APOE ε4 alters docosahexaenoic acid associations with preclinical markers of Alzheimer disease

[a,b] Gillian Coughlan, [c] Ryan Larsen, [d] Min Kim, [e] David White, [a] Rachel Gillings, [a] Michael Irvine, [e] Andrew Scholey, [c] Neal Cohen, [d] Cristina Legido Quigley, [a] Michael Hornberger, [a] Anne-Marie Minihane

[a] *Norwich Medical School, University of East Anglia, Norwich, United Kingdom*
[b] *Rotman Research Institute, Baycrest, Toronto, Canada*
[c] *Decision Neuroscience Laboratory, Beckman Institute for Advanced Science and Technology, University of Illinois, USA.*
[d] *Franklin-Wilkins Building, Stamford Street, Kings College London, London, United Kingdom*
Centre for Human Psychopharmacology, Swinburne University, Australia.

Correspondence:
Professor Anne-Marie Minihane
Norwich Medical School,
University of East Anglia,
Norwich,
United Kingdom
a.mininhane@uea.ac.uk

Abstract word count: 278

Main text word count: 4265

ABSTRACT

Docosahexaenoic acid (DHA) is the main long chain omega-3 polyunsaturated fatty acids in the brain and accounts for 30% to 40% of fatty acids in the grey matter of the human cortex. Although the influence of DHA on memory function is widely researched, its association with brain volumes is under investigated and its association with spatial navigation is virtually unknown. This is despite the fact that spatial navigation deficits are a new cognitive fingerprint for symptomatic and asymptomatic Alzheimer's disease (AD). We investigated the relationship between DHA levels and the major structural and cognitive markers of preclinical AD, namely hippocampal volume, entorhinal volume, and spatial navigation ability. Fifty-three cognitively normal adults underwent volumetric magnetic resonance imaging, measurements of serum DHA (including serum lysophosphatidylcholine DHA (LPC DHA)) and *APOE* ε4 genotyping. Relative regional brain volumes were calculated and linear regression models were fitted to examine DHA associations with brain volume. *APOE* genotype modulated serum DHA associations with entorhinal cortex volume and hippocampal volume. Linear models showed that greater serum DHA was associated with increased entorhinal cortex volume, but not hippocampal volume, in *APOE* ε4 non-carriers. *APOE* also interacted with serum LPC DHA to predict hippocampal volume. After testing interactions between DHA and *APOE* ε4 on brain volume, we investigated whether DHA and *APOE* interact to predict spatial navigation performance on a novel virtual reality diagnostic test for AD in an independent population of *APOE* genotyped adults (n = 46). Crucially, the *APOE* genotype modulated DHA associations with spatial navigation performance, showing that DHA was inversely associated with path integration in *APOE* ε4 carriers only. Interventions aiming to increase DHA status to protect against cognitive decline must consider *APOE* ε4 carrier status, and focus on higher doses of supplementary DHA to ensure adequate brain delivery.

Abbreviations

AD= Alzheimer's disease

APOE= Apolipoprotein E

DHA= Docosahexaenoic

DNA= Deoxyribonucleic acid

BMI=body mass index

HDL=high-density lipoprotein

BDNF=Brain-derived neurotrophic factor

LPC= Lysophosphatidylcholine

TG=triglyceride

FAs=fatty acids

CVLT= California Verbal Learning Test

MOCA= Montreal Cognitive Assessment

ACE=Addenbrookes cognitive examination

ROCF=Rey–Osterrieth Complex Figure

FAs=fatty acids

CI= confidence interval

GLM=General linear model

MRI=magnetic resonance imaging

PCR= Polymerase chain reaction

TIV= total intracranial volume

ω-3 PUFA= omega-three polyunsaturated fatty acids

np2=Partial eta squared

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia with increasing world-wide prevalence. In the absence of any licensed drugs to treat or reverse cognitive decline associated with AD, dietary behaviours which prevent or slow brain atrophy in the entorhinal cortex and the hippocampus hold tremendous potential (Larson *et al.*, 2013; Lewis *et al.*, 2014; Vauzour *et al.*, 2017). Higher long chain omega-3 polyunsaturated fatty acids (LC ω-3 PUFA) have been linked to better memory function and lower the risk of developing AD (Lim *et al.*, 2006; He *et al.*, 2009; Yassine *et al.*, 2016; Ammann *et al.*, 2017). However, the effect of LC ω-3 PUFA on spatial navigation is virtually unknown, despite evidence that spatial disorientation may appear in conjunction with, or prior to, episodic memory loss in AD (Tu *et al.*, 2015; Lester *et al.*, 2017; Coughlan *et al.*, 2018). Therefore, elucidating the effect of LC ω-3 PUFA on spatial navigation and on its associated brain regions is of high interest.

Docosahexaenoic acid (DHA), the main ω-3 PUFA in the brain which accounts for 30% to 40% of fatty acids in the grey matter of the human cortex and is especially enriched at the synapse (Lacombe *et al.*, 2018). Disturbances in brain DHA metabolism have been implicated in a host of neurodegenerative diseases, particularly AD. This may be because the beneficial properties of DHA appear to be concentrated in the hippocampus (Pottala *et al.*, 2014) (particularly the CA1 subfield (He *et al.*, 2009)) and the entorhinal cortex (Arsenault *et al.*, 2011; Yassine *et al.*, 2016). Major animal studies show long-term DHA supplementation in mice with preclinical AD reverses amyloid accumulation, protects against neuronal loss associated with AD pathology, and critically, improves overall navigation performance (Oksman M, Iivonen H, 2006; He *et al.*, 2009). Conversely, reduced ω-3 PUFA levels has been shown to impair hippocampal plasticity and reduce navigation function (Lim *et al.*, 2005; Fedorova *et al.*, 2007), suggesting that DHA may be beneficial in prevention AD.

Despite brain DHA levels being 10-fold higher in specific brain regions relative to most body tissues, and strong evidence for neurocognitive benefits proposed by animal models, the neuronal benefits in humans are less consistent. In the Framingham Heart Study, serum phosphatidylcholine DHA levels were associated with a 47% reduction in risk of dementia over 9 years of follow-up (Schaefer *et al.*, 2006), but in a similar dementia-free Dutch cohort, dietary DHA intake was not associated with relative risk for AD (Devore *et al.*, 2009). Moreover, the AD Cooperative Study reported no effect of DHA supplementation (18

months) on composite measures of cognition in adults with mild to moderate AD (Tomaszewski *et al.*, 2020), but a subset of the Ageing Brain Study led by the University of Southern California shows higher serum DHA levels are associated with lower cerebral amyloidosis and preservation of entorhinal and hippocampal volumes (Yassine *et al.*, 2016).

The unexamined role of the apolipoprotein (*APOE*) genotype is also a major factor behind the mixed results from human studies (Chouinard-Watkins and Plourde, 2014; Zamroziewicz *et al.*, 2015; Yassine, 2017). The *APOE ε4* isoform is the most important prevalent genetic determinant of AD risk (Corder *et al.*, 1993) and disrupts blood-brain barrier function in the hippocampus and wider medial temporal lobe, compared to the other *APOE* isoforms (ε2/ε3) (Montagne *et al.*, 2020). This then suggests that a faulty blood brain barrier system in *APOE ε4* carriers may impair the transport of circulating DHA to the brain, which has been shown in older APOE ε4 mice (Vandal *et al.*, 2014). Therefore, we hypothesized that the *APOE* genotype would modulate circulating blood DHA associations with brain volume and spatial navigation. We further hypothesized that higher DHA levels would be positively related to preserved brain entorhinal and hippocampal volume, as well as spatial navigation performance in ε4 non-carriers only. We expected that these associations would be non-significant or negative in ε4 carriers.

METHODS

This is a cross-sectional study examining the effect of *APOE* ε4 on DHA associations with entorhinal cortex volume, hippocampal volume and spatial navigation performance across two non-demented cohorts.

Setting

Non-demented adults were drawn from the Cognitive Ageing, Nutrition and Neurogenesis study and formed cohort one (Irvine *et al.*, 2018). Recruitment and screening began in 2015 and all neuroimaging data was collected by March 2017 across two data collection sites; the Swinburne University of Technology (Melbourne, Australia) and the University of East Anglia (UEA, Norwich, United Kingdom). At screening, cognitive status was pre-classified with a modified telephone interview for cognitive status and Montreal Cognitive Assessment tool (Nasreddine, 2005). Participants were invited for baseline neuropsychological testing and a baseline MRI scan. Blood samples were taken immediately following cognitive testing.

To investigate DHA associations with spatial navigation, we recruited a second cohort. Between February 2017 and June 2017, participants from this cohort were recruited to participate in a research study at Norwich Medical School, UEA and invited for spatial navigation and neuropsychological testing. Blood samples were taken immediately following testing.

Standard protocol approvals, registrations, and consents

Cohort one was acquired from the Cognitive Ageing, Nutrition and Neurogenesis study (ClinicalTrials.gov NCT02525198) and obtained ethical approval from the Swinburne University Human Research Ethics Committee (Study identifier SHR Project 2015-208) for the Swinburne University of Technology site and the National Research Ethics Service Committee for the University of East Anglia site (Study identifier 14/EE/0189). Ethical approval for the second navigation study (cohort two) was obtained from the Faculty of Medicine and Health Sciences Ethics Committee at UEA, UK, (Reference FMH/2016/2017–11). All participants from both studies provided informed signed consent before participating.

Participants

Participants from cohort one were a mean age of 64.7 (SD 7.6) years. Participants from cohort two were 61.3 (SD 5.6) years. Exclusion criteria for both samples included diagnosis of mild cognitive impairment, clinical dementia, significant neurologic/ psychiatric disorder, MRI evidence of brain damage, previous vascular disorders including infarction or stroke and history of alcohol or drug dependency within the last 2 years. In addition, homozygous *APOE* ε4 carriers (2% of the population) and *APOE* ε2 carriers (13% of the UK population) were excluded, due to their low population prevalence. Included were (1) *APOE* ε3ε4 allele carriers, who are at a 3-fold increased risk of developing AD and represent 23% of the population (moderate risk, high prevalence), and (2) age-gender matched ε3ε3 carriers, who represent the population wild-type genotype (60% of the population). (Liu *et al.*, 2013) All participants had normal or corrected-to-normal vision.

***APOE* genotyping**

In both cohorts, DNA was extracted and used for APOE genotyping. In cohort one, DNA was extracted from the buffy coat layer (containing the white cell layer) of each participant blood sample, and placed in a ethylenediaminetetraacetic acid tube (BD Biosciences, San Diego, CA, USA). In cohort two, DNA was extracted from a Darcon tip buccal swab LE11 5RG;

Fisher Scientific), using a commercial DNA extraction kit (Qiagen, Hildenberg, Germany). DHA from both samples underwent PCR amplification and plate read analysis using Applied Biosystems 7500 Fast Real-Time PCR System (TN23 4FD; Thermo Fisher Scientific) to determine participants' APOE genotype status. Further details for each cohort are detailed elsewhere (Irvine *et al.*, 2018; Coughlan *et al.*, 2019).

DHA measurements

Cohort one

Free/non-esterified fatty acid DHA and lysophosphatidylcholine (LPC) DHA was measured from a fasted blood samples, and 20µl of serum was utilised for analysis. Ten microlitres of high purity water and 40µl of MS-grade methanol were added, followed by a 2 min vortex mix to precipitate proteins. 200 µl of methyl t-butyl ether was added, and the samples were mixed via vortex at room temperature for 1 h. After the addition of 50 µl of high purity water, a final sample mixing was performed before centrifugation at 10000 g for 10 min. The upper, lipid-containing, methyl t-butyl ether phase was then extracted and analysed by liquid chromatography-mass spectrometry. The analytical method is detailed elsewhere (Whiley *et al.*, 2012). The single molecule integrated peak areas under the exact mass chromatographs of LPC-DHA were obtained by using Skyline by setting up an integration parameter file using its mass charge ration (m/z) and retention time (510.35 m/z and 3.0 minutes) (MacLean *et al.*, 2010; Peng and Ahrends, 2016). LPC DHA is the most important lipid pool to deliver DHA to the brain via the blood–brain barrier (Sugasini *et al.*, 2017, 2019, 2020).

Cohort two

Erythrocyte DHA was measured from a non-fasted blood samples collected using a single drop of whole blood obtained via a finger prick collection kit (Faculty of Natural Sciences Institute of Aquaculture, University of Stirling). Blood samples were immobilised on a specially made card and sent to the University of Stirling (Stirling, UK) for analysis. Please see Carboni et al., (2019) for a full description of the Blood Spot PUFA analysis used to derive fatty acid erythrocyte concentrations (Carboni *et al.*, 2019). In cohort 2, fasting status was not needed as DHA was measured in the erythrocyte phospholipid fraction, which is reflective or long term (up to 3 months) fatty acid intake and little influenced by recent DHA or overall fatty acid intake.

Volumetric MRI

Structural T1-weighted images were obtained using either a three-dimensional fast spoiled gradient echo brain volume imaging sequence in the sagittal orientation, repetition time (TR)/ echo time (TE)/inversion time (TI) = 7,040/2.612/900 ms, 0.9 mm isotropic resolution, field of view (FOV) = 230 × 230 mm, number of excitations (NEX) = 0.5, or a using a three-dimensional magnetization prepared rapid gradient echo (sequence, TR/TE/TI = 1,900/2.32/900 ms, 0.9 mm isotropic resolution, FOV = 230 × 230 mm, generalized autocalibrating partial parallel acquisition, acceleration factor of 2 depending on site. Full acquisition details are documented elsewhere (Irvine *et al.*, 2018).

Cortical surface reconstruction and segmentation was performed with FreeSurfer image analysis suite (version 6.0.0) (http://freesurfer.net/). The automised processing stream includes motion correction, removal of non-brain tissue, automated Talairach transformation, intensity correction, volumetric segmentation, cortical surface reconstruction, and parcellation. Quality checks included skull stripping and pial surface errors, intensity normalisation, white matter segmentation errors and were conducted on Freeview after processing and before statistical analysis. Entorhinal volume was derived from the Desikan-Killiany atlas (Desikan *et al.*, 2006). Hippocampal volumes were derived based on an atlas derived from combining high-resolution ex vivo data and in vivo data.

Cognitive assessments

In cohort one, intact cognitive status was pre-determined. Details on the cognitive assessment are outlined elsewhere (Irvine *et al.*, 2018). Participants were assessed on the California Verbal Learning Task, the Montreal Cognitive Assessment and Digital Span to test for cognitive differences between *APOE ε4* carriers and non-carriers (Elwood, 1995; Nasreddine, 2005). In cohort two, the Addenbrooke's cognitive evaluation and the Rey-Osterrieth complex figure test were available to confirm the sample was free of cognitive impairment and that there were no differences between *APOE ε4* carrier and non-carriers. Spatial navigation performance was measured using the Virtual Supermarket Test adopted by the European Prevention of Alzheimer's Dementia Consortium to assess the efficacy of potentially AD modifying treatments. Details for the spatial navigation task can be found elsewhere (Tu *et al.*, 2015, 2017; Coughlan *et al.*, 2020).

Statistical analysis

The data were analysed using RStudio (version 1.0.153). Linear regression models were specified with entorhinal and hippocampal volume as outcome variables, and DHA and *APOE* genotype as predictors (including an interaction term). Models were adjusted for age, sex, education, test centre and total estimated intracranial volume. Additional dietary variables such as total intake of green vegetables and fruit did not contribute to overall model fit based on the bayesian information criterion criteria and were not retained in the final models. In the spatial navigation dataset, adjustments for test centre and intracranial volume were dropped as volumetric MRI was not the outcome variable and data collection took place at one site only. Standardized residuals were extracted and plotted against fitted values to examine underlining assumption of normal distribution and heteroscedasticity. In the case of significant *APOE* x DHA interactions, post-hoc linear models were specified with *APOE ε4* carriers (ε3ε4) and non-carriers (ε3ε3) separately. All statistical tests are two-tailed: $P < 0.05$. Partial eta squared (n_p^2) was used as a measure of effect sizes and was derived from lmSupport package in R (https://cran.r-project.org/web/packages/lmSupport). n_p^2 is the ratio of variance associated with an effect plus that effect and its associated error variance ($n_p^2 = SS_{effect} / SS_{effect} + SS_{error}$).

Data availability

The authors have documented all data, methods, and materials used to conduct the research in this article and agree to share anonymized data by request from the first author or corresponding author.

RESULTS

The participant characteristics for cohorts one and two are summarized in Tables 1 and 2.

Table 1. Participant characteristics in cohort one. Data are presented as mean (SD) for normally distributed data or median for non-normal distributions. The two groups were compared by an independent sample t test. Serum free DHA is measured as total DHA in serum, in the free/non-esterified fatty acid form.

	Mean (SD)			
		APOE genotype		
Characteristic	Total (n=53)	ε3ε4 carriers (n=15)	ε3ε3 carriers (n=38)	*P* value
Age, (y)	64.2 (7.2)	65.0 (7.9)	64.0 (6.9)	.65
Sex (male/female)	21/33	7/9	15/22	
Education, (y)	14.2 (2.9)	14.5 (2.8)	14.1 (3.0)	.63
Serum free DHA (ug/mL)	1.23 (.64)	1.22 (.59)	1.23 (.66)	.96
Serum LPC DHA (ug/mL)	2.81 (1.39)	2.23 (1.49)	2.10 (1.26)	.43
Blood pressure				
Systolic (mm Hg)	133 (17)	121 (23)	126 (14)	.61
Diastolic (mm Hg)	77 (8.8)	72 (7.3)	75 (7.8)	.10
BMI (kg/m^2)	26.9 (4.0)	27.1 (4.9)	26.9 (3.8)	.87
Serum glucose (mmol/l)	5.19 (0.57)	5.26 (0.38)	5.17 (0.63)	.63
Serum cholesterol (mmol/l)				
Total	5.11 (0.9)	5.04 (1.2)	5.12 (0.8)	.82
HDL	1.51 (4.5)	1.39 (5.2)	1.44 (3.9)	.81
Serum TG (mmol/l)	1.21 (0.5)	1.59 (0.6)	1.47 (.4)	.48
Serum BDNF (pg/mL)	18958 (4676)	19359 (4702)	18144 (4589)	.13
Brain volume				
Hippocampal volume (ratio of total intracranial volume)	.0045 (.00038)	.0044 (.00046)	.0045 (.00035)	.36
Entorhinal volume (ratio of total intracranial volume)	.0024 (.00031)	.0023 (.00025)	.0024 (.00033)	.29
Cognition				
CVLT (delayed free recall)	10.99 (2.3)	10.43 (2.7)	11.16 (2.3)	.28
MOCA (delayed recall)	3.11 (1.3)	3.07 (1.2)	3.24 (1.4)	.68
MOCA (total)	27.87 (1.7)	27.73 (1.7)	27.93 (1.8)	.72
Digital Span (total score)	19.01 (3.4)	18.80 (3.2)	19.79 (3.6)	.37

Table 2. Participant characteristics in cohort two.

Data are presented as mean (SD) for normally distributed data or median (IQR) for non-normal distributions. The two groups were compared by an independent sample t-test.

	Mean (SD)			
		APOE genotype		
Characteristic	Total (n=46)	ε3ε4 carriers (n=22)	ε3ε3 carriers (n=24)	*P* value
Socio-demographic				
Age, (y)	61.30 (5.6)	60.82 (5.7)	61.75 (5.7)	.58
Sex (male/female)	15/31	4/18	11/13	
Education, (y)	14.4 (5.4)	14.5 (2.9)	14.4 (3.6)	.72
Erythrocyte DHA (% of total FA)	2.64 (.71)	2.76 (.73)	2.52 (.62)	.25
Blood pressure (missing=3)				
Not medicated	36	18	18	.61
Medicated	7	3	1	.10
Cholesterol (missing=3)				
Not medicated	39	19	20	.55
Medicated	4	2	2	.81
Cognition				
ACE total score	94 (3.7)	93 (5.4)	94 (2.1)	.55
ROCF				
Copy	32 (2.8)	32 (2.8)	32 (2.9)	.55
Recall	19 (5.8)	17 (5.2)	20 (6.1)	.08

Serum DHA associations with entorhinal and hippocampal volume

Serum free DHA (in the free/non-esterified fatty acid form) predicted right (t= 2.15, p=0.03, n_p^2=0.09) and left (t=2.33, p= 0.02, n_p^2=0.11; Figure 1 A-B) entorhinal volume. There was a significant interaction between serum free DHA and *APOE* genotype status on the left entorhinal volume (t=-2.20, p=0.03, n_p^2=0.10), with a trend evident for the right side (t=-2.00, p=0.05, n_p^2= 0.09). Independent linear models for *APOE* ε3ε3 and *APOE* ε3ε4 carrier groups revealed a positive association between serum free DHA levels and entorhinal volume in ε3ε3 carriers only (for both hemispheres: left t=2.67, p=0.01; right t=2.28, p=0.02). The DHA × *APOE* interaction was not significant for hippocampal volume, although there was a trend toward significance (right hemisphere: t=1.72, p=0.09; see Figure 1 C-D).

Serum lysophosphatidylcholine associations with entorhinal and hippocampal volume

LPC data was available from one of the two research sites in cohort one (n=30). LPC DHA predicted right hippocampal volume (t=2.31, p=0.03, np^2=0.22), with a significant LPC DHA

× *APOE* interaction (t= -2.24, p=0.03, np^2= 0.19) and a positive and negative association trend was evident in *APOE ε3ε3* carriers and *APOE ε3ε4* carrier's receptivity, but was not significant. Against predictions, there was no main effect of LPC DHA and entorhinal volume, suggesting the serum free DHA is associated with entorhinal cortex, but the LPC fraction is more strongly associated to the hippocampus in this sample.

APOE effects on brain volume

We also investigated if the *APOE* genotype effects entorhinal cortex and hippocampus volume by removing the DHA predictor from the model which may have pulled variance from the *APOE* genotype. No main effects of *APOE* were found on entorhinal cortex volume (left: t=-1.71 p=0.09; right: t=-0.47, p=0.64) or hippocampus volume (left: t=-0.76, p=0.44; right t=-1.44, p=0.15), adjusting for age, sex, education, test site and total intracranial volume. See supplementary Table 1 for a summary of the DHA effects on brain volume.

DHA associations with spatial navigation

In cohort two, we examined associations between erythrocyte DHA (the available blood DHA measure), and navigation processes known to be vulnerable to early AD. Specifically, we tested DHA associations with boundary-based place memory and egocentric path integration (n=46). Both processes tap into grid-cell mechanisms in the entorhinal cortex, which translate information to place cells in the hippocampus (Shine *et al.*, 2019). There was a main effect of DHA on boundary-based place memory, which was marginally significant (t=-2.017, p=0.058). *APOE* genotype modulated DHA associations with egocentric path integration (t=-2.06, p=0.04), with DHA inversely associated with path integration (*b*=-.834, t=2.69, p=0.01) in ε3ε4, but not in ε3ε3 (*b*=.31, t=1.487, p=.153; see Figure 2). These findings imply that higher circulating DHA predicts worse path integration performance in the ε4 carrier group only. See supplementary Table 1 for a summary of effects on spatial navigation.

DHA associations with brain regions beyond the medial temporal lobe

Finally, we investigated if the *APOE* genotype and DHA interact to predict volumes of other AD vulnerable brain regions in the human spatial navigation network, namely the precuneus and posterior cingulate cortex. No significant interactions (or main effects of serum free DHA or LPC DHA) on brain volume were found (see supplementary Table 2) suggesting that the effects of DHA are concentrated in the entorhinal cortex and hippocampus.

DISCUSSION

Our findings imply that the *APOE* ε4 allele alters associations between circulating DHA and volumes of the entorhinal cortex and hippocampus in non-dementia adults, almost a decade before the expected age of AD onset. Circulating serum DHA predicted greater entorhinal cortex volume, with a significant interaction between DHA and APOE genotype. As predicted, the positive association between DHA and entorhinal volume was evident in *APOE* ε4 non-carriers only. Our results also show that in *APOE ε4* carriers, serum DHA was inversely correlated with path integration (path integration is a process used in spatial navigation). We propose that the impaired blood brain barrier function and reduced DHA transport to the entorhinal cortex and hippocampus as an explanation for this. Unexpectedly, serum DHA was not positively associated with path integration in *APOE* ε4 non-carriers, which could be due to the small-moderate sample size. Together, the results imply that disrupted DHA absorption from the blood to the brain may exist in the genetically-at-risk of AD adult population, and may mediate navigation deficits seen in adults genetically-at-risk or preclinical AD cohorts.

Serum DHA predicted greater entorhinal volume in non-demented older adults, consistent with previous findings for a beneficial influence of circulating DHA (from serum or erythrocytes) on brain health. (Tan *et al.*, 2012; Yassine *et al.*, 2016; Ammann *et al.*, 2017; Yassine, 2017; Zhang *et al.*, 2017) The entorhinal cortex has one of the highest concentrations of lipoprotein receptors in the brain (due to the presence of APOE receptors LRP1) which are involved in DHA tissue delivery to neurons and clearance of amyloid βeta (Lane-Donovan et al., 2014), potentially explaining why DHA was associated with both these regions and not the precuneus cortex or the posterior cingulate cortex. However, the beneficial association of DHA with brain volume was exclusive to ε4 non-carriers in our study, consistent with two similar observational studies (Barberger-Gateau *et al.*, 2007; Whalley *et al.*, 2008). Daiello and colleagues previously showed that DHA supplementation predicted the preservation of the cerebral cortex gray matter and the hippocampus in *ε4* non-carriers only, (Daiello *et al.*, 2015) pointing to a neuroprotective effect of DHA that is at least partially exclusive to adults who do not bare the risk of the *ε4* allele.

The spatial navigation study from cohort two supported this theory. Among adults who did bare the risk of the *ε4* allele, circulating DHA predicted worse navigation proficiency. To the best of our knowledge, this is the first report of a significant association between circulating

DHA and AD vulnerable spatial navigation performance. Path integration, a sub-process involved in navigation ability, involves the capacity to use self-motion cues (or movements cues) to update and learn spatial location information in relation to a start location (Etienne and Jeffery, 2004) and is particularly vulnerable to early AD pathophysiology (Howett *et al.*, 2019). This process relies crucially on the structural integrity of the entorhinal cortex and hippocampus that were notably associated with serum DHA here (Hasselmo, 2008; Banino *et al.*, 2018). Almost a decade ago, He et al., demonstrated that increased brain DHA (via supplementation) significantly increased number of proliferating hippocampal cells and subsequently improved spatial learning performance in the Morris water maze (He *et al.*, 2009). In our *APOE* ε4 group, increased circulating DHA was associated with decreased navigation performance, supporting evidence that *APOE* ε4 disrupts blood-brain barrier function predicting cognitive decline (Chouinard-Watkins and Plourde, 2014; Yassine *et al.*, 2017; Montagne *et al.*, 2020).

A landmark paper by Montague and colleagues provides important insights into a deficit blood brain barrier transport system in *APOE* ε4 carriers. The authors report that *APOE ε4* carriers present with blood–brain barrier breakdown in the hippocampus and medial temporal lobes leading to cognitive decline. Lower brain uptake of DHA in older *APOE ε4* mice has also been shown to limit the accumulation of DHA in cerebral tissues, providing a potential mechanistic explanation for the inverse association between DHA and spatial navigation in *APOE ε4* shown here (Vandal *et al.*, 2014). Other explanations for APOE related changes in DHA metabolism beside blood brain barrier function include i) ε4 carrier status results in physiological dysregulation that is associated with both lower brain DHA uptake (and resultant higher blood levels) and deleterious changes to medial temporal lobe physiology and function, or ii) there is greater DHA uptake in adipose tissue for storage in *APOE ε4* carriers with less available for brain tissue absorption via the blood brain barrier. Other potential explanations for the increase in free serum DHA among *APOE ε4* may be that this reflects greater activation of phospholipase A2, which liberates esterified DHA from phospholipid (Gungor *et al.*, 2012), suggesting that the increase in DHA is a biomarker of another process such enhanced vascular inflammation, as opposed to being directly linked to AD pathology. All mechanisms warrant further investigation.

We examine the effect of *APOE ε4* on the association between circulating DHA, entorhinal cortex, hippocampal volume and spatial navigation, which is uncommon as most studies focus on memory or other cognitive functions. Strengths of our study include a rigours DHA

analysis, including the serum LPC-DHA lipid fraction, a comprehensive phenotyping of participants, adjusting for confounders, as well as the inclusion of a virtual reality spatial navigation diagnostic test of AD. The findings produced in this study have the following limitations however: 1) Although a unified model with an interaction term is the optimum method to test effect modification, an important limitation is that more statistical power is required than for association testing, and thus false-negative results may be seen in smaller samples. There were fewer ε4 carriers in cohort one, compared to cohort two, which may account for why in in *APOE ε4* carriers we found a significant inverse association between DHA and navigation performance, but a null association between DHA and entorhinal-hippocampal brain volume. 2) Likewise, the small sample sizes do not preclude the possibility that our findings could be observed by chance. These results will nevertheless play an important role in hypothesis-generating for future cross-sectional studies and RCTs. 3) Non-fasted blood samples from cohort two mean that if participant had a meal that was very high in DHA prior to testing that this may influence their DHA measurement, compared to a fasted sample. 4) While spatial navigation crucially relies on the integrity and function of the entorhinal cortex and the hippocampus, we cannot directly relate the participants across cohort one and two. Future studies should thus examine if entorhinal and hippocampal brain volume directly mediates the relationships between DHA and spatial navigation. 5) Given the observational nature of the study, and that DHA (fish intake) is a component of an overall healthy diet (Weichselbaum *et al.*, 2013), we cannot preclude the possibility of confounding residual and that DHA-brain phenotype associations were attributable to other dietary factors. To address this potential, confound, we tested various other dietary factors such as vegetable and fruit intake, which did not contribute to a significant amount of variance in the outcome variables of interest and therefore, where not retained in the downstream analysis. Given that brain and serum DHA has been previously linked to a range of neuro-protective processes in animal and human models, it is unlikely that the association are due to other diet derived bioactives.

In conclusion, we provide novel evidence that *APOE* genotype modifies DHA associations with brain volume and spatial navigation ability, typically affected in the first stages of AD. Future studies should examine the mechanisms behind the *APOE* genotype modulating effect of DHA, brain volume and cognitive function associations, particularly blood brain barrier integrity. Future positron emission tomography studies needed to measure rates of DHA incorporation from plasma into the brain (Yassine *et al.*, 2017), which would confirm if DHA

uptake to the brain is reduced in older *APOE ε4* carriers, leading to spatial navigation impairment. As over 50% of *APOE ε4* carriers do not develop clinical AD, longitudinal studies are clearly required to determine whether *APOE ε4,* coupled with disrupted DHA absorption to the brain, has diagnostic utility, and can predict conversion to clinical AD with a high degree of accuracy (Henderson *et al.*, 1995). Another important line of research will be to examine a therapeutic target in *APOE ε4* carriers to mitigate the negative effect on the allele on brain health. For example, it is possible that cyclophilin A inhibitors might suppress the pathway that is believed to cause blood brain barrier breakdown in the cerebral blood vessels of *APOE ε4* carriers, and thereby may slow spatial navigation impairment and other cognitive functions that rely on blood supply to the brain. (Stanciu *et al.*, 2019; Montagne *et al.*, 2020). Moreover, supplementation trials should focus on higher doses of DHA to ensure adequate brain delivery in *APOE ε4* carriers, as previous suggested (Arellanes *et al.*, 2020). Finally, understanding whether different blood lipid fractions differentially supply DHA to the entorhinal cortex and hippocampus in humans may refine DHA intervention approaches in treatment trials for AD.

ACKNOWLEDGEMENTS

We would like the Centre for Nutrition Learning and Memory, University of Illinois, US for funding the Cognitive Ageing Nutrition and Neurogenesis (CANN) trial, from where cohort one was sourced (funding grant UIUC Project #: 2013-04949). We would also like to thank the Faculty of Medical and Health Science, at University of East Anglia, UK who funded the collection of data for cohort two. Finally, we would like to thank the many participants who contributed their time and efforts to the research endeavours outlined in this article.

FUNDING INFORMATION

The Centre for Nutrition Learning and Memory, University of Illinois, US funded the Cognitive Ageing Nutrition and Neurogenesis (CANN) trial, from where the neuroimaging database was sourced (funding grant UIUC Project #: 2013-04949). The Faculty of Medical and Health Science, at University of East Anglia, UK funded the collection of data for the spatial navigation dataset.

DISCLOSURES

DJW has received research funding from Abbott Nutrition, Arla Foods, Bayer Healthcare, Fonterra and consultancy/speaker honoraria from Neurobrands, Naturex and Bayer Healthcare. Drs A.M.M., G.C., A.S., R.L., M.K., M.I., R.G., C.L., and M.H., report no disclosure

REFERENCES

Ammann EM, Pottala J V., Robinson JG, Espeland MA, Harris WS. Erythrocyte omega-3 fatty acids are inversely associated with incident dementia: Secondary analyses of longitudinal data from the Women's Health Initiative Memory Study (WHIMS). Prostaglandins Leukot Essent Fat Acids 2017; 121: 68–75.

Arellanes IC, Choe N, Solomon V, He X, Kavin B, Martinez AE, et al. Brain delivery of supplemental docosahexaenoic acid (DHA): A randomized placebo-controlled clinical trial. EBioMedicine 2020; 59: 1–8.

Arsenault D, Julien C, Tremblay C, Calon F. DHA improves cognition and prevents dysfunction of entorhinal cortex neurons in 3xTg-AD mice. PLoS One 2011; 6

Banino A, Barry C, Uria B, Blundell C, Lillicrap T, Mirowski P, et al. Vector-based navigation using grid-like representations in artificial agents. Nature 2018; 557: 429–433.

Barberger-Gateau P, Raffaitin C, Letenneur L, Berr C, Tzourio C, Dartigues JF, et al. Dietary patterns and risk of dementia: The Three-City cohort study. Neurology 2007; 69: 1921–1930.

Carboni S, Kaur G, Pryce A, McKee K, Desbois AP, Dick JR, et al. Mussel Consumption as a 'Food First' Approach to Improve Omega-3 Status. Nutrients 2019; 11: 1–11.

Chouinard-Watkins R, Plourde M. Fatty acid metabolism in carriers of apolipoprotein e epsilon 4 allele: Is it contributing to higher risk of cognitive decline and coronary heart disease? Nutrients 2014; 6: 4452–4471.

Corder E, Saunders A, Strittmatter W, Schmechel D, Gaskell P, Small G, et al. E Type 4 Allele Gene Dose of Apolipoprotein and the Risk of Alzheimer ' s Disease in Late Onset Families. Science (80-) 1993; 261: 921–923.

526 Coughlan G, Coutrot A, Khondoker M, Minihane A-M, Spiers H, Hornberger M. Toward
527 personalized cognitive diagnostics of at-genetic-risk Alzheimer's disease. Proc Natl Acad Sci
528 2019: 201901600.

529 Coughlan G, Laczó J, Hort J, Minihane A-M, Hornberger M. Spatial navigation deficits —
530 overlooked cognitive marker for preclinical Alzheimer disease? Nat Rev Neurol 2018; 14:
531 496–506.

532 Coughlan G, Zhukovsky P, Puthusseryppady V, Gillings R, Minihane AM, Cameron D, et al.
533 Functional connectivity between the entorhinal and posterior cingulate cortices underpins
534 navigation discrepancies in at-risk Alzheimer's disease. Neurobiol Aging 2020; 90: 110–118.

535 Daiello LA, Gongvatana A, Dunsiger S, Cohen RA, Ott BR. Association of fish oil
536 supplement use with preservation of brain volume and cognitive function. Alzheimer's
537 Dement 2015; 11: 226–235.

538 Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, et al. An automated
539 labeling system for subdividing the human cerebral cortex on MRI scans into gyral based
540 regions of interest. Neuroimage 2006; 31: 968–980.

541 Devore EE, Grodstein F, Van Rooij FJA, Hofman A, Rosner B, Stampfer MJ, et al. Dietary
542 intake of fish and omega-3 fatty acids in relation to long-term dementia risk. Am J Clin Nutr
543 2009; 90: 170–176.

544 Elwood RW. The California Verbal Learning Test: Psychometric characteristics and clinical
545 application. Neuropsychol Rev 1995; 5: 173–201.

546 Etienne AS, Jeffery KJ. Path integration in mammals. Hippocampus 2004; 14: 180–192.

547 Fedorova I, Hussein N, Martino C. An n-3 Fatty Acid Deficient Diet Affects Mouse Spatial
548 Learning in the Barnes Circular Maze. Prostaglandins Leukot Essent Fat Acids 2007; 77:
549 269–277.

550 Gungor Z, Anuurad E, Enkhmaa B, Zhang W, Kim K, Berglund L. Apo E4 and lipoprotein-
551 associated phospholipase A2 synergistically increase cardiovascular risk. Atherosclerosis
552 2012; 223: 230–234.

553 Hasselmo ME. Grid cell mechanisms and function: Contributions of entorhinal persistent
554 spiking and phase resetting. Hippocampus 2008; 18: 1213–1229.

He C, Qu X, Cui L, Wang J, Kang JX. Improved spatial learning performance of fat-1 mice is associated with enhanced neurogenesis and neuritogenesis by docosahexaenoic acid. Proc Natl Acad Sci U S A 2009; 106: 11370–11375.

Henderson AS, Jorm AF, Korten AE, Christensen H, Jacomb PA, Easteal S, et al. Apolipoprotein E allele ∈4, dementia, and cognitive decline in a population sample. Lancet 1995; 346: 1387–1390.

Howett D, Castegnaro A, Krzywicka K, Hagman J, Marchment D, Henson R, et al. Differentiation of mild cognitive impairment using an entorhinal cortex-based test of virtual reality navigation [Internet]. Brain 2019Available from: https://academic.oup.com/brain/advance-article/doi/10.1093/brain/awz116/5497752

Irvine MA, Scholey A, King R, Gillings R, Vauzour D, Demichele SJ, et al. The Cognitive Ageing, Nutrition and Neurogenesis (CANN) trial: Design and progress. Alzheimer's Dement Transl Res Clin Interv 2018; 4: 591–601.

Lacombe RJS, Chouinard-Watkins R, Bazinet RP. Brain docosahexaenoic acid uptake and metabolism. Mol Aspects Med 2018; 64: 109–134.

Larson EB, Yaffe K, Langa KM. New Insights into the Dementia Epidemic. N Engl J Med 2013; 71: 233–236.

Lester AW, Moffat SD, Wiener JM, Barnes CA, Wolbers T. The Aging Navigational System. Neuron 2017; 95: 1019–1035.

Lewis F, Schaffer SK, Sussex J, O'Neill P, Cockcroft L. The trajectory of dementia in the UK – making a difference. Rep Alzheimer's Res UK by OHE Consult 2014

Lim SY, Hoshiba J, Moriguchi T, Salem N. N-3 fatty acid deficiency induced by a modified artificial rearing method leads to poorer performance in spatial learning tasks. Pediatr Res 2005; 58: 741–748.

Lim WS, Gammack JK, Van Niekerk J, Dangour a D. Omega 3 fatty acid for the prevention of dementia. Cochrane Database Syst Rev 2006: CD005379.

Liu C-C, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat Rev Neurol 2013; 9: 106–118.

583 MacLean B, Tomazela DM, Shulman N, Chambers M, Finney GL, Frewen B, et al. Skyline:
584 An open source document editor for creating and analyzing targeted proteomics experiments.
585 Bioinformatics 2010; 26: 966–968.

586 Montagne A, Nation DA, Sagare AP, Barisano G, Sweeney MD, Chakhoyan A, et al. APOE4
587 leads to blood–brain barrier dysfunction predicting cognitive decline. Nature 2020: 1–6.

588 Nasreddine Z. The Montreal Cognitive Assessment, MoCA: A Brief Screening Tool For
589 Mild Cognitive Impairment. Water Resour Res 2005; 53: 695–699.

590 Oksman M, Iivonen H HE. Impact of different saturated fatty acid, polyunsaturated fatty acid
591 and cholesterol containing diets on beta-amyloid accumulation in APP/PS1 transgenic mice.
592 Neurobiol Disord 2006; 23: 563–572.

593 Peng B, Ahrends R. Adaptation of Skyline for Targeted Lipidomics. J Proteome Res 2016;
594 15: 291–301.

595 Pottala J V., Yaffe K, Robinson JG, Espeland MA, Wallace R, Harris WS. Higher RBC EPA
596 1 DHA corresponds with larger total brain and hippocampal volumes: WHIMS-MRI Study.
597 Neurology 2014; 82: 435–442.

598 Schaefer EJ, Bongard V, Beiser AS, Lamon-Fava S, Robins SJ, Au R, et al. Plasma
599 phosphatidylcholine docosahexaenoic acid content and risk of dementia and alzheimer
600 disease: The framingham heart study. Arch Neurol 2006; 63: 1545–1550.

601 Shine JP, Valdés-Herrera JP, Tempelmann C, Wolbers T. Evidence for allocentric boundary
602 and goal direction information in the human entorhinal cortex and subiculum. Nat Commun
603 2019; 10: 1–10.

604 Stanciu C, Trifan A, Muzica C, Sfarti C. Efficacy and safety of alisporivir for the treatment
605 of hepatitis C infection. Expert Opin Pharmacother 2019; 20: 379–384.

606 Sugasini D, Thomas R, Yalagala PCR, Tai LM, Subbaiah P V. Dietary docosahexaenoic acid
607 (DHA) as lysophosphatidylcholine, but not as free acid, enriches brain DHA and improves
608 memory in adult mice. Sci Rep 2017; 7: 1–11.

609 Sugasini D, Yalagala PCR, Goggin A, Tai LM, Subbaiah P V. Enrichment of brain
610 docosahexaenoic acid (DHA) is highly dependent upon the molecular carrier of dietary DHA:
611 lysophosphatidylcholine is more efficient than either phosphatidylcholine or triacylglycerol. J

612 Nutr Biochem 2019; 74: 108231.

613 Sugasini D, Yalagala PCR, Subbaiah P V. Plasma BDNF is a more reliable biomarker than
614 erythrocyte omega-3 index for the omega-3 fatty acid enrichment of brain. Sci Rep 2020; 10:
615 1–10.

616 Tan ZS, Harris WS, Beiser AS, Au R, Himali JJ, Debette S, et al. Red blood cell omega-3
617 fatty acid levels and markers of accelerated brain aging. Neurology 2012; 78: 658–664.

618 Tomaszewski N, He X, Solomon V, Lee M, Mack WJ, Quinn JF, et al. Effect of APOE
619 Genotype on Plasma Docosahexaenoic Acid (DHA), Eicosapentaenoic Acid, Arachidonic
620 Acid, and Hippocampal Volume in the Alzheimer's Disease Cooperative Study-Sponsored
621 DHA Clinical Trial. J Alzheimer's Dis 2020; 74: 975–990.

622 Tu S, Spiers HJ, Hodges JR, Piguet O, Hornberger M. Egocentric versus Allocentric Spatial
623 Memory in Behavioral Variant Frontotemporal Dementia and Alzheimer's Disease. J
624 Alzheimer's Dis 2017; 59: 883–892.

625 Tu S, Wong S, Hodges JR, Irish M, Piguet O, Hornberger M. Lost in spatial translation - A
626 novel tool to objectively assess spatial disorientation in Alzheimer's disease and
627 frontotemporal dementia. Cortex 2015; 67: 83–94.

628 Vandal M, Alata W, Tremblay C, Rioux-Perreault C, Salem N, Calon F, et al. Reduction in
629 DHA transport to the brain of mice expressing human APOE4 compared to APOE2. J
630 Neurochem 2014; 129: 516–526.

631 Vauzour D, Camprubi-Robles M, Miquel-Kergoat S, Andres-Lacueva C, B??n??ti D,
632 Barberger-Gateau P, et al. Nutrition for the ageing brain: Towards evidence for an optimal
633 diet. Ageing Res Rev 2017; 35: 222–240.

634 Weichselbaum E, Coe S, Buttriss J, Stanner S. Fish in the diet: A review. Nutr Bull 2013; 38:
635 128–177.

636 Whalley LJ, Deary IJ, Starr JM, Wahle KW, Rance KA, Bourne VJ, et al. n-3 Fatty acid
637 erythrocyte membrane content, APOE ε4, and cognitive variation: An observational follow-
638 up study in late adulthood. Am J Clin Nutr 2008; 87: 449–454.

639 Whiley L, Godzien J, Ruperez FJ, Legido-quigley C, Barbas C. In-Vial Dual Extraction for
640 Direct LC-MS Analysis of Plasma for Comprehensive and Highly Reproducible Metabolic

Fingerprinting. Anal Chem 2012; 84: 5992−5999.

Yassine. Association of Docosahexaenoic Acid Supplementation With Alzheimer Disease Stage in Apolipoprotein E ε4 Carriers: A Review [Internet]. JAMA Neurol 2017Available from: https://academic.oup.com/qjmed/article-lookup/doi/10.1093/qjmed/94.10.521

Yassine HN, Croteau E, Rawat V, Hibbeln JR, Rapoport SI, Cunnane SC, et al. DHA brain uptake and APOE4 status: a PET study with [1-11C]-DHA. Alzheimers Res Ther 2017; 9: 23.

Yassine HN, Feng Q, Azizkhanian I, Rawat V, Castor K, Fonteh AN, et al. Association of Serum Docosahexaenoic Acid With Cerebral Amyloidosis. JAMA Neurol 2016; 73: 1208–1216.

Zamroziewicz MK, Paul EJ, Rubin RD, Barbey AK. Anterior cingulate cortex mediates the relationship between O3PUFAs and executive functions in APOE e4 carriers. Front Aging Neurosci 2015; 7: 1–7.

Zhang YP, Miao R, Li Q, Wu T, Ma F. Effects of DHA Supplementation on Hippocampal Volume and Cognitive Function in Older Adults with Mild Cognitive Impairment: A 12-Month Randomized, Double-Blind, Placebo-Controlled Trial. J Alzheimer's Dis 2017; 55: 497–507.

FIGURE LEGENDS

Figure 1. **Serum DHA associations with entorhinal and hippocampal brain volume from cohort one (n=53).**

A-B There was a significant interaction between *APOE* genotype and DHA on left entorhinal volume. In ε3ε3 carriers, serum free DHA was significantly associated with right entorhinal volume and explained 20% of volume variability (R^2 = .20, p=.005). Serum free DHA explained 8% of the variability in the left entorhinal volume (R^2 = .75, p=100). **C-D** No interaction between serum free DHA levels and *APOE* genotype on hippocampal volume was found, and no main effects of serum fre/ DHA on hippocampal volume were found, although there was a trend toward significance. Confidence intervals represented by dotted curve lines are shown in for associations in the ε3ε3 groups.

Figure 2. **DHA association with spatial navigation performance from cohort two (n=46).** There was a significant interaction between *APOE* genotype and DHA on left egocentric path integration. Total DHA in erythrocytes was inversely related to egocentric path integration in cognitively intact *APOE ε3ε4* carriers only. No significant association was found in the ε3ε3 carrier group.