.72 ± 0.08	1.68 ± 0.19	1.59 ± 0.14	1.72 ± 0.10	NS	**0.011**	NS	NS	NS	NS	NS
18:0DMA	3.33 ± 0.16	3.34 ± 0.19	3.19 ± 0.20	3.42 ± 0.20	3.30 ± 0.16	3.19 ± 0.13	3.45 ± 0.15	3.33 ± 0.16	2.89 ± 0.21	3.22 ± 0.27	3.27 ± 0.17	2.85 ± 0.18	NS	**0.015**	NS	NS	NS	NS	NS
18:1n-9DMA	0.77 ± 0.05	0.95 ± 0.07	0.93 ± 0.08	0.86 ± 0.05	0.95 ± 0.06	0.97 ± 0.05	0.83 ± 0.06	0.96 ± 0.07	0.95 ± 0.10	0.75 ± 0.11	0.95 ± 0.07	1.00 ± 0.08	NS	**0.003**	NS	NS	NS	NS	NS
18:1n-7DMA	0.68 ± 0.05	1.28 ± 0.11	1.39 ± 0.14	0.76 ± 0.04	1.22 ± 0.08	1.39 ± 0.08	0.72 ± 0.05	1.21 ± 0.10	1.53 ± 0.15	0.66 ± 0.09	1.21 ± 0.09	1.48 ± 0.12	**0.000**	**0.000**	NS	NS	NS	NS	NS
Total SFA	43.61 ± 0.63	42.44 ± 0.20	41.71 ± 0.32	43.02 ± 0.24	42.71 ± 0.46	42.77 ± 0.54	43.63 ± 0.31	41.97 ± 0.42	42.52 ± 0.60	42.87 ± 0.51	42.58 ± 0.58	42.55 ± 0.63	NS	**0.017**	NS	NS	NS	NS	NS
Total MUFA	20.31 ± 0.80	20.65 ± 0.59	21.17 ± 1.12	19.00 ± 0.39	21.84 ± 1.30	21.12 ± 0.86	20.86 ± 1.12	21.24 ± 0.89	23.58 ± 1.36	22.69 ± 2.05	21.68 ± 0.78	22.49 ± 0.92	0.080	**0.025**	NS	**0.013**	NS	NS	NS
Total n-6 PUFA	14.41 ± 0.49	13.75 ± 0.41	13.60 ± 0.47	14.76 ± 0.20	13.53 ± 0.64	13.87 ± 0.50	13.81 ± 0.42	13.83 ± 0.61	13.73 ± 0.44	13.22 ± 0.83	13.48 ± 0.46	13.74 ± 0.45	NS	NS	NS	NS	NS	NS	NS
Total n-3 PUFA	15.14 ± 0.44	14.63 ± 0.60	14.84 ± 0.54	15.63 ± 0.42	14.87 ± 0.74	15.09 ± 0.65	14.86 ± 0.60	14.77 ± 1.01	13.07 ± 1.03	13.27 ± 1.87	15.22 ± 0.54	14.17 ± 0.68	NS	NS	NS	NS	NS	NS	NS
n3/n6	1.05 ± 0.01	1.06 ± 0.02	1.09 ± 0.02	1.06 ± 0.02	1.10 ± 0.02	1.09 ± 0.01	1.07 ± 0.02	1.06 ± 0.04	0.94 ± 0.05	0.98 ± 0.11	1.13 ± 0.01	1.03 ± 0.02	**0.010**	**0.041**	NS	NS	NS	**0.010**	NS
DHA/AA	1.42 ± 0.01	1.43 ± 0.02	1.49 ± 0.02	1.42 ± 0.02	1.48 ± 0.02	1.45 ± 0.01	1.45 ± 0.01	1.44 ± 0.04	1.34 ± 0.05	1.52 ± 0.00	1.55 ± 0.02	1.39 ± 0.02	**0.001**	**0.002**	**0.003**	NS	**0.032**	**0.000**	**0.008**
Total DMA	6.53 ± 0.34	7.22 ± 0.44	7.12 ± 0.50	6.93 ± 0.37	7.05 ± 0.40	7.15 ± 0.31	6.84 ± 0.33	7.14 ± 0.42	7.10 ± 0.49	6.31 ± 0.65	7.03 ± 0.45	7.05 ± 0.46	NS	NS	NS	NS	NS	NS	NS

Results are presented as means ± SEM (n=6-7 per group). NS, non-significant. F, female, M, male, Mo, months, SFA, saturated fatty acid, MUFA, monounsaturated fatty acid, PUFA, polyunsaturated fatty acid, DHA, docosahexaenoic acid, AA, arachidonic acid and DMA, dimethyl acetal fatty acid. P-values are shown for age, sex, genotype, two-way interactions and at a group level (comparing each group with each other). ANOVAs (1 way and 2 ways) were used and non-parametric equivalent tests (e.g. Kruskal-Wallis) were used where the data were not compliant with ANOVA requirements, followed by Tukey Honest Significant Differences with $p<0.05$ indicating significant differences.

Supplementary data to:

Altered SPMs and age-associated decrease in brain DHA in APOE4 female mice

Anneloes Martinsen[1], Noemi Tejera[1], David Vauzour[1], Glenn Harden[1], James Dick[2], Sujata Shinde[3], Anne Barden[3], Trevor A Mori[3], Anne Marie Minihane[1]

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Supplemental data 4: Detailed fatty acid composition in µg/mg of total lipid in cortex and hippocampus of female and male 2-, 9- and 18-month of age *APOE3* and *APOE4* mice fed on chow diet (n=6-7 per group).

Cortex	APOE3									APOE4														
	Female			Male						Female						Male								
Compound (µg/mg TL)	APOE3F2Mo	APOE3F9Mo	APOE3F18Mo	APOE3M2Mo	APOE3M9Mo	APOE3M18Mo	APOE4F2Mo	APOE4F 9 Mo	APOE4F18Mo	APOE4M2Mo	APOE4M9Mo	APOE4M18Mo	P-group	P-age	P-sex	P-apoe	P-age:sex	P-age:apoe	P-sex:apoe
14:0	2.27 ± 0.98	1.54 ± 0.52	2.29 ± 1.15	3.12 ± 1.86	1.15 ± 0.21	0.95 ± 0.11	1.40 ± 0.20	1.05 ± 0.23	1.27 ± 0.23	1.69 ± 0.25	1.32 ± 0.22	2.66 ± 1.44	NS	NS	NS	NS	NS	NS	NS
16:0	87.46 ± 2.92	81.99 ± 2.57	80.20 ± 3.07	91.34 ± 2.85	85.06 ± 2.61	79.57 ± 2.33	92.71 ± 3.76	88.24 ± 3.57	82.57 ± 4.46	93.02 ± 2.68	87.87 ± 3.54	83.07 ± 3.18	**0.019**	**0.000**	NS	NS	NS	NS	NS
16:1n-7	2.05 ± 0.13	2.04 ± 0.10	2.13 ± 0.11	2.27 ± 0.15	2.21 ± 0.09	2.16 ± 0.10	2.14 ± 0.11	2.20 ± 0.09	2.56 ± 0.14	2.35 ± 0.10	2.48 ± 0.12	2.52 ± 0.11	**0.014**	NS	**0.026**	**0.001**	NS	NS	NS
16:1n-9	2.61 ± 0.68	2.47 ± 0.64	2.55 ± 0.69	2.41 ± 0.52	2.35 ± 0.47	1.86 ± 0.32	3.01 ± 0.64	2.16 ± 0.49	2.63 ± 0.50	2.88 ± 0.67	2.61 ± 0.53	2.62 ± 0.57	NS	NS	NS	NS	NS	NS	NS
16:1	4.66 ± 0.68	4.51 ± 0.68	4.68 ± 0.75	4.68 ± 0.62	4.57 ± 0.46	4.02 ± 0.31	5.14 ± 0.58	3.74 ± 0.75	5.18 ± 0.54	5.23 ± 0.64	5.09 ± 0.51	5.13 ± 0.54	NS	NS	NS	NS	NS	NS	NS
18:0	80.78 ± 3.48	78.55 ± 2.54	75.64 ± 3.28	81.72 ± 3.65	80.54 ± 2.47	75.98 ± 2.45	83.69 ± 3.47	82.05 ± 1.96	75.56 ± 3.05	80.44 ± 2.36	78.42 ± 2.37	74.36 ± 2.74	NS	**0.008**	NS	NS	NS	NS	NS
18:1n-7	12.53 ± 0.46	12.45 ± 0.37	12.31 ± 0.51	13.47 ± 0.49	12.98 ± 0.39	12.64 ± 0.40	13.33 ± 0.47	13.19 ± 0.22	13.12 ± 0.46	12.91 ± 0.38	12.81 ± 0.36	12.72 ± 0.44	NS	NS	NS	NS	NS	NS	**0.044**
18:1n-9	51.90 ± 2.03	56.18 ± 2.01	54.99 ± 2.27	54.49 ± 1.95	57.57 ± 1.57	55.08 ± 1.80	56.13 ± 1.82	59.26 ± 1.35	60.57 ± 2.71	56.83 ± 1.60	58.14 ± 1.83	58.70 ± 1.81	NS	NS	NS	**0.004**	NS	NS	NS
18:1	64.43 ± 2.46	68.63 ± 2.35	67.30 ± 2.75	67.96 ± 2.43	70.54 ± 1.94	67.72 ± 2.18	69.46 ± 2.26	62.09 ± 10.43	73.69 ± 3.07	69.74 ± 1.85	70.95 ± 2.14	71.42 ± 2.08	NS	NS	NS	**0.009**	NS	NS	NS
18:2n-6	2.62 ± 0.17	2.27 ± 0.10	2.33 ± 0.15	2.91 ± 0.20	2.74 ± 0.15	2.40 ± 0.14	3.11 ± 0.23	2.89 ± 0.47	3.14 ± 0.43	4.11 ± 0.52	3.12 ± 0.34	3.33 ± 0.49	**0.003**	**0.017**	**0.017**	**0.000**	NS	NS	NS
20:1n-9	3.18 ± 0.23	4.56 ± 0.21	4.27 ± 0.25	3.31 ± 0.16	4.41 ± 0.28	4.23 ± 0.20	3.17 ± 0.07	4.33 ± 0.19	4.28 ± 0.24	3.31 ± 0.12	3.85 ± 0.20	3.90 ± 0.23	**0.000**	**0.000**	NS	NS	NS	NS	NS
20:2n-6	0.56 ± 0.02	0.37 ± 0.01	0.38 ± 0.02	0.59 ± 0.04	0.49 ± 0.04	0.38 ± 0.02	0.48 ± 0.09	0.45 ± 0.03	0.39 ± 0.03	0.60 ± 0.03	0.43 ± 0.04	0.37 ± 0.07	**0.000**	**0.000**	NS	NS	NS	NS	NS
20:4n-6	37.63 ± 1.37	35.06 ± 0.96	33.14 ± 1.66	37.51 ± 1.48	35.24 ± 1.17	33.58 ± 1.20	38.41 ± 1.69	35.54 ± 1.24	34.62 ± 1.54	35.49 ± 1.27	33.63 ± 0.99	33.42 ± 1.56	NS	**0.001**	NS	NS	NS	NS	NS
22:4n-6	7.99 ± 0.29	8.94 ± 0.30	8.05 ± 0.51	7.63 ± 0.44	8.61 ± 0.35	8.32 ± 0.32	7.87 ± 0.29	8.70 ± 0.34	8.11 ± 0.37	7.34 ± 0.28	7.89 ± 0.21	7.82 ± 0.32	NS	**0.005**	NS	NS	NS	NS	NS
24:1n-9	2.32 ± 0.44	3.45 ± 0.55	3.22 ± 0.52	1.77 ± 0.26	2.98 ± 0.52	3.32 ± 0.59	2.21 ± 0.35	3.84 ± 0.44	4.02 ± 0.79	2.43 ± 0.36	3.70 ± 0.74	4.16 ± 0.93	**0.004**	**0.000**	NS	NS	NS	NS	NS
22:6n-3	71.72 ± 2.95	68.01 ± 1.86	66.84 ± 2.99	75.03 ± 3.44	72.71 ± 2.66	67.84 ± 2.62	78.55 ± 3.05	71.01 ± 4.05	58.21 ± 7.09	71.89 ± 5.74	72.26 ± 2.43	65.17 ± 3.61	**0.050**	**0.001**	NS	NS	NS	NS	NS
16:0DMA	7.75 ± 0.41	6.66 ± 0.22	6.40 ± 0.45	8.02 ± 0.45	6.75 ± 0.29	6.29 ± 0.24	8.42 ± 0.41	6.98 ± 0.35	6.52 ± 0.37	8.15 ± 0.45	6.87 ± 0.32	6.40 ± 0.33	**0.000**	**0.000**	NS	NS	NS	NS	NS
18:0DMA	13.76 ± 1.05	13.90 ± 0.76	13.00 ± 0.86	13.72 ± 1.11	14.31 ± 0.72	13.05 ± 0.68	14.92 ± 0.67	14.39 ± 0.85	12.56 ± 0.82	14.25 ± 0.98	13.63 ± 0.79	11.95 ± 0.77	NS	**0.009**	NS	NS	NS	NS	NS
18:1n-9DMA	3.26 ± 0.24	4.00 ± 0.25	3.90 ± 0.28	3.42 ± 0.28	4.15 ± 0.28	4.07 ± 0.23	3.65 ± 0.20	4.21 ± 0.31	4.04 ± 0.34	3.43 ± 0.27	4.03 ± 0.24	4.28 ± 0.32	NS	**0.001**	NS	NS	NS	NS	NS
18:1n-7DMA	3.01 ± 0.25	5.63 ± 0.37	6.09 ± 0.42	3.11 ± 0.24	5.68 ± 0.39	6.34 ± 0.39	3.26 ± 0.18	5.60 ± 0.44	6.42 ± 0.60	3.09 ± 0.26	5.40 ± 0.34	6.27 ± 0.47	**0.000**	NS	NS	NS	NS	NS	NS
Total SFA	174.97 ± 6.41	166.11 ± 5.41	162.02 ± 6.19	180.75 ± 5.77	170.73 ± 4.81	160.17 ± 4.90	182.70 ± 7.41	175.28 ± 5.47	163.90 ± 7.40	180.16 ± 4.47	171.68 ± 5.73	164.25 ± 5.37	NS	**0.000**	NS	NS	NS	NS	NS
Total MUFA	75.04 ± 3.08	81.65 ± 3.19	79.91 ± 3.33	78.24 ± 2.74	83.01 ± 2.30	79.79 ± 2.88	80.49 ± 2.42	85.46 ± 1.95	87.64 ± 4.04	81.13 ± 1.97	84.03 ± 2.80	85.04 ± 2.53	NS	**0.031**	NS	**0.007**	NS	NS	NS
Total n-6 PUFA	51.52 ± 1.90	48.82 ± 1.43	45.85 ± 2.29	51.51 ± 2.00	49.52 ± 1.72	46.80 ± 1.68	52.66 ± 2.28	49.85 ± 1.72	48.46 ± 2.05	50.35 ± 1.74	47.43 ± 1.40	47.13 ± 2.16	NS	**0.005**	NS	NS	NS	NS	NS
Total n-3 PUFA	72.47 ± 3.01	68.88 ± 1.95	67.68 ± 3.01	76.02 ± 3.40	73.92 ± 2.69	69.05 ± 2.72	79.45 ± 3.14	72.10 ± 4.19	59.29 ± 7.19	73.01 ± 5.86	73.35 ± 2.55	66.30 ± 3.74	NS	**0.002**	NS	NS	NS	NS	NS
n3/n6	1.41 ± 0.01	1.41 ± 0.02	1.48 ± 0.02	1.47 ± 0.02	1.49 ± 0.01	1.48 ± 0.02	1.51 ± 0.02	1.44 ± 0.05	1.23 ± 0.14	1.45 ± 0.10	1.55 ± 0.01	1.40 ± 0.03	**0.010**	**0.048**	**0.016**	NS	NS	**0.001**	NS
DHA/AA	1.90 ± 0.01	1.94 ± 0.02	2.02 ± 0.03	2.00 ± 0.02	2.06 ± 0.01	2.02 ± 0.02	2.05 ± 0.03	1.99 ± 0.07	1.68 ± 0.19	2.02 ± 0.12	2.15 ± 0.02	1.95 ± 0.03	**0.001**	**0.030**	**0.004**	NS	NS	**0.001**	NS
Total DMA	27.77 ± 1.92	30.20 ± 1.54	29.39 ± 1.96	28.28 ± 2.04	30.88 ± 1.60	29.75 ± 1.49	30.25 ± 1.40	31.19 ± 1.85	29.54 ± 1.95	28.92 ± 1.91	29.92 ± 1.63	28.89 ± 1.78	NS	NS	NS	NS	NS	NS	NS

Hippocampus	APOE3									APOE4																	
	Female						Male			Female						Male											
Compound (µg/mg TL)	APOE3 F 2 Mo		APOE3 F 9 Mo		APOE3 F 18 Mo		APOE3 M 2 Mo		APOE3 M 9 Mo		APOE3 M 18 Mo		APOE4 F 2 Mo		APOE4 F 9 Mo		APOE4 F 18 Mo		APOE4 M 2 Mo		APOE4 M 9 Mo		APOE4 M 18 Mo		P-group	P-age	P-sex	P-apoe	P-age:sex	P-age:apoe	P-sex:apoe
14:0	2.51	0.70	4.52	2.73	5.88	4.13	3.25	1.67	3.12	1.27	1.95	0.59	3.18	0.87	2.35	0.93	2.23	0.39	3.42	1.14	2.36	0.51	2.48	0.55	NS	NS	NS	NS	NS	NS	NS
15:0	1.76	0.62	1.49	0.43	1.28	0.38	1.03	0.30	2.08	0.85	1.53	0.73	2.56	0.91	1.70	0.65	1.59	0.40	2.27	0.94	1.64	0.49	1.64	0.48	NS	NS	NS	NS	NS	NS	NS
16:0	76.96	3.34	74.52	3.53	74.52	2.50	80.16	5.22	83.18	2.71	78.49	3.65	92.51	4.88	79.32	5.62	81.63	5.67	84.38	3.95	77.17	3.34	79.59	4.55	NS	NS	NS	NS	NS	NS	NS
16:1n-7	1.97	0.15	1.78	0.12	1.98	0.14	1.83	0.13	2.44	0.39	1.99	0.09	2.62	0.42	1.90	0.11	2.40	0.17	2.11	0.15	2.02	0.07	2.51	0.22	**0.042**	NS	NS	**0.012**	**0.048**	NS	NS
16:1n-9	5.77	2.00	4.58	1.42	4.19	1.49	3.50	1.02	6.75	3.09	5.05	2.42	8.39	3.63	5.43	2.00	5.55	1.61	7.64	3.30	5.26	1.71	5.36	1.78	NS	NS	NS	NS	NS	NS	NS
16:1	7.74	2.07	6.35	1.48	6.17	1.59	5.33	1.08	9.19	3.44	7.04	2.42	11.01	3.63	7.33	1.99	7.96	1.63	9.75	3.37	7.29	1.70	7.86	1.83	NS	NS	NS	NS	NS	NS	NS
18:0	69.59	4.26	67.99	3.80	67.79	3.75	73.63	5.67	74.63	3.18	73.62	5.40	80.25	5.37	71.63	5.40	70.12	3.68	69.12	7.19	68.28	3.74	67.48	3.77	NS	NS	NS	NS	NS	NS	**0.044**
18:1n-7	10.02	0.67	9.63	0.53	9.94	0.50	11.03	0.83	11.09	0.38	10.71	0.71	12.03	0.93	10.13	0.78	11.20	0.51	10.08	1.09	9.86	0.50	10.65	0.66	NS	NS	NS	NS	NS	NS	**0.016**
18:1n-9	49.18	3.92	49.49	3.14	52.20	3.64	49.32	3.70	58.10	2.46	53.84	2.44	58.50	3.12	53.59	3.17	61.85	6.77	56.50	2.57	54.23	3.72	56.96	3.83	NS	NS	NS	**0.020**	NS	NS	NS
18:1	59.21	4.53	59.12	3.61	62.14	3.93	60.35	4.50	69.19	2.60	64.55	3.10	70.52	3.78	63.72	3.86	73.05	7.08	66.58	3.00	64.09	4.01	67.61	4.37	NS	NS	NS	**0.033**	NS	NS	NS
18:2n-6	3.49	0.64	2.21	0.20	3.07	0.97	2.87	0.24	3.06	0.19	2.42	0.14	3.60	0.43	3.31	0.53	3.53	0.54	4.72	0.70	3.64	0.54	3.55	0.57	**0.009**	**0.034**	NS	**0.001**	NS	NS	NS
20:1n-9	2.71	0.46	3.37	0.32	3.25	0.27	2.65	0.28	3.73	0.37	3.32	0.33	3.26	0.35	3.11	0.37	3.54	0.36	2.85	0.26	3.02	0.21	3.31	0.28	NS	NS	NS	NS	NS	NS	NS
20:3n-6	1.68	0.12	1.36	0.08	1.26	0.08	1.93	0.12	1.74	0.08	1.48	0.12	1.79	0.14	1.39	0.11	1.67	0.13	1.79	0.24	1.61	0.11	1.78	0.18	**0.016**	**0.010**	**0.015**	NS	NS	NS	NS
20:4n-6	37.08	2.33	35.05	2.05	34.88	2.32	39.98	3.14	38.23	2.23	38.53	3.31	42.08	2.80	37.06	3.54	36.77	1.93	34.82	4.64	34.67	2.34	36.16	2.77	NS	NS	NS	NS	NS	NS	**0.046**
22:0	1.54	0.10	1.64	0.12	1.51	0.06	1.62	0.10	1.73	0.10	1.81	0.20	1.97	0.17	1.47	0.09	1.85	0.12	1.80	0.15	1.48	0.07	1.60	0.11	NS	NS	NS	NS	NS	**0.018**	NS
22:4n-6	8.09	0.63	8.83	0.58	8.52	0.67	8.50	0.75	9.09	0.60	9.23	0.83	9.31	0.64	9.10	0.91	8.85	0.55	7.79	1.06	8.26	0.57	8.37	0.64	NS	NS	NS	NS	NS	NS	NS
24:0	1.67	0.20	1.25	0.15	1.18	0.11	1.44	0.12	1.51	0.23	1.38	0.19	2.13	0.28	1.30	0.15	1.60	0.20	1.89	0.24	1.35	0.10	1.36	0.13	**0.022**	**0.001**	NS	NS	NS	NS	NS
24:1n-9	2.45	0.51	3.14	0.81	3.25	0.53	1.93	0.30	2.84	0.53	2.86	0.57	2.24	0.39	3.34	0.84	4.12	0.94	2.53	0.41	3.15	0.53	2.74	0.41	NS	**0.026**	NS	NS	NS	NS	NS
22:6n-3	52.66	3.24	50.10	3.10	51.75	2.96	57.00	4.60	56.62	3.13	55.79	4.96	61.01	3.91	53.84	5.64	47.45	4.16	50.17	8.77	53.65	3.45	50.52	4.07	NS	NS	NS	NS	NS	NS	NS
16:0DMA	6.28	0.54	5.77	0.45	5.76	0.51	7.11	0.71	6.20	0.46	6.09	0.59	7.76	0.63	6.13	0.65	6.49	0.44	6.46	0.99	5.82	0.63	6.36	0.61	NS	NS	NS	NS	NS	NS	NS
18:0DMA	11.98	1.10	11.75	0.98	11.38	0.91	12.89	1.43	12.90	0.69	12.06	1.05	14.45	0.67	12.44	1.22	10.95	1.00	12.26	1.59	11.86	0.92	10.50	0.97	NS	NS	NS	NS	NS	NS	NS
18:1n-9DMA	2.77	0.30	3.35	0.34	3.31	0.34	3.23	0.35	3.72	0.29	3.67	0.36	3.49	0.28	3.60	0.44	3.61	0.44	2.92	0.53	3.47	0.33	3.71	0.44	NS	NS	NS	NS	NS	NS	NS
18:1n-7DMA	2.46	0.28	4.52	0.48	4.97	0.55	2.85	0.32	4.78	0.37	5.28	0.54	3.03	0.21	4.58	0.58	5.81	0.65	2.55	0.46	4.41	0.41	5.50	0.66	**0.000**	**0.000**	NS	NS	NS	NS	NS
Total SFA	155.11	7.13	152.43	7.26	153.17	5.75	162.22	10.77	167.35	5.21	159.84	7.93	183.92	9.79	158.84	9.72	160.23	8.14	164.06	7.79	153.24	5.41	155.24	7.62	NS	NS	NS	NS	NS	NS	**0.039**
Total MUFA	72.69	5.54	72.54	5.06	75.30	5.06	70.79	5.17	85.63	6.03	78.32	2.73	87.70	5.80	78.03	4.54	89.18	8.07	82.38	4.43	78.10	4.13	82.01	4.75	NS	NS	NS	**0.017**	NS	NS	NS
Total n-6 PUFA	51.53	3.78	48.09	2.86	48.45	2.90	54.96	4.07	52.98	2.90	52.42	4.37	58.16	3.38	51.53	4.62	51.55	2.40	50.11	5.52	48.79	3.11	50.57	3.94	NS	NS	NS	NS	NS	NS	NS
Total n-3 PUFA	53.98	3.36	51.13	3.15	52.79	2.97	58.28	4.68	58.18	3.24	57.11	5.03	62.56	3.78	54.94	5.67	48.89	4.10	51.59	8.93	55.04	3.54	52.07	4.19	NS	NS	NS	NS	NS	NS	NS
n3/n6	1.05	0.01	1.06	0.02	1.09	0.02	1.06	0.02	1.10	0.02	1.09	0.01	1.07	0.02	1.06	0.04	0.94	0.05	0.98	0.11	1.13	0.01	1.03	0.02	**0.010**	**0.041**	NS	NS	NS	**0.010**	NS
DHA/AA	1.42	0.01	1.43	0.02	1.49	0.02	1.42	0.02	1.48	0.02	1.45	0.01	1.45	0.01	1.45	0.04	1.28	0.07	1.39	0.13	1.55	0.02	1.39	0.02	**0.001**	**0.013**	NS	NS	NS	**0.002**	NS
Total DMA	23.48	2.19	25.38	2.20	25.42	2.25	26.09	2.80	27.60	1.76	27.10	2.50	28.73	1.72	26.75	2.86	26.86	2.38	24.19	3.55	25.57	2.25	26.07	2.66	NS	NS	NS	NS	NS	NS	NS

Results are presented as means ± SEM (n=6-7 per group). NS, non-significant. F, female, M, male, Mo, months, SFA, saturated fatty acid, MUFA, monounsaturated fatty acid, PUFA, polyunsaturated fatty acid, DHA, docosahexaenoic acid, AA, arachidonic acid and DMA, dimethyl acetal fatty acid. P-values are shown for age, sex, genotype, two-way interactions and at a group level (comparing each group with each other). ANOVAs (1 way and 2 ways) were used and non-parametric equivalent tests (e.g. Kruskal-Wallis) were used where the data were not compliant with ANOVA requirements, followed by Tukey Honest Significant Differences with $p<0.05$ indicating significant differences.

Supplementary data to:

Altered SPMs and age-associated decrease in brain DHA in APOE4 female mice

Anneloes Martinsen[1], Noemi Tejera[1], David Vauzour[1], Glenn Harden[1], James Dick[2], Sujata Shinde[3], Anne Barden[3], Trevor A Mori[3], Anne Marie Minihane[1]

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Supplemental data 5: Phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylcholine (PC) composition in % of total lipid in cortex of female and male 2-, 9- and 18-month of age *APOE3* and *APOE4* mice fed on chow diet.

Cortex	APOE3				APOE4											
	Female		Male		Female		Male									
Compound (%TL)	APOE3 F 2 Mo	APOE3 F 18 Mo	APOE3 M 2 Mo	APOE3 M 18 Mo	APOE4 F 2 Mo	APOE4 F 18 Mo	APOE4 M 2 Mo	APOE4 M 18 Mo	P-group	P-age	P-sex	P-apoe	P-age:sex	P-age:apoe	P-sex:apoe
PE fraction															
16:0	7.20 ± 0.14	6.57 ± 0.53	6.70 ± 0.36	6.36 ± 0.14	6.61 ± 0.26	6.57 ± 0.39	6.66 ± 0.15	6.31 ± 0.21	NS	0.099	NS	NS	NS	NS	NS
16:1	0.35 ± 0.01	0.38 ± 0.04	0.48 ± 0.12	0.58 ± 0.08	0.33 ± 0.01	0.44 ± 0.04	0.52 ± 0.09	0.56 ± 0.10	**0.043**	0.051	**0.003**	NS	NS	NS	NS
18:0	22.52 ± 0.08	19.11 ± 0.31	21.99 ± 0.27	20.49 ± 0.30	21.29 ± 0.26	18.91 ± 0.27	21.38 ± 0.12	20.47 ± 0.36	**0.000**	**0.000**	**0.006**	**0.005**	**0.000**	**0.017**	NS
18:1n-7	1.89 ± 0.06	2.27 ± 0.10	1.88 ± 0.17	2.31 ± 0.10	1.69 ± 0.08	2.24 ± 0.06	1.71 ± 0.05	2.18 ± 0.11	**0.000**	**0.000**	NS	0.062	NS	NS	NS
18:1n-9	7.96 ± 0.18	9.27 ± 0.29	8.44 ± 0.76	9.95 ± 0.55	7.54 ± 0.27	9.87 ± 0.23	8.18 ± 0.28	9.96 ± 0.46	**0.000**	**0.000**	NS	NS	NS	NS	NS
18:1	9.85 ± 0.23	11.54 ± 0.38	10.31 ± 0.92	12.26 ± 0.64	9.22 ± 0.34	12.10 ± 0.27	9.89 ± 0.32	12.14 ± 0.57	**0.000**	**0.000**	NS	NS	NS	NS	NS
18:2n-6	0.41 ± 0.07	0.32 ± 0.05	0.43 ± 0.14	0.38 ± 0.09	0.33 ± 0.04	0.32 ± 0.03	0.49 ± 0.08	0.38 ± 0.07	NS	NS	NS	NS	NS	NS	NS
20:1n-9	1.12 ± 0.08	1.83 ± 0.09	1.12 ± 0.05	1.99 ± 0.08	0.94 ± 0.04	1.69 ± 0.20	1.07 ± 0.03	1.94 ± 0.20	**0.000**	**0.000**	0.066	**0.037**	NS	NS	NS
20:3n-6	0.42 ± 0.01	0.37 ± 0.01	0.48 ± 0.05	0.41 ± 0.04	0.42 ± 0.02	0.40 ± 0.02	0.49 ± 0.05	0.44 ± 0.03	**0.050**	**0.006**	**0.037**	NS	NS	NS	NS
20:4n-6	11.70 ± 0.10	11.11 ± 0.25	11.52 ± 0.14	10.40 ± 0.10	11.61 ± 0.07	11.14 ± 0.35	10.75 ± 0.22	10.66 ± 0.38	**0.004**	**0.002**	**0.002**	NS	NS	0.090	NS
22:4n-6	3.80 ± 0.03	3.92 ± 0.11	3.61 ± 0.08	3.96 ± 0.04	3.55 ± 0.04	3.89 ± 0.04	3.45 ± 0.02	3.95 ± 0.12	**0.000**	**0.000**	NS	**0.008**	**0.024**	**0.020**	NS
22:6n-3	27.04 ± 0.42	27.82 ± 0.55	26.96 ± 0.98	25.47 ± 0.58	30.02 ± 0.68	26.66 ± 0.29	28.32 ± 0.27	25.55 ± 0.72	**0.000**	**0.001**	**0.005**	0.080	NS	**0.005**	NS
16:0DMA	4.19 ± 0.14	3.72 ± 0.05	4.42 ± 0.17	3.69 ± 0.10	4.39 ± 0.05	3.94 ± 0.08	4.44 ± 0.17	3.63 ± 0.19	**0.000**	**0.000**	NS	NS	NS	NS	NS
18:0DMA	6.92 ± 0.17	6.81 ± 0.27	7.26 ± 0.26	7.16 ± 0.11	7.10 ± 0.22	6.83 ± 0.27	7.69 ± 0.30	6.83 ± 0.15	NS	**0.044**	0.052	NS	NS	NS	NS
18:1n-7 DMA	1.55 ± 0.06	3.43 ± 0.05	1.71 ± 0.07	3.58 ± 0.04	1.54 ± 0.05	3.78 ± 0.26	1.74 ± 0.05	3.56 ± 0.17	**0.000**	**0.000**	**0.043**	NS	**0.029**	NS	NS
18:1n-9 DMA	1.69 ± 0.04	2.21 ± 0.05	1.86 ± 0.08	2.30 ± 0.02	1.72 ± 0.04	2.39 ± 0.14	1.91 ± 0.04	2.45 ± 0.09	**0.000**	**0.000**	**0.004**	0.075	0.084	NS	NS
Total SFA	30.09 ± 0.30	25.86 ± 0.26	29.01 ± 0.24	27.14 ± 0.42	28.08 ± 0.47	25.68 ± 0.58	28.40 ± 0.14	27.14 ± 0.30	**0.000**	**0.000**	NS	**0.006**	**0.002**	**0.011**	NS
Total MUFA	11.48 ± 0.30	13.77 ± 0.45	12.06 ± 1.09	14.93 ± 0.85	10.56 ± 0.38	14.26 ± 0.44	11.59 ± 0.43	14.79 ± 0.85	**0.000**	**0.000**	NS	NS	NS	NS	NS
Total n-6 PUFA	16.77 ± 0.15	16.05 ± 0.31	16.46 ± 0.19	15.41 ± 0.14	16.33 ± 0.15	16.02 ± 0.34	15.58 ± 0.33	15.69 ± 0.58	**0.045**	**0.026**	**0.020**	NS	NS	NS	NS
Total n-3 PUFA	27.32 ± 0.42	28.16 ± 0.56	27.23 ± 0.98	25.81 ± 0.60	30.29 ± 0.69	27.11 ± 0.28	28.65 ± 0.25	25.92 ± 0.74	**0.000**	**0.001**	**0.005**	**0.046**	NS	**0.004**	NS
n3/n6	1.63 ± 0.02	1.76 ± 0.06	1.66 ± 0.07	1.68 ± 0.05	1.86 ± 0.06	1.69 ± 0.04	1.84 ± 0.05	1.66 ± 0.09	**0.047**	NS	NS	**0.041**	NS	**0.005**	NS
DHA/AA	2.31 ± 0.03	2.51 ± 0.10	2.34 ± 0.09	2.45 ± 0.07	2.59 ± 0.06	2.40 ± 0.08	2.64 ± 0.06	2.41 ± 0.12	0.062	NS	NS	0.057	NS	**0.003**	NS
Total DMA	14.34 ± 0.38	16.17 ± 0.39	15.25 ± 0.48	16.72 ± 0.16	14.75 ± 0.27	16.94 ± 0.60	15.77 ± 0.54	16.47 ± 0.37	**0.001**	**0.000**	NS	NS	NS	NS	NS
PS fraction															
16:0	3.43 ± 0.18	4.05 ± 0.65	2.65 ± 0.31	2.93 ± 0.12	3.50 ± 0.32	4.42 ± 1.29	2.94 ± 0.08	2.88 ± 0.39	NS	NS	**0.004**	NS	NS	NS	NS
18:0	42.54 ± 0.58	38.74 ± 0.99	44.55 ± 0.94	43.98 ± 0.65	41.99 ± 0.72	40.44 ± 0.89	44.07 ± 0.80	43.84 ± 0.59	**0.000**	**0.011**	**0.000**	NS	0.055	NS	NS
18:1n-7	1.03 ± 0.01	1.16 ± 0.01	0.97 ± 0.06	1.10 ± 0.07	1.01 ± 0.05	1.15 ± 0.05	0.92 ± 0.07	1.14 ± 0.08	**0.025**	**0.000**	NS	NS	NS	NS	NS
18:1n-9	10.54 ± 0.29	14.15 ± 0.29	10.62 ± 0.51	13.67 ± 1.00	10.51 ± 0.49	14.71 ± 0.30	10.16 ± 0.70	14.62 ± 1.26	**0.000**	**0.000**	NS	NS	NS	NS	NS
18:1	11.57 ± 0.31	15.30 ± 0.29	11.59 ± 0.56	14.77 ± 1.07	11.53 ± 0.53	15.86 ± 0.32	11.08 ± 0.76	15.75 ± 1.33	**0.000**	**0.000**	NS	NS	NS	NS	NS
20:1n-9	0.61 ± 0.03	0.57 ± 0.04	0.66 ± 0.05	0.63 ± 0.06	0.53 ± 0.04	0.51 ± 0.06	0.56 ± 0.04	0.60 ± 0.05	**NS**	**NS**	NS	0.070	NS	NS	NS
20:4n-6	2.11 ± 0.08	3.22 ± 0.18	2.29 ± 0.07	2.94 ± 0.06	2.05 ± 0.05	3.03 ± 0.24	2.11 ± 0.08	2.93 ± 0.35	**0.000**	**0.000**	NS	0.086	0.068	NS	NS
22:4n-6	2.00 ± 0.02	2.22 ± 0.10	1.99 ± 0.07	2.26 ± 0.14	1.75 ± 0.08	2.24 ± 0.09	1.93 ± 0.04	2.16 ± 0.04	**0.001**	**0.000**	NS	0.079	NS	NS	NS
22:5n-6	0.58 ± 0.02	0.35 ± 0.02	0.51 ± 0.03	0.28 ± 0.02	0.53 ± 0.05	0.28 ± 0.04	0.48 ± 0.02	0.24 ± 0.01	**0.000**	**0.000**	**0.017**	**0.028**	NS	NS	NS
22:6n-3	34.64 ± 0.83	32.29 ± 0.86	32.58 ± 1.24	28.29 ± 1.59	35.48 ± 1.57	30.67 ± 1.53	33.71 ± 0.45	27.89 ± 1.62	**0.002**	**0.000**	**0.007**	NS	NS	NS	NS
Total SFA	47.07 ± 0.69	43.96 ± 0.82	48.61 ± 0.84	48.30 ± 0.77	46.62 ± 1.08	45.82 ± 1.81	48.33 ± 0.80	48.07 ± 0.59	**0.038**	NS	**0.002**	NS	NS	NS	NS
Total MUFA	12.66 ± 0.40	16.64 ± 0.32	12.97 ± 0.54	16.48 ± 1.08	12.61 ± 0.67	17.01 ± 0.37	12.53 ± 0.86	17.34 ± 1.44	**0.000**	**0.000**	NS	NS	NS	NS	NS
Total n-6 PUFA	5.40 ± 0.13	6.79 ± 0.43	5.64 ± 0.08	6.64 ± 0.15	5.09 ± 0.20	6.25 ± 0.35	5.24 ± 0.11	6.35 ± 0.77	**0.000**	**0.000**	NS	**0.011**	NS	NS	NS
Total n-3 PUFA	34.87 ± 0.81	32.61 ± 0.85	32.79 ± 1.25	28.59 ± 1.60	35.68 ± 1.56	30.93 ± 1.51	33.90 ± 0.45	28.24 ± 1.51	**0.002**	**0.000**	**0.006**	NS	NS	NS	NS
n3/n6	6.47 ± 0.17	4.86 ± 0.30	5.81 ± 0.21	4.31 ± 0.25	7.02 ± 0.23	4.97 ± 0.23	6.48 ± 0.22	4.68 ± 0.66	**0.000**	**0.000**	**0.019**	**0.022**	NS	NS	NS
DHA/AA	16.50 ± 0.55	10.13 ± 0.61	14.31 ± 0.93	9.64 ± 0.52	17.33 ± 0.50	10.22 ± 0.61	16.07 ± 0.82	10.06 ± 1.49	**0.000**	**0.000**	0.071	NS	NS	NS	NS
PC fraction															
14:0	0.28 ± 0.02	0.34 ± 0.04	0.30 ± 0.02	0.26 ± 0.01	0.28 ± 0.00	0.55 ± 0.16	0.29 ± 0.02	0.29 ± 0.01	NS	NS	NS	NS	**0.011**	NS	NS
16:0	48.23 ± 0.42	47.95 ± 0.10	48.23 ± 0.45	47.61 ± 0.49	48.92 ± 0.24	46.56 ± 0.55	49.27 ± 0.26	47.96 ± 0.27	**0.001**	**0.000**	NS	NS	NS	**0.009**	**0.040**
16:1n-7	0.67 ± 0.02	0.78 ± 0.02	0.66 ± 0.01	0.75 ± 0.01	0.70 ± 0.01	1.03 ± 0.09	0.69 ± 0.03	0.87 ± 0.03	**0.000**	**0.000**	NS	**0.000**	NS	NS	NS
16:1n-9	0.42 ± 0.01	0.40 ± 0.02	0.43 ± 0.02	0.47 ± 0.06	0.40 ± 0.01	0.43 ± 0.03	0.37 ± 0.00	0.42 ± 0.01	NS	NS	NS	NS	NS	NS	NS
16:1	1.09 ± 0.02	1.18 ± 0.03	1.09 ± 0.01	1.22 ± 0.05	1.09 ± 0.02	1.47 ± 0.11	1.06 ± 0.02	1.30 ± 0.03	**0.000**	**0.000**	NS	**0.026**	NS	0.067	NS
18:0	12.51 ± 0.08	11.96 ± 0.05	12.41 ± 0.27	12.72 ± 0.20	11.73 ± 0.25	12.57 ± 0.32	11.97 ± 0.04	12.31 ± 0.20	**0.016**	0.085	NS	0.058	NS	**0.014**	NS
18:1n-7	5.74 ± 0.09	5.58 ± 0.05	5.79 ± 0.10	5.65 ± 0.08	5.67 ± 0.09	5.68 ± 0.08	5.58 ± 0.03	5.64 ± 0.14	NS	NS	NS	NS	NS	NS	NS
18:1n-9	19.47 ± 0.29	20.42 ± 0.13	19.43 ± 0.32	20.72 ± 0.09	19.44 ± 0.13	20.47 ± 0.23	19.22 ± 0.11	20.65 ± 0.15	**0.000**	**0.000**	NS	NS	NS	NS	NS
18:1	25.20 ± 0.37	26.00 ± 0.15	25.23 ± 0.41	26.36 ± 0.16	25.11 ± 0.19	26.15 ± 0.30	24.80 ± 0.13	26.29 ± 0.21	**0.000**	**0.000**	NS	NS	NS	NS	NS
18:2n-6	0.66 ± 0.02	0.62 ± 0.04	0.61 ± 0.02	0.57 ± 0.03	0.69 ± 0.04	0.71 ± 0.03	0.72 ± 0.02	0.62 ± 0.02	**0.004**	**0.025**	NS	**0.001**	0.082	NS	NS
20:1n-9	0.60 ± 0.02	0.60 ± 0.02	0.61 ± 0.01	0.62 ± 0.01	0.56 ± 0.02	0.67 ± 0.08	0.57 ± 0.02	0.60 ± 0.03	NS	NS	NS	NS	NS	NS	NS
20:4n-6	5.65 ± 0.07	5.25 ± 0.13	5.34 ± 0.21	4.83 ± 0.13	5.48 ± 0.15	5.28 ± 0.34	5.03 ± 0.10	4.78 ± 0.12	**0.015**	**0.018**	**0.002**	NS	NS	NS	NS
22:0	0.10 ± 0.01	0.07 ± 0.01	0.12 ± 0.01	0.07 ± 0.01	0.10 ± 0.00	0.07 ± 0.00	0.11 ± 0.01	0.07 ± 0.00	**0.000**	**0.000**	NS	NS	0.087	NS	NS
22:6n-3	4.27 ± 0.07	4.91 ± 0.11	4.58 ± 0.39	4.53 ± 0.18	4.66 ± 0.10	4.68 ± 0.34	4.63 ± 0.12	4.44 ± 0.17	NS	NS	NS	NS	NS	NS	NS
Total SFA	61.39 ± 0.44	60.40 ± 0.13	61.36 ± 0.51	60.81 ± 0.46	61.30 ± 0.12	59.90 ± 0.60	61.94 ± 0.27	60.82 ± 0.44	**0.024**	**0.001**	0.071	NS	NS	NS	NS
Total MUFA	26.99 ± 0.36	27.82 ± 0.20	27.02 ± 0.41	28.24 ± 0.11	26.83 ± 0.19	28.37 ± 0.31	26.58 ± 0.11	28.29 ± 0.22	**0.000**	**0.000**	NS	NS	NS	0.088	NS
Total n-6 PUFA	7.32 ± 0.09	6.82 ± 0.11	7.00 ± 0.26	6.36 ± 0.17	7.15 ± 0.19	6.98 ± 0.42	6.77 ± 0.12	6.38 ± 0.17	**0.032**	**0.012**	**0.005**	NS	NS	NS	NS
Total n-3 PUFA	4.30 ± 0.05	4.97 ± 0.12	4.63 ± 0.40	4.60 ± 0.18	4.73 ± 0.09	4.76 ± 0.34	4.70 ± 0.13	4.52 ± 0.17	NS	NS	NS	NS	NS	NS	NS
n3/n6	0.59 ± 0.01	0.73 ± 0.03	0.66 ± 0.04	0.72 ± 0.01	0.66 ± 0.02	0.69 ± 0.05	0.69 ± 0.01	0.71 ± 0.01	**0.015**	**0.001**	NS	NS	NS	**0.041**	NS
DHA/AA	0.75 ± 0.01	0.94 ± 0.04	0.85 ± 0.04	0.94 ± 0.01	0.85 ± 0.03	0.89 ± 0.06	0.92 ± 0.01	0.93 ± 0.02	**0.012**	**0.003**	0.057	NS	NS	**0.037**	NS

Results are presented as means ± SEM (n=6-7 per group). NS, non-significant. F, female, M, male, Mo, months, PE, phosphatidylethanolamine, PS, phosphatidylserine, PC, phosphatidylcholine, SFA, saturated fatty acid, MUFA, monounsaturated fatty acid, PUFA, polyunsaturated fatty acid, DHA, docosahexaenoic acid, AA, arachidonic acid and DMA, dimethyl acetal fatty acid. P-values are shown for age, sex, genotype, two-way interactions and at a group level (comparing each group with each other). ANOVAs (1 way and 2 ways) were used and non-parametric equivalent tests (e.g. Kruskal-Wallis) were used where the data were not compliant with ANOVA requirements, followed by Tukey Honest Significant Differences with $p<0.05$ indicating significant differences.

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Supplemental data 6: Detailed specialized proresolving mediators composition in pg/mg in cortex and hippocampus of female and male 2-, 9- and 18-month of age *APOE3* and *APOE4* mice fed on chow diet.

Cortex	APOE3									APOE4								
	Female			Male						Female			Male					
Compound (pg/mg)	APOE3F 2Mo	APOE3F 9Mo	APOE3F 18Mo	APOE3M 2Mo	APOE3M 9Mo	APOE3M 18Mo	APOE4F 2Mo	APOE4F 9Mo	APOE4F 18Mo	APOE4M 2Mo	APOE4M 9 Mo	APOE4M 18Mo	P-group	P-age	P-sex	P-apoe	P-age:sex	P-age:apoe	P-sex:apoe
(±) 18 HEPE	0.17 ± 0.04	0.14 ± 0.03	0.23 ± 0.08	0.22 ± 0.04	0.14 ± 0.02	0.24 ± 0.08	0.10 ± 0.01	0.14 ± 0.04	0.11 ± 0.02	0.12 ± 0.04	0.09 ± 0.02	0.22 ± 0.10	NS	NS	NS	**0.005**	NS	NS	NS
RvD1	0.51 ± 0.07	0.41 ± 0.02	0.64 ± 0.10	0.58 ± 0.06	0.47 ± 0.03	0.55 ± 0.08	0.66 ± 0.13	0.63 ± 0.16	0.70 ± 0.18	0.53 ± 0.07	0.50 ± 0.07	0.44 ± 0.05	NS	NS	NS	NS	NS	NS	0.097
RvD3	0.44 ± 0.05	0.42 ± 0.03	0.59 ± 0.13	0.42 ± 0.03	0.41 ± 0.02	0.41 ± 0.03	0.37 ± 0.01	0.44 ± 0.01	0.43 ± 0.03	0.37 ± 0.03	0.39 ± 0.02	0.42 ± 0.05	NS	NS	NS	NS	NS	NS	NS
RvD5	0.33 ± 0.03	0.32 ± 0.02	0.51 ± 0.16	0.37 ± 0.05	0.33 ± 0.02	0.35 ± 0.03	0.30 ± 0.02	0.34 ± 0.01	0.33 ± 0.02	0.28 ± 0.01	0.32 ± 0.02	0.34 ± 0.02	NS	NS	NS	NS	NS	NS	NS
17R-RvD1	0.46 ± 0.08	0.35 ± 0.05	0.41 ± 0.08	0.52 ± 0.16	0.30 ± 0.03	0.27 ± 0.02	0.32 ± 0.10	0.28 ± 0.03	0.29 ± 0.05	0.31 ± 0.05	0.25 ± 0.02	0.31 ± 0.07	NS	NS	NS	**0.009**	NS	NS	NS
10S,17S-diHDHA	0.18 ± 0.02	0.16 ± 0.01	0.21 ± 0.04	0.19 ± 0.03	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.02	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.15 ± 0.02	NS	NS	NS	**0.026**	NS	NS	NS
PD1	0.41 ± 0.05	0.28 ± 0.02	0.41 ± 0.10	0.28 ± 0.02	0.30 ± 0.02	0.30 ± 0.02	0.34 ± 0.04	0.34 ± 0.05	0.59 ± 0.19	0.27 ± 0.02	0.29 ± 0.01	0.31 ± 0.05	NS	NS	**0.014**	NS	NS	NS	NS
7R-Maresin	0.67 ± 0.10	0.57 ± 0.13	1.06 ± 0.52	0.53 ± 0.11	0.45 ± 0.05	0.43 ± 0.05	0.39 ± 0.03	0.51 ± 0.02	0.54 ± 0.04	0.40 ± 0.04	0.41 ± 0.02	0.42 ± 0.04	0.062	NS	**0.005**	0.056	NS	NS	NS
17S-HDHA	36.79 ± 8.66	9.27 ± 1.04	11.20 ± 2.62	11.21 ± 0.96	10.49 ± 2.15	11.35 ± 2.63	12.30 ± 1.77	11.90 ± 1.45	14.44 ± 2.00	10.07 ± 0.62	10.06 ± 0.94	9.38 ± 0.68	**0.010**	**0.026**	**0.020**	NS	NS	**0.028**	NS
14S-HDHA	29.48 ± 6.46	8.22 ± 0.57	7.20 ± 1.05	9.20 ± 0.66	8.45 ± 2.24	9.31 ± 2.32	9.48 ± 1.76	8.84 ± 1.26	9.40 ± 0.90	8.94 ± 0.66	7.30 ± 0.70	7.10 ± 0.45	**0.005**	**0.003**	**0.046**	NS	NS	0.068	NS

Hippocampus	APOE3						APOE4												
	Female			Male			Female			Male									
Compound (pg/mg)	APOE3F 2Mo	APOE3F 9Mo	APOE3F 18Mo	APOE3M 2Mo	APOE3M 9Mo	APOE3M 18Mo	APOE4F 2Mo	APOE4F 9Mo	APOE4F 18Mo	APOE4M 2Mo	APOE4M 9 Mo	APOE4M 18Mo	P-group	P-age	P-sex	P-apoe	P-age:sex	P-age:apoe	P-sex:apoe
(±) 18 HEPE	0.29 ± 0.05	0.21 ± 0.07	0.25 ± 0.07	0.18 ± 0.02	0.25 ± 0.06	0.17 ± 0.08	0.13 ± 0.04	0.17 ± 0.03	0.24 ± 0.07	0.26 ± 0.12	0.22 ± 0.06	0.19 ± 0.04	NS	NS	NS	NS	NS	NS	NS
RvD1	1.04 ± 0.16	1.04 ± 0.09	1.22 ± 0.20	0.89 ± 0.05	0.85 ± 0.06	1.01 ± 0.13	0.97 ± 0.11	1.13 ± 0.17	1.09 ± 0.18	1.13 ± 0.15	1.19 ± 0.29	0.99 ± 0.15	NS	NS	NS	NS	NS	NS	NS
RvD3	1.10 ± 0.13	0.88 ± 0.06	1.06 ± 0.16	0.87 ± 0.08	0.89 ± 0.07	0.87 ± 0.06	0.96 ± 0.12	0.94 ± 0.08	0.90 ± 0.07	0.93 ± 0.06	0.80 ± 0.03	0.84 ± 0.03	NS	NS	NS	NS	NS	NS	NS
RvD5	0.88 ± 0.12	0.71 ± 0.06	0.83 ± 0.11	0.73 ± 0.08	0.67 ± 0.05	0.69 ± 0.05	0.76 ± 0.09	0.74 ± 0.08	0.70 ± 0.05	0.70 ± 0.05	0.62 ± 0.02	0.65 ± 0.02	NS	NS	0.057	NS	NS	NS	NS
17R-RvD1	0.71 ± 0.10	0.65 ± 0.05	0.71 ± 0.12	0.68 ± 0.15	0.58 ± 0.11	0.68 ± 0.09	0.66 ± 0.12	0.54 ± 0.07	0.68 ± 0.16	0.79 ± 0.15	0.54 ± 0.06	0.56 ± 0.05	NS	NS	NS	NS	NS	NS	NS
10S,17S-diHDHA	0.38 ± 0.05	0.32 ± 0.02	0.37 ± 0.06	0.32 ± 0.04	0.30 ± 0.03	0.32 ± 0.02	0.34 ± 0.05	0.31 ± 0.03	0.33 ± 0.05	0.35 ± 0.04	0.28 ± 0.01	0.29 ± 0.01	NS	NS	NS	NS	NS	NS	NS
PD1	0.79 ± 0.11	0.61 ± 0.05	0.71 ± 0.10	0.72 ± 0.08	0.57 ± 0.04	0.75 ± 0.11	0.75 ± 0.05	0.61 ± 0.05	0.64 ± 0.08	0.75 ± 0.11	0.56 ± 0.03	0.57 ± 0.03	NS	**0.021**	NS	NS	NS	NS	NS
7R-Maresin	1.30 ± 0.24	1.00 ± 0.09	1.05 ± 0.16	1.03 ± 0.10	1.02 ± 0.10	0.85 ± 0.05	0.97 ± 0.11	0.96 ± 0.09	0.94 ± 0.07	1.08 ± 0.20	0.92 ± 0.08	0.84 ± 0.04	NS	NS	NS	NS	NS	NS	NS
17S-HDHA	5.97 ± 0.91	4.55 ± 1.08	5.24 ± 1.53	4.09 ± 1.08	3.31 ± 0.56	4.42 ± 0.97	4.23 ± 1.69	4.72 ± 1.27	6.41 ± 1.15	3.58 ± 0.86	5.13 ± 0.64	3.74 ± 0.69	NS	NS	NS	NS	NS	NS	NS
14S-HDHA	4.68 ± 0.78	2.71 ± 0.65	4.04 ± 0.98	3.04 ± 0.67	2.70 ± 0.56	3.72 ± 0.65	3.19 ± 1.34	4.73 ± 0.73	5.68 ± 1.68	3.36 ± 0.37	2.72 ± 0.34	3.59 ± 0.22	NS	NS	NS	NS	NS	NS	NS

Results are presented as means ± SEM (n=5 per group). NS, non-significant. F, female, M, male, Mo, months. P-values are shown for age, sex, genotype, two-way interactions and at a group level (comparing each group with each other). 18R/S-hydroxy-5Z,8Z,11Z,14Z,16E-eicosapentaenoic acid ((±) 18-HEPE), 17S-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid (17S-HDHA), 14S-hydroxy-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid (14S-HDHA), 7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid (RvD1, resolvin D1), RvD3 (4S,11R,17S-trihydroxy-docosa-5Z,7E,9E,13Z,15E,19Z-hexaenoic acid, resolvin D3), RvD5 (7S,17S-dihydroxy-5Z,8E,10Z,13Z,15E,19Z-docosahexaenoic acid, resolvin D5), 7S,8R,17R-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid (17R-RvD1), 7R,14S-dihydroxy-4Z,8E,10E,12Z,16Z,19Z-docosahexaenoic acid (MaR1, 7R-Maresin), 10S,17S-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid (10S,17S-diHDHA) and 10R,17S-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid (PD1, protectin D1). ANOVAs (1 way and 2 ways) were used and non-parametric equivalent tests (e.g. Kruskal-Wallis) were used where the data were not compliant with ANOVA requirements, followed by Tukey Honest Significant Differences with $p<0.05$ indicating significant differences.

Supplementary data to:

Altered SPMs and age-associated decrease in brain DHA in APOE4 female mice

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