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Associations of erythrocyte omega-3 fatty acids with cognition, brain imaging and biomarkers in the Alzheimer's disease neuroimaging initiative: cross-sectional and longitudinal retrospective analyses

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ABSTRACT

Background: The association between omega-3 (ω-3) PUFAs and cognition, brain imaging and biomarkers is still not fully established.

Objectives: The aim was to analyze the cross-sectional and retrospective longitudinal associations between erythrocyte ω-3 index and cognition, brain imaging, and biomarkers among older adults.

Methods: A total of 832 Alzheimer's Disease Neuroimaging Initiative 3 (ADNI-3) participants, with a mean (SD) age of 74.0 (7.9) y, 50.8% female, 55.9% cognitively normal, 32.7% with mild cognitive impairment, and 11.4% with Alzheimer disease (AD) were included. A low ω-3 index (%EPA + %DHA) was defined as the lowest quartile ($\leq$3.70%). Cognitive tests [composite score, AD Assessment Scale Cognitive (ADAS-Cog), Wechsler Memory Scale (WMS), Trail Making Test, Category Fluency, Mini-Mental State Examination, Montreal Cognitive Assessment] and brain variables [hippocampal volume, white matter hyperintensities (WMHs), positron emission tomography (PET) amyloid-β (Aβ) and tau] were considered as outcomes in regression models.

Results: Low ω-3 index was not associated with cognition, hippocampal, and WMH volume or brain Aβ and tau after adjustment for demographics, *ApoEε4*, cardiovascular disease, BMI, and total intracranial volume in the cross-sectional analysis. In the retrospective analysis, low ω-3 index was associated with greater Aβ accumulation (adjusted $\beta = 0.02$; 95% CI: 0.01, 0.03; $P = 0.003$). The composite cognitive score did not differ between groups; however, low ω-3 index was significantly associated with greater WMS-delayed recall cognitive decline (adjusted $\beta = -1.18$; 95% CI: –2.16, –0.19; $P = 0.019$), but unexpectedly lower total ADAS-Cog cognitive decline. Low ω-3 index was cross-sectionally associated with lower WMS performance (adjusted $\beta = -1.81$, SE = 0.73, $P = 0.014$) and higher tau accumulation among *ApoE ε4* carriers.

Conclusions: Longitudinally, low ω-3 index was associated with greater Aβ accumulation and WMS cognitive decline but unexpectedly with lower total ADAS-Cog cognitive decline. Although no associations were cross-sectionally found in the whole population, low ω-3 index was associated with lower WMS cognition and higher tau accumulation among *ApoE ε4* carriers. The Alzheimer's Disease Neuroimaging Initiative (ADNI) is registered at clinicaltrials.gov as NCT00106899. *Am J Clin Nutr* 2022;116:1492–1506.

Keywords: omega-3, cognition, brain imaging, biomarkers, Alzheimer disease, mild cognitive impairment, docosahexaenoic acid, eicosapentaenoic acid

Introduction

Omega-3 (ω-3) PUFAs, mainly DHA and EPA, have received substantial attention due to their beneficial effect on brain functioning (1). Several studies reported associations between higher ω-3 PUFA intake or blood concentrations and lower cognitive decline, lower risk of dementia, or preserved brain

structure (2); however, much less is known about the association of erythrocyte ω-3 fatty acids with brain imaging and biomarkers of cognitive function.

Erythrocyte ω-3 index, defined as the sum of EPA and DHA proportion from the total membrane lipid content, represents a more reliable measurement of nutritional status since fatty acids are stable in the erythrocytes' membranes for up to 3 mo (3). In the Multidomain Alzheimer Preventive Trial (MAPT), dementia-free older adults with a low ω-3 index were at greater risk of cognitive decline (4). Consistent results were found among dementia-free Framingham Study participants, with lower erythrocyte DHA being associated with smaller brain volumes and a vascular pattern of cognitive impairment (5). In animal studies, DHA and EPA have also been shown to reduce amyloid-β (Aβ) peptide production, particularly by altering amyloid precursor protein (APP) processing (6). In the MAPT study, multidomain interventions both with and without ω-3 PUFA supplementation were associated with lower cerebral Aβ (7). To our knowledge, only 1 study investigated the association between serum DHA concentration and Aβ positron emission tomography (PET) imaging, and reported an inverse correlation with brain Aβ load (8). Although even less is known, DHA could also additionally contribute to the inhibition of tau phosphorylation (9). Finally, the beneficial effects of ω-3 PUFA on cognition have been most often seen in cognitively healthy individuals or individuals with mild cognitive impairment (MCI) (10). A recent Cochrane meta-analysis found no convincing evidence for the efficacy of ω-3 PUFA supplements in the treatment of mild to moderate Alzheimer disease (AD) (11). Interestingly, previous studies also showed that the presence of the *ApoE ε4* allele might modify the relation between ω-3 PUFAs and brain functioning, but results are still conflicting (12–14). Overall, in the literature, most studies were only cross-sectional and investigated ω-3 intake or plasma/serum measurements and their association with cognitive function, with very few analyses on brain imaging biomarkers, especially PET tau, and on the potential modifying effect of *ApoE ε4* genotype.

We therefore aimed to assess the cross-sectional and retrospective longitudinal associations between erythrocyte ω-3 index, cognition, and neuroimaging biomarkers [hippocampal and white matter hyperintensity (WMH) volume, amyloid and tau PET] among community-dwelling older adults. Subgroup analyses were also conducted according to cognitive status [cognitively normal (CN), MCI, and AD] and *ApoE ε4* genotype. We hypothesized that participants with low erythrocyte ω-3 index would present lower cognitive function, lower hippocampal volume, higher WMH volume, and brain amyloid and tau accumulation.

Methods

Study design and population

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI, a multicenter observational study conducted in 60 sites in the United States and Canada (15), was launched in 2003 as a public–private partnership, led by principal investigator Michael W. Weiner, MD. The primary goal of ADNI was to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessment could be combined to measure the progression of MCI and early AD. After 3 waves [ADNI-1, ADNI-Grand Opportunity (ADNI-GO), and ADNI-2], the ongoing ADNI-3 wave began on 1 August 2016. Older adults aged 55 to 97 y with CN status (with or without subjective memory concerns), MCI (both early and late MCI), or AD, newly enrolled in ADNI-3 or previously recruited in other ADNI waves, were included in our study. Main exclusion criteria were having a major neurologic or psychiatric illness; a history of substance abuse; or a screening MRI showing evidence of infection, infarction, or other focal lesions (including multiple lacunes, or lacunes in a critical memory structure). Detailed inclusion and exclusion criteria for joining ADNI according to each of these 3 cognitive status categories are described elsewhere (16). A study flowchart is presented in **Figure 1**. Among the 849 participants with fatty acid measurements during the ADNI-3 wave, 832 were included in our cross-sectional analysis (cognition, brain imaging, and biomarkers measured simultaneously in the ADNI-3 wave). After exclusion of 482 participants without ADNI-1, ADNI-GO, or ADNI-2 data, 350 participants were included in our retrospective longitudinal analysis (cognition, brain imaging, and biomarkers assessed over time using data from all waves). ADNI-3 was registered in www.ClinicalTrials.gov under the protocol NCT02854033. All subjects were informed about the aims of the study and signed a consent form.

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Supplemental Figures 1–3 and Supplemental Tables 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: Aβ, amyloid-β; AD, Alzheimer disease; ADAS-Cog, Alzheimer's Disease Assessment Scale cognitive subscale; ADNI, Alzheimer's Disease Neuroimaging Initiative; ADNI-GO, ADNI-Grand Opportunity; CDR-SB, Clinical Dementia Rating Sum of Boxes; CN, cognitively normal; MAPT, Multidomain Alzheimer Preventive Trial; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; PET, positron emission tomography; RCT, randomized controlled trial; SUVr, standard uptake value ratio; TIV, total intracranial volume; TMT, Trail Making Test; WMH, white matter hyperintensity; WMS, Wechsler Memory Scale; 3C, Three-City.

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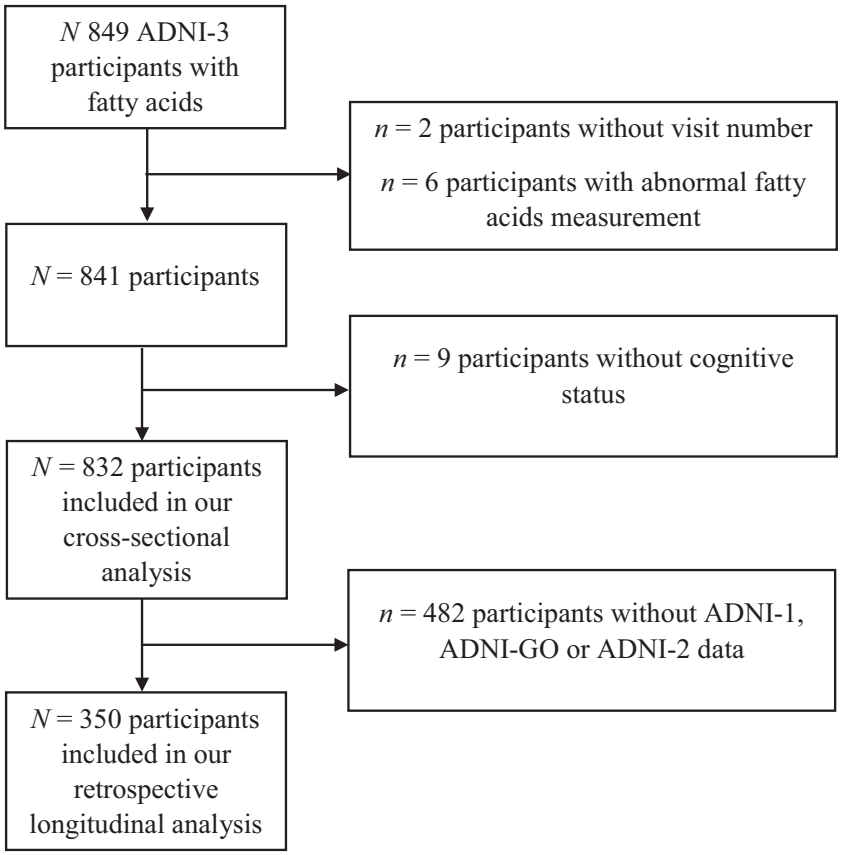

FIGURE 1 Study flowchart. ADNI, Alzheimer's Disease Neuroimaging Initiative; ADNI-GO, ADNI-Grand Opportunity.

Isolation of erythrocyte and fatty acid measurement

Fatty acids were measured during the ADNI-3 wave. RBCs were isolated from whole blood (10 mL) collected into EDTA-coated tubes, according to the standardized procedures at ADNI sites (ω-3 PUFA measurement is not affected by the presence of anticoagulant). The blood was centrifuged at 3000 × *g* for 15 min at 4°C. This results in the formation of an RBC pellet, an intermediate layer containing the leukocytes and platelets (buffy coat) and an upper phase comprising plasma. Following removal of the plasma and buffy coat, RBCs were stored immediately at –80°C in EDTA-coated tubes. Fatty acid analysis was performed at the biochemical laboratory of Institut Agro Rennes, France, according to a previous publication (17). Briefly, lipids were extracted twice from RBC (475 to 525 mg) samples with a mixture of hexane/isopropanol (3:2 vol:vol), after acidification with 1 mL HCl 3 M (18), containing 5 ppm butylated hydroxytoluene (BHT). Margaric acid (C17:0) was added as internal standard. Total lipid extracts were saponified with 1 mL of 0.5 M sodium hydroxide (NaOH) in methanol for 30 min at 70°C and methylated with 1 mL boron trifluoride (BF3) methanol solution (12% wt:vol in methanol) for 15 min at 70°C. FAMEs were extracted twice with pentane. Solvent was removed under nitrogen and FAMEs were redissolved in 200 μL hexane. Analyses were performed using an Agilent Technologies 7890 N gas chromatograph (Bios Analytic) fitted with a split injector (10:1) at 250°C (injection volume 1 μL) and a bonded silica capillary column (BPX 70, 60-m long, 250-μm inner diameter and 0.25-μm film thickness; SGE). Helium was used as a carrier gas (constant flow: 1.8 mL/min, 36 cm/s). The column temperature program started at 170°C, increased by 4°C/min to 250°C, and held at 250°C for 2 min. Transfer line was at 270°C. Mass spectra were obtained with an Agilent 5975C spectrometer used In electron impact mode (EI) with 70 eV energy; source temperature was set at 150°C and quadrupole at 230°C. Acquisition was performed in the full scan mode, ranging from 50 to 500 atomic mass units (amu; 3 scans/s). Identification of FAMEs was based on retention times obtained for FAMEs prepared from fatty acid standards and confirmed by comparison of their MS spectra with those of the NIST (National Institute of Standards and Technology) bank (V.2.2,2014). Quantification was achieved by determining the area under the peaks with Mass Hunter software (version B.07.00 SP2, 2015; Agilent).

Results of EPA and DHA are expressed as percentage of total identified fatty acids. ω-3 index was calculated as the sum of %EPA and %DHA, and thus also expressed in percentages from total fatty acids. A low ω-3 index was characterized by the lowest quartile within the populations investigated ($\leq$3.70% for the cross-sectional analysis and $\leq$3.62% for the retrospective longitudinal analysis). The distribution of the ω-3 index is shown in **Supplemental Figure 1**.

Cognition, brain imaging, and biomarkers

Cognitive, brain imaging, and biomarker variables were collected over time in the different ADNI waves. The primary outcome was cognitive function, which was assessed using a modified form of the Alzheimer's Disease Cooperative Study Preclinical Alzheimer Cognitive Composite (ADCS-PACC) (19). The modified composite score included the delayed word recall from the Alzheimer's Disease Assessment Scale–Cognitive subscale (ADAS-Cog) (20), the Trail Making Test B (TMT-B) (21), the Mini-Mental State Examination (MMSE) total score (22), and the Wechsler Memory Scale (WMS) delayed recall (23). The modified composite score was determined using standardized z scores. These were calculated by dividing each of the component scores by the sample SD of that component and by subtracting each of the component scores by the mean of that component. The z scores of delayed recall from the ADAS-Cog and from the TMT-B were then multiplied by −1 to account for the fact that, differently than the other tests, higher scores mean worse cognitive performance. All 4 z scores were then summed to form the composite score. For all subjects, the MMSE total score, WMS-delayed recall, WMS-immediate recall, Category Fluency test (24), Montreal Cognitive Assessment (MoCA) (25) (higher scores mean better cognitive function for these 5 tests) and delayed word recall ADAS-Cog, total ADAS-Cog, TMT-A, TMT-B, Clinical Dementia Rating Sum of Boxes (CDR-SB) (26) (higher scores mean worse cognitive function for these 5 tests) were also examined. Total MMSE, ADAS-Cog, MOCA, and CDR-SB were used to assess global cognition; ADAS-Cog delayed recall and WMS immediate and delayed recall were used to assess memory and category fluency, TMT-A and TMT-B tests were used to assess executive function.

Secondary outcomes were brain imaging and biomarker variables and included hippocampal subcortical volume (in the left hemisphere, right hemisphere, and total), WMH volume (27), assessed by 3 Tesla (3T) MRI, and brain Aβ and hyperphosphorylated paired helical filaments (PHF) tau, measured by PET. MRI was performed using unified parameter scanning protocols available on https://adni.loni.usc.edu/methods/documents/mri-protocols/. Amyloid positive status (Aβ+) was defined as ^{18}F-florbetapir PET cortical standardized uptake value ratio (SUVr) normalized by the whole cerebellum reference region (SUVr) >1.11 for the cross-sectional analysis and SUVr based on the composite reference region (SUVr >0.78) for the longitudinal analysis (28). Tau PET was assessed during the ADNI-3 wave in the meta-temporal region and in Braak stages 1 to 6 (29) after intravenous bolus injection of [^{18}F]-radiolabeled AV1451.

Potential confounders

Potential confounders included the following: age (years), education (years), BMI (calculated as body weight in kg divided by height in m^2), *ApoE ε4* genotype (carriers, noncarriers), history of cardiovascular disease (yes, no), and total intracranial volume (TIV; cm^3); this last potential confounder was only for WMH and hippocampal volume.

Statistical analysis

Characteristics of the population are presented as mean (SD) for continuous variables and as frequencies and percentages for categorical variables. They were compared according to ω-3 index (first quartile vs. others) using Student's t-test for continuous variables (with log transformation if necessary) with normal distribution (or nonparametric Kruskal–Wallis test for other quantitative variables) and Pearson's chi-square test for qualitative variables. In cross-sectional analyses, linear regression models were performed to assess the association between ω-3 index (first quartile vs. others); cognitive, brain imaging, and biomarker variables; and whether the associations differed according to cognitive status (CN, MCI, and AD) or ApoE4 genotype (carriers vs. noncarriers). For this purpose, an interaction in each model was introduced between ω-3 index and cognitive status or *ApoE ε4* genotype. If interactions were significant or marginally significant ($P < 0.10$), within-group estimates were further explored using model estimates with interaction. Linear mixed models with random intercepts and slopes were used in the longitudinal analyses. Differences in retrospective change in cognitive, brain imaging, and biomarker variables between participants in the lowest ω-3 index quartile and those in the other 3 quartiles were assessed at 24, 36, 60, and 120 mo, except for MoCA, WMH volume, and amyloid PET (whose maximum retrospective follow-up was 6 y). Analyses were adjusted for potential confounders [age, education, BMI, *ApoE ε4* genotype, history of cardiovascular disease, and TIV (the last one only for WMH and hippocampal volume)]. Given the exploratory nature of our study, we did not correct for multiplicity. We finally conducted cross-sectional and longitudinal sensitivity analyses by excluding participants with AD and also investigated among CN and individuals with MCI whether the associations differed according to amyloid status [Aβ+ vs. amyloid negative status (Aβ−)]. Analyses were performed using the SAS version 9.4 (SAS Institute), with a significance level established as 5%.

Results

We included 832 individuals [mean (SD) age: 74.0 (7.9) y; 50.8% female; 37.7% *ApoE ε4* carriers]: 465 (55.9%) had a CN status, 272 (32.7%) had MCI, and 95 (11.4%) had AD. The mean ω-3 index was 4.85% (SD = 1.52). The distribution was as follows: median, 4.68%; IQR, 3.70–5.83%; minimum, 1.77%; maximum, 11.37%. Among fish-oil supplements users, only 45 (13.2%) were in the lowest ω-3 index quartile compared with 162 (33.1%) among nonusers ($P < 0.001$). Participants in the lowest ω-3 index quartile (≤3.70%) were less educated [mean (SD) years of education: 15.8 (2.4) vs. 16.8 (2.4); $P < 0.0001$] than those in the 3 other quartiles. No other differences were found in terms of population characteristics. The proportion of participants with low ω-3 index did not significantly differ according to cognitive status or ApoE ε4 genotype (**Table 1**).

In our cross-sectional analysis, low ω-3 index was not significantly associated with cognitive function (composite cognitive score or any cognitive test), hippocampal volume, WMH, brain Aβ and tau accumulation in unadjusted models, or after adjustment for age, education, ApoE ε4 genotype, history of cardiovascular disease, BMI, and TIV (the last one only

TABLE 1 Population characteristics and cognitive, brain imaging, and biomarker variables according to ω-3 index status (cross-sectional analysis)[1]

	Total (n = 832)	Lowest ω-3 index quartile (≤3.70%) (n = 209)	Three highest ω-3 index quartiles (>3.70%) (n = 623)	P	
				Unadjusted	Adjusted
Female sex, n (%)	423 (50.8%)	109 (52.2%)	314 (50.4%)	0.661***	—
Age, y	74.0 (7.9)	73.4 (8.6)	74.3 (7.7)	0.200*	—
Education, y	16.5 (2.5)	15.8 (2.4)	16.8 (2.4)	<0.0001**	—
BMI (n = 822), kg/m^2	27.3 (5.2)	27.8 (5.6)	27.1 (5.1)	0.077*	—
Cognitive status, n (%)					—
Cognitively normal	465 (55.9%)	117 (56.0%)	348 (55.9%)	0.682***	
Mild cognitive impairment	272 (32.7%)	65 (31.1%)	207 (33.2%)		
Alzheimer disease	95 (11.4%)	27 (12.9%)	68 (10.9%)		
ApoE ε4 genotype carriers (n = 794), n (%)	299 (37.7%)	69 (34y.2%)	230 (38.9%)	0.235***	—
History of cardiovascular disease (n = 820), n (%)	573 (69.9%)	141 (68.5%)	432 (70.4%)	0.605***	—
Cognitive tests					
Composite cognitive score[2] (n = 801)	0.00 (3.31)	− 0.13 (3.29)	0.04 (3.31)	0.534*	0.882
MMSE score (n = 828)	27.8 (3.0)	27.9 (2.9)	27.8 (3.1)	0.907**	0.350
ADAS-Cog delayed recall (n = 831)	3.9 (2.6)	3.8 (2.5)	3.9 (2.6)	0.987**	0.552
Total ADAS-Cog (n = 823)[3]	17.2 (8.6)	17.5 (8.7)	17.1 (8.6)	0.465*	0.968
Wechsler Memory Scale-delayed recall (n = 826)	10.5 (5.6)	10.0 (5.5)	10.7 (5.6)	0.109*	0.293
Wechsler Memory Scale-immediate recall (n = 828)	12.1 (5.2)	11.8 (5.0)	12.2 (5.2)	0.349*	0.949
Category Fluency test (words) (n = 829)	19.4 (6.1)	18.8 (5.4)	19.6 (6.3)	*0.089**	0.574
TMT-A (n = 825),[3] s	37.4 (21.2)	38.1 (21.1)	37.2 (21.3)	0.351*	0.513
TMT-B (n = 808),[3] s	97.4 (62.9)	98.5 (63.5)	97.0 (62.8)	0.714*	0.768
MoCA total score (n = 810)	24.2 (4.5)	24.0 (4.3)	24.2 (4.5)	0.508*	0.819
CDR sum of boxes (n = 829)	1.15 (2.05)	1.18 (2.09)	1.15 (2.04)	0.759**	0.995
CDR score >0 (n = 829)	362 (43.7)	93 (25.7)	269 (74.3)	0.780***	0.892
Brain imaging and biomarker variables					
Total hippocampal volume (n = 651), mm^3	7168.8 (1090.8)	7203.7 (1034.1)	7156.9 (1110.1)	0.634*	0.699
Left hippocampal subcortical volume, mm^3	3525.2 (546.5)	3547.3 (515.7)	3517.7 (556.8)	0.548*	0.504
Right hippocampal subcortical volume, mm^3	3643.5 (571.2)	3656.4 (546.8)	3639.2 (579.7)	0.739*	0.933
White matter hyperintensity volume (n = 773),[3] cm^3	5.6 (10.6)	5.1 (11.4)	5.7 (10.4)	0.294*	0.422
Cortical SUVr (n = 472)[3]	1.18 (0.24)	1.18 (0.24)	1.18 (0.24)	0.896*	0.561
Amyloid status (n = 472), n (%)					
Aβ positive (SUVr >1.11)	218 (46.2%)	57 (44.2%)	161 (46.9%)	0.593***	0.837
Aβ negative (SUVr ≤1.11)	254 (53.8%)	72 (55.8%)	182 (53.1%)		
Tau ^{18}F-AV1451 meta-temporal region (n = 693)[3]	1.93 (0.51)	1.94 (0.51)	1.93 (0.52)	0.761*	0.947
Tau ^{18}F-AV1451 Braak stage 1 (n = 693)[3]	2.19 (0.71)	2.18 (0.69)	2.19 (0.72)	0.955*	0.812
Tau ^{18}F-AV1451 Braak stage 2 (n = 693)	1.46 (0.27)	1.46 (0.27)	1.46 (0.27)	0.819*	0.650
Tau ^{18}F-AV1451 Braak stages 3 and 4 (n = 693)[3]	1.76 (0.39)	1.77 (0.39)	1.76 (0.39)	0.727*	0.919
Tau ^{18}F-AV1451 Braak stages 5 and 6 (n = 693)	1.79 (0.37)	1.82 (0.43)	1.78 (0.34)	0.285*	0.806

[1] Values are means (SDs) except where otherwise specified. Aβ, amyloid-β; ADAS, Alzheimer's Disease Assessment Scale; ADAS-Cog, Alzheimer's Disease Assessment Scale cognitive subscale; CDR, Clinical Dementia Rating; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; TMT, Trail Making Test; SUVr, standard uptake value ratio normalized by whole cerebellum.

[2] The modified composite score included the delayed word recall from the ADAS-Cog, the TMT-B, the MMSE total score, and the Wechsler Memory Scale delayed recall; ω-3 index defined as erythrocyte %DHA + %EPA; P values based on Student's t-test (∗), Kruskal–Wallis test (∗∗), or chi-square test (∗∗∗), after log transformation when needed.

[3] Variables with log transformation. Analyses adjusted for age, education, ApoE ε4 genotype, history of cardiovascular disease, BMI, and total intracranial volume (the last one only for white matter hyperintensities and hippocampal volume) with a linear regression model for the quantitative outcomes and with a logistic regression model for the qualitative outcomes.

for WMH and hippocampal volume) (Table 1). Interactions were found between low ω-3 index and *ApoE ε4* genotype (**Table 2**). Among the subgroup of *ApoE ε4* carriers, low ω-3 index was significantly associated in unadjusted models with lower performance on WMS-delayed and -immediate recall and with higher tau accumulation in several regions. After adjustment, a similar result with lower performance on WMS-delayed recall ($\beta = -1.81$, SE = 0.73, $P = 0.014$) was found and a tendency was observed with higher tau accumulation (**Table 3**). Interactions were also found between low ω-3 index and cognitive status on MMSE, MoCA, hippocampal volume, and Tau accumulation (**Supplemental Table 1**), with significantly better cognitive, brain imaging, and biomarker conditions among the subgroup of AD if ω-3 index was low (**Supplemental Table 2**). In our cross-sectional sensitivity analyses after exclusion of participants with AD, low ω-3 index was not significantly associated with cognitive function, WMH, brain Aβ load (SUVr), and tau accumulation after adjustment, but was significantly associated with lower right hippocampal volume ($\beta = -85.64$, SE = 43.03, $P = 0.047$). Interactions were found between low ω-3 index, *ApoE ε4* genotype, and amyloid status but not cognitive status. Among *ApoE ε4* carriers only, low ω-3 index was significantly associated with lower right hippocampal volume after adjustment ($\beta = -212.50$, SE = 73.39, $P = 0.004$) and with higher tau accumulation in several regions. Among Aβ+ participants only, we observed a trend toward statistical significance after adjustment between low ω-3 index and higher tau accumulation (Tau ^{18}F-AV1451 Braak stage 2: $\beta = 0.08$, SE = 0.04, $P = 0.078$).

In our retrospective longitudinal analysis, consistent across all time periods ($P = 0.003$), individuals in the lowest ω-3 index quartile had significantly greater Aβ accumulation over time compared with those in the 3 other quartiles [adjusted $\beta = 0.02$; 95% CI: 0.01, 0.03; $P = 0.003$ between M-60 and M0 (M) months] (**Table 4** and **Figure 2**). No significant differences were observed between the 2 ω-3 index groups for WMH volume or the composite cognitive score. WMS-delayed recall cognitive trajectories tended to differ (global $P = 0.066$) between individuals in the lowest ω-3 index quartile versus those in the 3 other quartiles (Table 4 and **Supplemental Figure 2**). In both groups, cognitive performance declined between M-60 and M0 months (lowest ω-3 index quartile: unadjusted $\beta = -1.25$; 95% CI: –2.12, –0.38; 3 highest ω-3 index quartiles: unadjusted $\beta = -0.08$; 95% CI: –0.59, 0.42). Compared with those in the 3 highest ω-3 index quartiles, individuals in the lowest ω-3 index quartile had a significant greater cognitive decline (adjusted $\beta = -1.18$; 95% CI: –2.16, –0.19; $P = 0.019$). Total ADAS-Cog cognitive trajectories were also different between the 2 ω-3 index groups, with a low ω-3 index unexpectedly associated with lower cognitive decline across certain time periods (Table 4 and Supplemental Figure 2). In our retrospective longitudinal analysis, cognitive, brain imaging, and biomarker trajectories between participants in the 2 ω-3 index groups did not statistically differ according to *ApoE ε4* genotype (**Supplemental Table 3**). Total MMSE, TMT-A, TMT-B, and MoCA cognitive trajectories between participants in the 2 ω-3 index groups significantly differed according to cognitive status, particularly in participants with AD compared with those with a CN status (**Supplemental Table 4** and **Supplemental Figure 3**). Unlike individuals with MCI or a CN status, whose cognitive trajectories did not significantly differ between ω-3 index groups, participants with AD in the lowest ω-3 index quartile compared with those in the 3 highest quartiles had a significant lower cognitive decline in our retrospective analysis (Supplemental Table 4 and Supplemental Figure 3). In our retrospective longitudinal sensitivity analyses after exclusion of participants with AD, consistent across all time periods ($P = 0.038$), individuals in the lowest ω-3 index quartile had significantly greater Aβ accumulation (adjusted $\beta = 0.01$; 95% CI: 0.00, 0.03; $P = 0.038$ between M-60 and M0 months). MOCA trajectories also differed between the 2 ω-3 index groups ($P = 0.053$ across all time periods). Individuals in the lowest ω-3 index quartile had a borderline significant greater cognitive decline between M-60 and M0 months (adjusted $\beta = -0.53$; 95% CI: –1.07, 0.01; $P = 0.053$). Although not significant across all time periods, individuals in the lowest ω-3 index quartile had a significant greater cognitive decline between M-60 and M0 months on the WMS-immediate recall (adjusted $\beta = -1.00$; 95% CI: –1.88, –0.13; $P = 0.025$) and on the WMS-delayed recall (adjusted $\beta = -0.94$; 95% CI: –1.87, –0.00; $P = 0.050$). Although not significant across all time periods, individuals in the lowest ω-3 index quartile had an unexpected lower cognitive decline on total ADAS-Cog between M-60 and M0 months (adjusted $\beta = -0.11$; 95% CI: –0.22, –0.01; $P = 0.030$). No interactions were found between low ω-3 index and cognitive or amyloid status. However, unlike noncarriers, *ApoE ε4* carriers in the lowest ω-3 index quartile experienced a significant higher cognitive decline on MMSE between M-60 and M0 months (adjusted $\beta = -0.56$; 95% CI: –1.12, –0.01; $P = 0.045$).

Discussion

Our study explored the cross-sectional and retrospective longitudinal associations between erythrocyte ω-3 index and cognitive tests, MRI data, and PET Aβ and tau accumulation among community-dwelling older adults. In the cross-sectional analysis, we did not find any significant association between low ω-3 index and cognitive or brain outcomes in the whole population. In the retrospective longitudinal analysis, low ω-3 index was significantly associated with greater Aβ accumulation over time. Although no differences were observed on the composite cognitive scores, individuals with a low ω-3 index had greater cognitive decline on the WMS-delayed recall but unexpectedly lower cognitive decline on the total ADAS-Cog.

Several studies explored the association between erythrocyte ω-3 index and cognitive outcomes (**Table 5**); however, to our knowledge, our study is the first to investigate the association between erythrocyte ω-3 index and both PET Aβ and tau accumulation. Only 1 cross-sectional study in 61 older adults reported an inverse correlation between serum DHA concentrations and brain amyloid load on PET imaging (8); however, the longitudinal association had never been investigated. Although there is evidence suggesting that DHA could confer neuro-protection, in part through the direct inhibition of tau phosphorylation (9, 30), we did not find any significant association between erythrocyte ω-3 index and tau PET. Further longitudinal studies on ω-3 fatty acids and tau PET are needed to assess this important relation.

Studies investigating the relation between ω-3 PUFAs, brain morphology, and volumes have also provided mixed findings (10). Higher erythrocyte ω-3 index was correlated with larger

TABLE 2 Interactions between ω-3 index (lowest vs. 3 highest quartiles) and *ApoE ε4* genotype (carriers vs. noncarriers) on cognitive, brain imaging, and biomarker variables among community-dwelling older adults (cross-sectional analysis)[1]

	n	*ApoE ε4* noncarrier	ω-3 Index × *ApoE ε4* carrier		
			β	SE	*P*
Model 1 (unadjusted)					
Composite cognitive score	764	Ref.	−0.92	0.56	0.104
MMSE score	790	Ref.	−0.37	0.52	0.479
Total ADAS-Cog[2]	785	Ref.	0.02	0.08	0.758
ADAS-Cog delayed recall	793	Ref.	0.39	0.44	0.373
Wechsler Memory Scale-delayed recall	788	Ref.	−2.07	0.94	0.028
Wechsler Memory Scale-immediate recall	790	Ref.	−1.49	0.87	0.088
Category Fluency test (words)	791	Ref.	−0.21	1.04	0.836
TMT-A (s)[2]	787	Ref.	0.02	0.07	0.769
TMT-B (s)[2]	771	Ref.	0.08	0.09	0.366
MoCA total score	775	Ref.	−0.23	0.76	0.764
CDR sum of boxes	791	Ref.	0.32	0.34	0.346
Total hippocampal volume (mm^3)	624	Ref.	−62.43	210.92	0.767
Left hippocampal subcortical volume (mm^3)	624	Ref.	−8.00	105.81	0.940
Right hippocampal subcortical volume (mm^3)	624	Ref.	−54.43	110.34	0.622
White matter hyperintensities volume (cm^3)[2]	736	Ref.	0.15	0.28	0.591
Cortical SUVr[2]	459	Ref.	0.02	0.04	0.608
Tau 18F-AV1451 meta-temporal region[2]	660	Ref.	0.10	0.04	0.010
Tau 18F-AV1451 Braak stage 1[2]	660	Ref.	0.12	0.05	0.019
Tau 18F-AV1451 Braak stage 2	660	Ref.	0.10	0.05	0.038
Tau 18F-AV1451 Braak stages 3 and 4[2]	660	Ref.	0.09	0.03	0.009
Tau 18F-AV1451 Braak stages 5 and 6	660	Ref.	0.17	0.07	0.011
Model 2 (adjusted)					
Composite cognitive score	745	Ref.	−0.92	0.53	0.081
MMSE score	769	Ref.	−0.35	0.51	0.494
Total ADAS-Cog[2]	765	Ref.	0.05	0.07	0.543
ADAS-Cog delayed recall	773	Ref.	0.47	0.42	0.267
Wechsler Memory Scale-delayed recall	769	Ref.	−2.09	0.91	0.022
Wechsler Memory Scale-immediate recall	771	Ref.	−1.47	0.84	0.081
Category Fluency test (words)	771	Ref.	−0.47	0.97	0.630
TMT-A (s)[2]	767	Ref.	0.04	0.07	0.560
TMT-B (s)[2]	751	Ref.	0.09	0.08	0.263
MoCA total score	754	Ref.	−0.33	0.72	0.652
CDR sum of boxes	770	Ref.	0.38	0.34	0.263
Total hippocampal volume (mm^3)	608	Ref.	−85.33	182.39	0.640
Left hippocampal subcortical volume (mm^3)	608	Ref.	−13.10	93.45	0.889
Right hippocampal subcortical volume (mm^3)	608	Ref.	−72.24	95.04	0.448
White matter hyperintensities (cm^3)[2]	718	Ref.	0.18	0.25	0.478
Cortical SUVr[2]	454	Ref.	0.01	0.04	0.783
Tau ^{18}F-AV1451 meta-temporal region[2]	648	Ref.	0.09	0.04	0.023
Tau ^{18}F-AV1451 Braak stage 1[2]	648	Ref.	0.11	0.05	0.023
Tau ^{18}F-AV1451 Braak stage 2	648	Ref.	0.09	0.05	*0.060*
Tau ^{18}F-AV1451 Braak stages 3 and 4[2]	648	Ref.	0.08	0.03	0.022
Tau ^{18}F-AV1451 Braak stages 5 and 6	648	Ref.	0.14	0.07	0.040

[1]Results from linear regression models. The modified composite score included the delayed word recall from the ADAS-Cog, the TMT-B, the MMSE total score, and the Wechsler Memory Scale delayed recall; ω-3 index defined as erythrocyte %DHA + %EPA. Model 2 was adjusted for age, education, history of cardiovascular disease, BMI, and total intracranial volume (this last only for white matter hyperintensities and hippocampal volume). ADAS, Alzheimer's Disease Assessment Scale; ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive subscale; CDR, Clinical Dementia Rating; MoCA, Montreal Cognitive Assessment; MMSE, Mini-Mental State Examination; Ref., reference; TMT, Trail Making Test; SUVr, standard uptake value ratio normalized by whole cerebellum.

[2]Variable with log transformation.

TABLE 3 Associations between ω-3 index (lowest vs. 3 highest quartiles) and cognitive and brain biomarker variables among community-dwelling older adults *ApoE ε4* carriers and noncarriers (within-group estimates according to the interaction between ω-3 index and *ApoE ε4* status presented in Table 2) (cross-sectional analysis)[1]

	ApoE ε4 noncarriers			*ApoE ε4* carriers		
	β	SE	*P*	β	SE	*P*
Model 1 (unadjusted)						
Wechsler Memory Scale-delayed recall	−0.22	0.56	0.699	−2.29	0.76	0.003
Wechsler Memory Scale-immediate recall	0.01	0.52	0.985	−1.48	0.70	0.035
Tau 18F-AV1451 meta-temporal region[2]	−0.03	0.02	0.294	0.08	0.03	0.014
Tau 18F-AV1451 Braak stage 1[2]	−0.04	0.03	0.236	0.08	0.04	0.040
Tau 18F-AV1451 Braak stage 2	−0.04	0.03	0.196	0.06	0.04	0.105
Tau 18F-AV1451 Braak stages 3 and 4[2]	−0.02	0.02	0.306	0.07	0.03	0.013
Tau 18F-AV1451 Braak stages 5 and 6	−0.02	0.04	0.686	0.15	0.05	0.004
Model 2 (adjusted)						
Wechsler Memory Scale-delayed recall	0.28	0.55	0.613	−1.81	0.73	0.014
Wechsler Memory Scale-immediate recall	0.55	0.51	0.279	−0.92	0.68	0.178
Tau 18F-AV1451 meta-temporal region[2]	−0.03	0.02	0.192	0.06	0.03	0.066
Tau 18F-AV1451 Braak stage 1[2]	−0.04	0.03	0.242	0.08	0.04	0.052
Tau 18F-AV1451 Braak stage 2	−0.02	0.03	0.448	0.06	0.04	0.078
Tau 18F-AV1451 Braak stages 3 and 4[2]	−0.03	0.02	0.198	0.05	0.03	0.061
Tau 18F-AV1451 Braak stages 5 and 6	−0.04	0.04	0.303	0.09	0.05	0.076

[1] ω-3 index defined as erythrocyte %DHA + %EPA. Model 2 was adjusted for age, education, history of cardiovascular disease, BMI, and total intracranial volume (this last only for white matter hyperintensities and hippocampal volume). Significant *P* values are presented in bold, and marginally significant p-values are presented in italics. Results from linear regression models.

[2] Variable with log transformation.

total normal brain volume and hippocampal volume in females after menopause from the Women's Health Initiative Memory Study (31). However, no relations were observed between erythrocyte ω-3 index and total hippocampal volume among dementia-free Framingham Study participants (5). Although accumulating evidence has reported a beneficial role of ω-3 PUFAs on cognitive aging, previous studies have examined different exposures and findings have been mixed overall (32). Consistent with our findings on WMS-delayed recall, higher levels of plasma ω-3 index were associated with lower cognitive decline and risk of dementia among older adults from the Three-City (3C) Study (2). Conversely, in a recent meta-analysis including 181,580 participants from 21 prospective studies (33), DHA intake (but not blood DHA) was associated with lower risk of cognitive impairment or dementia, and total blood ω-3 PUFAs were not related to dementia outcomes. Finally, our unexpected results on the total ADAS-Cog need further investigation. The ADAS-Cog is not an optimal outcome measure for pre-dementia studies and is limited in measuring progression of cognitive impairment over the course of the disease progression (34). Its low sensitivity is primarily due to most of its items suffering from either floor or ceiling effects.

Although we did not find any association between low ω-3 index and cognitive or brain outcomes in the whole population, our cross-sectional (but not retrospective longitudinal) findings, reported a significant interaction between low ω-3 index and *ApoE ε4* genotype on certain cognitive tests and tau accumulation. Accumulating evidence also suggests that ApoE genotype could be one of the most important moderators of ω-3 PUFA effectiveness in the aging brain. Some studies reported associations of better cognitive effectiveness in *ApoE ε4* noncarriers (35–38), whereas others showed benefits among *ApoE ε4* carriers only (13). In the 3C study, greater plasma DHA and EPA were associated with lower cognitive decline in *ApoE ε4* carriers (39). Similar findings were observed in secondary analyses from the Rush Memory and Aging Project, with *ApoE ε4* carriers exhibiting slower rates of cognitive decline upon weekly seafood consumption (40). This is consistent with the study by Morris et al. (41), in which consumption of α-linolenic acid (ALA, 18:3n−3) was only protective towards incident AD in *ApoE ε4* carriers. In cognitively unimpaired ApoE ε4 homozygotes from the Alzheimer and Families Study (ALFA), dietary DHA intake also related to structural patterns that may result in greater resilience to AD pathology (42). Finally, very recent findings from the Framingham Offspring Cohort showed that risk for incident AD in the highest RBC DHA quintile was 49% lower compared with the lowest quintile and that *ApoE ε4* carriers may benefit more from higher DHA concentrations than noncarriers (43). There is a biological rationale for a vulnerability of *ApoE ε4* carriers to lower ω-3 PUFA status, especially in early AD, since several mechanisms linked ApoE ε4 with reduced brain DHA metabolism (13). It has been suggested that *ApoE ε4* carriers β-oxidize DHA at greater rates than noncarriers (44), and are more likely to exhibit dysfunctions in the blood–brain barrier (45), which can impair the DHA delivery to the brain (44, 46).

Despite the aforementioned interesting findings, we observed in our cross-sectional study an unexpected association between low ω-3 index and better cognition, higher total hippocampal volume, and lower tau accumulation in patients with AD only. Similarly, participants with AD in the lowest ω-3 index quartile exhibited lower cognitive decline than those in the 3 highest quartiles in our retrospective longitudinal analysis. Given the distributions of the cognitive tests among participants with AD, we ruled out the possibility of a floor effect, which might have partly explained some of these unexpected findings. The existing literature shows that the association between ω-3 PUFAs

TABLE 4 Differences in retrospective change in cognitive, brain imaging, and biomarker variables between the lowest ω-3 index quartile and the 3 highest quartiles (longitudinal analysis)[1]

		Unadjusted						Adjusted[2]		
		Lowest ω-3 index quartile			Three highest ω-3 index quartiles			Lowest vs. highest ω-3 index quartiles		
Outcome	Time	*n*	β	[95% CI]	*n*	β	[95% CI]	β	[95% CI]	*P*
Composite cognitive score	M0	83			250					
	M0–M–24	44	−0.88	[−1.28, −0.47]	120	−0.68	[−0.91, −0.44]	−0.19	[−0.67, 0.29]	0.436
	M0–M–36	31	−1.25	[−1.82, −0.68]	94	−0.95	[−1.28, −0.62]	−0.27	[−0.93, 0.39]	0.426
	M0–M–60	34	−1.87	[−2.68, −1.05]	125	−1.37	[−1.85, −0.90]	−0.40	[−1.33, 0.54]	0.408
	M0–M–120	14	−2.66	[−3.84, −1.48]	28	−1.69	[−2.37, −1.00]	−0.53	[−1.90, 0.83]	0.440
	Whatever time									0.701
Total MMSE	M0	88			259					
	M0–M–24	46	−0.47	[−0.76, −0.18]	127	−0.43	[−0.59, −0.26]	0.00	[−0.34, 0.35]	0.985
	M0–M–36	34	−0.68	[−1.09, −0.27]	97	−0.60	[−0.84, −0.37]	−0.01	[−0.49, 0.48]	0.977
	M0–M–60	52	−1.04	[−1.62, −0.47]	167	−0.90	[−1.23, −0.56]	−0.05	[−0.74, 0.63]	0.880
	M0–M–120	18	−1.64	[−2.36, −0.92]	35	−1.24	[−1.65, −0.82]	−0.31	[−1.15, 0.53]	0.471
	Whatever time									0.379
Total ADAS-Cog[3]	M0	88			257					
	M0–M–24	44	0.32	[0.25, 0.38]	127	0.43	[0.39, 0.47]	−0.10	[−0.19, −0.02]	0.017
	M0–M–36	34	0.40	[0.32, 0.47]	97	0.52	[0.48, 0.57]	−0.12	[−0.21, −0.02]	0.018
	M0–M–60	52	0.45	[0.38, 0.53]	167	0.57	[0.52, 0.61]	−0.09	[−0.19, 0.01]	0.073
	M0–M–120	18	0.58	[0.43, 0.72]	35	0.50	[0.42, 0.58]	0.04	[−0.16, 0.24]	0.689
	Whatever time									0.092
ADAS-Cog delayed recall	M0	88			261					
	M0–M–24	46	0.14	[−0.06, 0.35]	127	0.29	[0.18, 0.41]	−0.11	[−0.35, 0.14]	0.398
	M0–M–36	34	0.22	[−0.06, 0.50]	97	0.41	[0.25, 0.57]	−0.14	[−0.47, 0.19]	0.400
	M0–M–60	52	0.39	[0.01, 0.77]	167	0.60	[0.38, 0.82]	−0.18	[−0.64, 0.27]	0.421
	M0–M–120	18	0.89	[0.23, 1.55]	35	0.75	[0.36, 1.14]	−0.11	[−0.92, 0.70]	0.787
	Whatever time									0.700
Wechsler Memory Scale-delayed recall	M0	87			258					
	M0–M–24	46	−0.73	[−1.18, −0.27]	123	−0.19	[−0.45, 0.08]	−0.61	[−1.13, −0.08]	0.024
	M0–M–36	34	−0.98	[−1.60, −0.35]	94	−0.21	[−0.57, 0.16]	−0.84	[−1.55, −0.13]	0.021
	M0–M–60	36	−1.25	[−2.12, −0.38]	125	−0.08	[−0.59, 0.42]	−1.18	[−2.16, −0.19]	0.019
	M0–M–120	14	−0.62	[−2.17, 0.93]	29	1.13	[0.21, 2.05]	−1.23	[−3.05, 0.58]	0.180
	Whatever time									0.066
Wechsler Memory Scale-immediate recall	M0	87			260					
	M0–M–24	46	−0.55	[−0.97, −0.14]	124	−0.11	[−0.35, 0.13]	−0.43	[−0.92, 0.05]	0.079
	M0–M–36	34	−0.77	[−1.34, −0.20]	95	−0.12	[−0.45, 0.21]	−0.62	[−1.29, 0.04]	0.065
	M0–M–60	36	−1.10	[−1.90, −0.30]	125	−0.02	[−0.49, 0.44]	−0.96	[−1.88, −0.04]	0.042
	M0–M–120	14	−1.26	[−2.55, 0.03]	29	0.81	[0.05, 1.56]	−1.52	[−3.04, 0.01]	0.052
	Whatever time									0.097
Category Fluency test (words)	M0	88			259					
	M0–M–24	46	−0.52	[−0.78, −0.26]	125	−0.45	[−0.60, −0.30]	−0.07	[−0.38, 0.24]	0.644
	M0–M–36	34	−0.78	[−1.17, −0.39]	96	−0.68	[−0.91, −0.45]	−0.11	[−0.58, 0.36]	0.644
	M0–M–60	52	−1.30	[−1.95, −0.65]	167	−1.13	[−1.51, −0.74]	−0.18	[−0.96, 0.59]	0.644
	M0–M–120	18	−2.61	[−3.90, −1.31]	35	−2.25	[−3.02, −1.49]	−0.37	[−1.92, 1.19]	0.644

(*Continued*)

TABLE 4 (*Continued*)

		Unadjusted						Adjusted[2]		
		Lowest ω-3 index quartile			Three highest ω-3 index quartiles			Lowest vs. highest ω-3 index quartiles		
Outcome	Time	*n*	β	[95% CI]	*n*	β	[95% CI]	β	[95% CI]	*P*
	Whatever time									0.644
TMT-A (seconds)[3]	M0	88			258					
	M0–M–24	46	0.05	[0.02, 0.09]	124	0.03	[0.01, 0.05]	0.02	[−0.02, 0.06]	0.354
	M0–M–36	34	0.07	[0.02, 0.12]	95	0.05	[0.02, 0.08]	0.02	[−0.03, 0.08]	0.395
	M0–M–60	52	0.09	[0.03, 0.16]	167	0.07	[0.03, 0.11]	0.02	[−0.05, 0.10]	0.529
	M0–M–120	18	0.07	[−0.03, 0.16]	35	0.10	[0.04, 0.15]	−0.03	[−0.14, 0.09]	0.637
	Whatever time									0.352
TMT-B (seconds)[3]	M0	84			254					
	M0–M–24	44	0.09	[0.05, 0.14]	121	0.06	[0.04, 0.09]	0.03	[−0.02, 0.08]	0.287
	M0–M–36	32	0.13	[0.07, 0.19]	96	0.09	[0.06, 0.13]	0.03	[−0.03, 0.10]	0.329
	M0–M–60	50	0.19	[0.11, 0.27]	167	0.14	[0.10, 0.19]	0.03	[−0.06, 0.13]	0.467
	M0–M–120	18	0.25	[0.14, 0.35]	33	0.22	[0.16, 0.28]	−0.04	[−0.17, 0.09]	0.558
	Whatever time									0.206
MoCA total score	M0	87			257					
	M0–M–24	46	−0.74	[−1.17, −0.31]	123	−0.51	[−0.76, −0.26]	−0.35	[−0.82, 0.11]	0.137
	M0–M–36	34	−1.02	[−1.58, −0.46]	96	−0.68	[−1.00, −0.36]	−0.47	[−1.07, 0.13]	0.122
	M0–M–60	51	−1.41	[−2.10, −0.72]	165	−0.85	[−1.25, −0.45]	−0.60	[−1.36, 0.17]	0.127
	Whatever time									0.288
CDR sum of boxes	M0	88			259					
	M0–M–24	46	0.53	[0.31, 0.74]	125	0.38	[0.25, 0.51]	0.20	[−0.06, 0.45]	0.134
	M0–M–36	35	0.68	[0.39, 0.97]	95	0.49	[0.33, 0.66]	0.23	[−0.11, 0.58]	0.180
	M0–M–60	52	0.83	[0.43, 1.23]	166	0.62	[0.39, 0.86]	0.22	[−0.24, 0.69]	0.349
	M0–M–120	17	1.12	[0.60, 1.63]	35	0.85	[0.55, 1.15]	0.03	[−0.57, 0.63]	0.927
	Whatever time									0.101
White matter hyperintensities (cm^3)[3]	M0	77			226					
	M0–M–24	43	−0.33	[−0.54, −0.11]	117	−0.34	[−0.46, −0.21]	0.00	[−0.23, 0.24]	0.970
	M0–M–36	24	−0.25	[−0.46, −0.03]	52	−0.30	[−0.42, −0.18]	0.06	[−0.18, 0.29]	0.638
	M0–M–60	36	−0.03	[−0.22, 0.15]	115	−0.13	[−0.24, −0.03]	0.11	[−0.09, 0.32]	0.279
	Whatever time									0.287
Cortical SUVr (brain Aβ load)	M0	74			215					
	M0–M–24	44	0.02	[0.01, 0.02]	120	0.01	[0.01, 0.01]	0.01	[0.00, 0.01]	0.003
	M0–M–36	24	0.02	[0.02, 0.03]	71	0.01	[0.01, 0.02]	0.01	[0.00, 0.02]	0.003
	M0–M–60	34	0.04	[0.03, 0.05]	77	0.02	[0.02, 0.03]	0.02	[0.01, 0.03]	0.003
	Whatever time									0.003

[1]Results from mixed linear regression models. Aβ, amyloid-β; ADAS, Alzheimer's Disease Assessment Scale; ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive subscale; CDR, Clinical Dementia Rating; M, Month; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; TMT, Trail Making Test; SUVr, standard uptake value ratio.

[2]Adjustments: age (years), participant education (years), ApoE4 (yes vs. no), history of cardiovascular disease (yes vs. no), BMI (kg/m^2) + intra-cranial volume (intra-cranial volume only for WMH), and their interaction with time.

[3]Variable with log transformation.

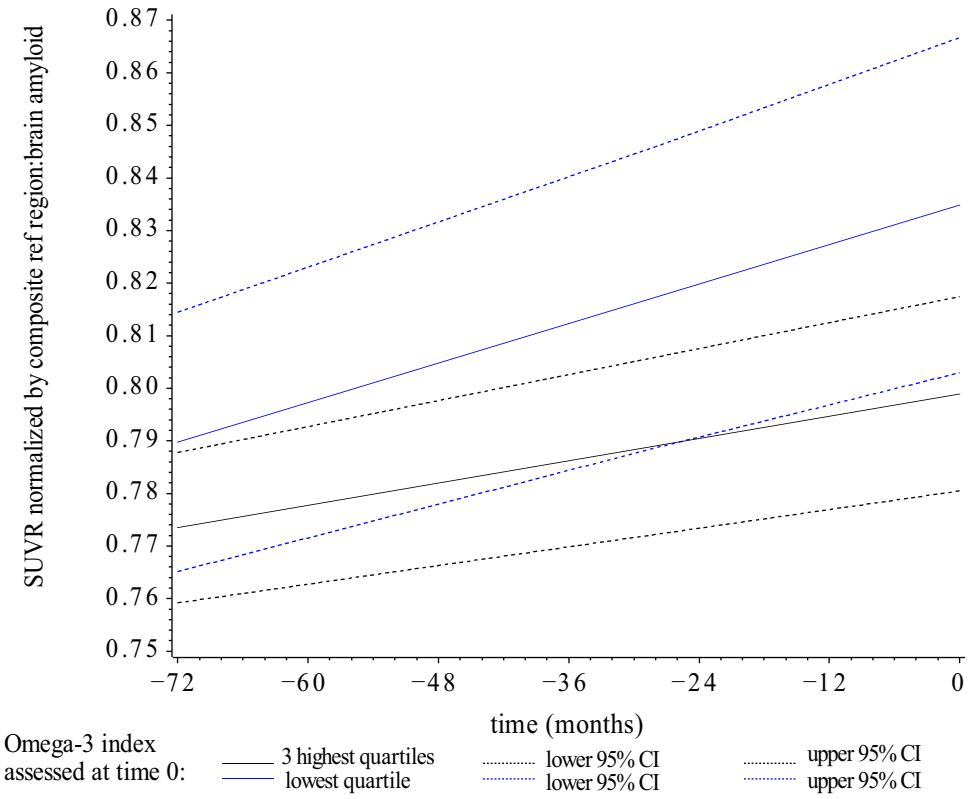

FIGURE 2 Aβ trajectories over time according to the lowest vs. highest ω-3 index quartiles (retrospective longitudinal analysis—whole population). Results are from mixed linear regression models (mean estimate and 95% CI). Aβ, amyloid-β; SUVR, standard uptake value ratio (brain amyloid-β load).

and brain outcomes may differ according to the cognitive status. A recent systematic review of clinical trials showed that interventions were promising for people with mild or subjective cognitive impairment but not in those already diagnosed with dementia (47). Further in-depth investigations of the differential effects of ω-3 PUFAs according to cognitive status are required.

Sensitivity analyses after exclusion of participants with AD did not notably change our findings. They strengthened the negative effects of low ω-3 index on cognitive health, including on Aβ accumulation, and confirmed the greater vulnerability of *ApoE4* *ε4* carriers to lower ω-3 PUFA status. We also observed a trend towards higher tau accumulation among Aβ+ participants with a low ω-3 index, which was not confirmed in the retrospective longitudinal analysis. Finally, a low ω-3 index was overall mostly associated with lower global cognitive performance and complex memory tests. Other studies (48) showed that EPA and DHA could benefit executive functions but not overall cognition. Further studies are needed to clarify the effects of ω-3 PUFAs on different cognitive domains.

The present study adds important elements to the field by evaluating several cognitive outcomes (including several clinical tests comprising different cognitive domains, MRI data, and brain Aβ and tau accumulation) and their association with ω-3 PUFA status among a large sample of older adults with different cognitive and *ApoE ε4* status. We were also able to analyze not only the cross-sectional but also the retrospective longitudinal associations between erythrocyte ω-3 index, cognition, and neuroimaging biomarkers. Another strength to be noted is the assessment of ω-3 status through erythrocyte fatty acid concentrations, considered a more reliable measurement of ω-3 PUFA intake compared with plasma or serum measurements (since fatty acids are stable in erythrocyte membranes over its life span, i.e., up to 3 mo) (3, 49), and known to reflect fatty acid concentrations of other tissues (50). However, some limitations must be considered. Our findings are based on an observational study, and residual confounding may still exist despite extensive efforts to account for possible confounders. ADNI participants were predominantly White, highly educated, with relatively few comorbidities, which may limit the generalizability of our findings. PET scan and MRI were not performed for all subjects; in addition, hippocampal volume and tau PET were not assessed in our retrospective longitudinal analysis because of differences in methods used over time, which could have affected the comparability of the results. Our subgroup analyses according to cognitive status included few individuals with AD in the longitudinal analysis; therefore, the results should be taken with caution. Erythrocyte fatty acid assessment was only performed once at baseline during the ADNI-3 wave, on de-freeze samples after blood collection, which might have underestimated ω-3 PUFA values due to partial oxidative degradation (51). However, the storage under –80°C might have minimized such possible occurrence. In addition, fish-oil consumption was associated with higher ω-3 index, supporting the reliability of our erythrocyte fatty acid assessment. However, although our analytical method, which is the most widely used in

TABLE 5 Studies exploring erythrocyte ω-3 index (% of DHA + EPA from total lipids in erythrocyte membranes) and cognitive outcomes

Publication	Population	Study design	Cognitive outcomes/main findings
Amen et al., 2017 (54)	166 individuals from a psychiatric referral clinical	Cross-sectional	Neurocognitive status assessed by computerized testing (WebNeuro); ω-3 index correlated to the feeling subsection of the WebNeuro ($r = 0.25$, $P = 0.01$).
Ammann et al., 2017 (55)	6706 women ≥65 y, participants of the Women's Health Initiative Memory Study (WHIMS)	Prospective observational study	Risk of dementia. After adjusting for demographic, clinical, and behavioral risk factors, 1-SD increase in ω-3 index was associated with a lower risk of dementia (HR = 0.92, 95% CI: 0.84, 1.00; $P < 0.05$).
Bigornia et al., 2018 (56)	1032 subjects (45–75 y)	Prospective observational study	Verbal fluency, executive function, memory, global cognitive function. Erythrocyte ω-3 index and ω-3 very-long-chain fatty acids (EPA + DHA + DPA) were associated with better executive function (P-trend < 0.02).
Coley et al., 2018 (4)	724 older adults (≥70 y) participants of the MAPT study	Secondary exploratory analysis of an RCT with ω-3 supplementation	Memory, lexical fluency, attention, executive function. Participants receiving placebo in the lowest quartile of baseline ω-3 index (≤4.83%) underwent more 3-y cognitive decline than the other quartiles (mean composite cognitive score difference 0.14; 95% CI: 0.00, 0.28; $P = 0.048$).
Cook et al., 2017 (57)	299 healthy women (18–35 y)	Cross-sectional	Impulsivity, attention, information processing, memory, and executive function. Adjustment for ω-3 index weakened the interaction effect between BMI and cognitive domains.
Cook et al., 2019 (58)	299 healthy women (18–35 y)	Cross-sectional	Impulsivity, attention, information processing, memory, and executive function. Participants in the low ω-3 index tertile group scored significantly lower on attention than the other tertile groups in adjusted analysis.
Coro et al., 2022 (59)	76 Australian survivors of breast and colorectal cancer, mean age 57.5 (±10.2) y	Cross-sectional	Visual memory, learning, visuospatial memory, executive function, attention, short-term visual memory, sustained attention, executive function, working memory; ω-3 index was not associated with cognitive outcomes.
Dretsch et al., 2014 (60)	78 American service members deployed to Iraq (18–55 y)	RCT with ω-3 supplementation	Reaction time, processing speed, cognitive flexibility, executive functioning, verbal memory, working memory. Changes in ω-3 index were not associated with changes in cognitive outcomes.
Erhardt et al., 2021 (61)	142 Australian community-dwelling older adults (60–85 y)	Cross-sectional	Memory, language, executive function, visuospatial skills. Erythrocyte ω-3 index was not associated with cognitive performance.
Johnston et al., 2013 (62)	78 American service members deployed to Iraq (20–54 y)	Cross-sectional	Processing speed, complex attention, reaction time, cognitive flexibility, and executive function. The ω-3 index was positively associated with cognitive flexibility ($P < 0.02$) and executive function ($P = 0.01$).
Lukaschek et al., 2016 (63)	720 older adults (68–92 y)	Cross-sectional	Cognitive status. In model adjusted by sex and age, subjects with a low ω-3 index were at a significantly higher risk for cognitive impairment (OR: 1.77; 95% CI: 1.15, 2.73; $P = 0.009$). The association remained stable after further adjusting for educational level, metabolic risk factors, and affective disorders.
Tan et al., 2012 (5)	1575 individuals aged 67 ± 9 y	Cross-sectional	Visual memory, executive function, abstract thinking. Participants with lower ω-3 index (quartile 1 vs. quartiles 2–4) had lower scores on tests of visual memory ($\beta \pm SE = -0.47 \pm 0.18$; $P = 0.008$), executive function ($\beta \pm SE = -0.07 \pm 0.03$; $P = 0.004$), and abstract thinking ($\beta \pm SE = -0.52 \pm 0.18$; $P = 0.004$).
Thomas et al., 2020 (2)[2]	1279 older adults from the Three-City (3C) Study	Prospective observational study	Risk of dementia, global cognition, memory. Higher levels of plasma ω-3 index were consistently associated with a lower risk of dementia (HR for 1 SD = 0.87, 0.76–0.98), and a lower decline in global cognition ($P = 0.04$ for change over time) and memory ($P = 0.06$).
Witte et al., 2014 (64)	65 subjects (50–75 y)	RCT with ω-3 supplementation	Executive function, memory performance, sensorimotor speed, attention. Changes in ω-3 index correlated positively with improvements in executive functions.

[1]MAPT, Multidomain Alzheimer Preventive Trial; RCT, randomized controlled trial.
[2]This study analyzed ω-3 index in plasma, not in erythrocytes.

the research setting, makes findings internally valid, they can only be compared with studies that used the same analytic approach. Finally, the analytical variability has been determined formerly in a precedent trial and was approximately 2%, which is very low and negligible compared with the patients' value variability (52).

In conclusion, cross-sectional analysis did not find any significant association between erythrocyte ω-3 index and cognitive tests, brain imaging, and biomarkers in the general sample of older adults with different cognitive status. In our retrospective longitudinal analysis, low ω-3 index was associated with greater Aβ accumulation over time and cognitive decline on the WMS test, but unexpectedly with lower cognitive decline on the total ADAS-Cog. Low ω-3 index was associated with lower cognition and higher tau accumulation in certain brain regions among *ApoE ε4* carriers in the cross-sectional analysis. Our findings are consistent with the vulnerability of *ApoE ε4* carriers to lower ω-3 PUFA status reported in the literature and reinforce the group of evidence supporting the potential use of ω-3 PUFAs for cognitive decline and AD prevention among *ApoE ε4* carriers. Identifying individuals who may benefit more from this dietary and/or supplementation approach is of high interest, given that increasing ω-3 intake is a generally safe, simple, and well-tolerated intervention. This is critical from a public health perspective, not only in terms of preserving cognition but also given the potential benefits on other health conditions such as inflammation and cardiovascular events (53). Our analysis was exploratory, and prospective longitudinal studies further evaluating the topic are encouraged. They should be conducted with large sample sizes, sufficient follow-ups, detailed dietary information, and a greater heterogeneity of participants, especially those with MCI and AD.

Authors' contributions

The authors' responsibilities were as follows: LR and KVG contributed equally. LR and KVG: interpreted data and wrote the manuscript; CC: performed the statistical analyses, interpreted the data, and revised the draft critically for important intellectual content; PL and DC: performed fatty acid analysis, interpreted analytical data, and revised the draft critically on this point; SG, JD, SA, PdSB, and BV: interpreted data and revised the draft critically for important intellectual content; MWW: is the Principal Investigator of the Alzheimer's Disease Neuroimaging Initiative, interpreted data, and revised the draft critically for important intellectual content; and all authors: read and approved the final manuscript. SA reports grants from EU programs (H2020, JPND, FP7), Fondation de l'Avenir; personal fees from Nestlé, Nestec SA, Sanofi, MSD; and nonfinancial support from Biogen, Pfizer; grants from France Alzheimer Association; and grants from AMPA Association outside the submitted work. BV is an investigator in clinical trials sponsored by Biogen, Lilly, Roche, Eisai Pharmaceuticals, and the Toulouse University Hospital (Inspire Geroscience Program). He has served as a Scientific Advisory Board member for Biogen, Alzheon, Green Valey, Norvo Nordisk, and Longeveron but received no personal compensation. He has served as consultant and/or SAB member for Roche, Lilly, Eisai, and TauX with personal compensation.

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Data Availability

The data set is owned by a third-party organization; the Alzheimer's Disease Neuroimaging Initiative (ADNI). Data are publicly available at http://adni.loni.usc.edu/data-samples/access-data/Institutional Data Access/Ethics Committee (contact via http://adni.loni.usc.edu/data-samples/access-data/) upon sending a request composed by proposed analysis plan and the named lead investigator.

References

1. Dyall SC. Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. Front Aging Neurosci 2015;7:52.
2. Thomas A, Baillet M, Proust-Lima C, Féart C, Foubert-Samier A, Helmer C, et al. Blood polyunsaturated omega-3 fatty acids, brain atrophy, cognitive decline, and dementia risk. Alzheimers Dement 2020;16(S10):
3. von Schacky C. Importance of EPA and DHA blood levels in brain structure and function. Nutrients 2021;13(4):1074
4. Coley N, Raman R, Donohue MC, Aisen PS, Vellas B, Andrieu S. Defining the optimal target population for trials of polyunsaturated fatty acid supplementation using the erythrocyte omega-3 index: a step towards personalized prevention of cognitive decline? J Nutr Health Aging 2018;22(8):982–8.
5. Tan ZS, Harris WS, Beiser AS, Au R, Himali JJ, Debette S, et al. Red blood cell ω-3 fatty acid levels and markers of accelerated brain aging. Neurology 2012;78(9):658–64.
6. Grimm MOW, Kuchenbecker J, Grösgen S, Burg VK, Hundsdörfer B, Rothhaar TL, et al. Docosahexaenoic acid reduces amyloid beta production via multiple pleiotropic mechanisms. J Biol Chem 2011;286(16):14028–39.
7. Hooper C, Coley N, De S, Barreto P, Payoux P, Salabert AS, et al.; MAPT/DSA Study Group. Cortical β-Amyloid in older adults is associated with multidomain interventions with and without omega 3 polyunsaturated fatty acid supplementation. J Prev Alzheimers Dis 2020;7:128–34.
8. 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(10):1208–16.
9. Vela S, Sainz N, Moreno-Aliaga MJ, Solas M, Ramirez MJ. DHA selectively protects SAMP-8-associated cognitive deficits through inhibition of JNK. Mol Neurobiol 2019;56(3):1618–27.

10. Macaron T, Giudici KV, Bowman GL, Sinclair A, Stephan E, Vellas B, et al. Associations of omega-3 fatty acids with brain morphology and volume in cognitively healthy older adults: a narrative review. Ageing Res Rev 2021;67:101300.
11. Burckhardt M, Herke M, Wustmann T, Watzke S, Langer G, Fink A. Omega-3 fatty acids for the treatment of dementia. Cochrane Database Syst Rev 2016;4:CD009002.
12. Daiello LA, Gongvatana A, Dunsiger S, Cohen RA, Ott BR, Alzheimer's disease neuroimaging initiative. Association of fish oil supplement use with preservation of brain volume and cognitive function. Alzheimers Dement 2015;11(2):226–35.
13. Yassine HN, Braskie MN, Mack WJ, Castor KJ, Fonteh AN, Schneider LS, et al. Association of docosahexaenoic acid supplementation with alzheimer disease stage in apolipoprotein e ε4 carriers: a review. JAMA Neurol 2017;74(3):339–47.
14. Coughlan G, Larsen R, Kim M, White D, Gillings R, Irvine M, et al. APOE ε4 alters associations between docosahexaenoic acid and preclinical markers of Alzheimer's disease. Brain Comm 2021;3(2):fcab085.
15. Weiner MW, Veitch DP, Aisen PS, Beckett LA, Cairns NJ, Green RC, et al. The Alzheimer's Disease Neuroimaging Initiative 3: continued innovation for clinical trial improvement. Alzheimers Dement 2017;13(5):561–71.
16. Aisen P. Inclusion and exclusion criteria for joining ADNI according to cognitive status. [Internet]. [Accessed 2022 Aug 1]. Available from: https://clinicaltrials.gov/ct2/show/NCT02854033.
17. Guillocheau E, Garcia C, Drouin G, Richard L, Catheline D, Legrand P, et al. Retroconversion of dietary trans-vaccenic (trans-C18:1 n-7) acid to trans-palmitoleic acid (trans-C16:1 n-7): proof of concept and quantification in both cultured rat hepatocytes and pregnant rats. J Nutr Biochem 2019;63:19–26.
18. Drouin G, Catheline D, Guillocheau E, Gueret P, Baudry C, Le Ruyet P, et al. Comparative effects of dietary n-3 docosapentaenoic acid (DPA), DHA and EPA on plasma lipid parameters, oxidative status and fatty acid tissue composition. J Nutr Biochem 2019;63:186–96.
19. Donohue MC, Sperling RA, Salmon DP, Rentz DM, Raman R, Thomas RG, et al. The preclinical Alzheimer cognitive composite: measuring amyloid-related decline. JAMA Neurol 2014;71(8):961–70.
20. Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. Am J Psychiatry 1984;141:1356–64.
21. Llinàs-Reglà J, Vilalta-Franch J, López-Pousa S, Calvó-Perxas L, Torrents Rodas D, Garre-Olmo J. The Trail Making Test. Assessment 2017;24(2):183–96.
22. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12(3):189–98.
23. D'Elia L, Satz P, Schretlen D. Wechsler Memory Scale: a critical appraisal of the normative studies. J Clin Exp Neuropsychol 1989;11(4):551–68.
24. Gladsjo JA, Schuman CC, Evans JD, Peavy GM, Miller SW, Heaton RK. Norms for letter and category fluency: demographic corrections for age, education, and ethnicity. Assessment 1999;6(2):147–78.
25. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCa: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 2005;53(4):695–9.
26. Lynch CA, Walsh C, Blanco A, Moran M, Coen RF, Walsh JB, et al. The clinical dementia rating sum of box score in mild dementia. Dement Geriatr Cogn Disord 2006;21(1):40–3.
27. Schwarz C, Fletcher E, DeCarli C, Carmichael O. Fully-automated white matter hyperintensity detection with anatomical prior knowledge and without FLAIR. Inf Process Med Imaging Proc Conf 2009;21:239–51.
28. Landau SM, Thomas BA, Thurfjell L, Schmidt M, Margolin R, Mintun M, et al. Amyloid PET imaging in Alzheimer's disease: a comparison of three radiotracers. Eur J Nucl Med Mol Imaging 2014;41(7):1398–407.
29. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol (Berl) [Internet] 2006;112(4):389–404. [Accessed 2021 Jun 18]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3906709/.
30. Green KN, Martinez-Coria H, Khashwji H, Hall EB, Yurko-Mauro KA, Ellis L, et al. Dietary docosahexaenoic acid and docosapentaenoic acid ameliorate amyloid-beta and tau pathology via a mechanism involving presenilin 1 levels. J Neurosci 2007;27(16):4385–95.
31. Pottala JV, Yaffe K, Robinson JG, Espeland MA, Wallace R, Harris WS. Higher RBC EPA + DHA corresponds with larger total brain and hippocampal volumes: WHIMS-MRI study. Neurology 2014;82(5):435–42.
32. Cederholm T, Salem N, Palmblad J. ω-3 Fatty acids in the prevention of cognitive decline in humans. Adv Nutr 2013;4(6):672–6.
33. Zhang Y, Chen J, Qiu J, Li Y, Wang J, Jiao J. Intakes of fish and polyunsaturated fatty acids and mild-to-severe cognitive impairment risks: a dose-response meta-analysis of 21 cohort studies. Am J Clin Nutr 2015;103(2):330–40.
34. Kueper JK, Speechley M, Montero-Odasso M. The Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog): modifications and responsiveness in pre-dementia populations. A narrative review. J Alzheimers Dis 2018;63(2):423–44.
35. Beydoun MA, Kaufman JS, Satia JA, Rosamond W, Folsom AR. Plasma n-3 fatty acids and the risk of cognitive decline in older adults: the Atherosclerosis Risk in Communities Study. Am J Clin Nutr 2007;85(4):1103–11.
36. 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(20):1921–30.
37. Whalley LJ, Deary IJ, Starr JM, Wahle KW, Rance KA, Bourne VJ, et al. n-3 Fatty acid erythrocyte membrane content, APOE varepsilon4, and cognitive variation: an observational follow-up study in late adulthood. Am J Clin Nutr 2008;87(2):449–54.
38. Huang TL, Zandi PP, Tucker KL, Fitzpatrick AL, Kuller LH, Fried LP, et al. Benefits of fatty fish on dementia risk are stronger for those without APOE epsilon4. Neurology 2005;65(9):1409–14.
39. Samieri C, Féart C, Proust-Lima C, Peuchant E, Dartigues J-F, Amieva H, et al. ω-3 Fatty acids and cognitive decline: modulation by Apoeε4 allele and depression. Neurobiol Aging 2011;32(12):2317.e13–22
40. van de Rest O, Wang Y, Barnes LL, Tangney C, Bennett DA, Morris MC. APOE ε4 and the associations of seafood and long-chain omega-3 fatty acids with cognitive decline. Neurology 2016;86(22):2063–70.
41. Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Wilson RS, et al. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 2003;60(7):940–6.
42. Sala-Vila A, Arenaza-Urquijo EM, Sánchez-Benavides G, Suárez-Calvet M, Milà-Alomà M, Grau-Rivera O, et al. DHA intake relates to better cerebrovascular and neurodegeneration neuroimaging phenotypes in middle-aged adults at increased genetic risk of Alzheimer disease. Am J Clin Nutr 2021;113(6):1627–35.
43. Sala-Vila A, Satizabal CL, Tintle N, Lent DM, Vasan RS, Beiser AS, et al. Red blood cell DHA is inversely associated with risk of incident Alzheimer's disease and all-cause dementia: Framingham Offspring Study. Nutrients[Internet] 2022;14(12):2408. [Accessed 2022 Aug 1]. Available from: https://pubmed.ncbi.nlm.nih.gov/35745137/.
44. 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(10):4452–71.
45. Montagne A, Nation DA, Sagare AP, Barisano G, Sweeney MD, Chakhoyan A, et al. APOE4 leads to blood-brain barrier dysfunction predicting cognitive decline. Nature 2020;581(7806):71–6.
46. Vandal M, Alata W, Tremblay C, Rioux-Perreault C, Salem N, Calon F, et al. Reduction in DHA transport to the brain of mice expressing human APOE4 compared to APOE2. J Neurochem 2014;129(3):516–26.
47. Gkotzamanis V, Panagiotakos D. Dietary interventions and cognition: a systematic review of clinical trials. Psychiatriki 2020;31:248–56.
48. Kosti RI, Kasdagli MI, Kyrozis A, Orsini N, Lagiou P, Taiganidou F, et al. Fish intake, n-3 fatty acid body status, and risk of cognitive decline: a systematic review and a dose-response meta-analysis of observational and experimental studies. Nutr Rev 2022;80(6):1445–58.
49. Arab L. Biomarkers of fat and fatty acid intake. J Nutr 2003;133(3):925S–32S.
50. Harris WS, Sands SA, Windsor SL, Ali HA, Stevens TL, Magalski A, et al. Omega-3 fatty acids in cardiac biopsies from heart transplantation patients: correlation with erythrocytes and response to supplementation. Circulation 2004;110(12):1645–9.

51. Metherel AH, Stark KD. The stability of blood fatty acids during storage and potential mechanisms of degradation: a review. Prostaglandins, Leukotrienes Essent Fatty Acids 2016;104:33–43.
52. Vellas B, Carrie I, Gillette-Guyonnet S, Touchon J, Dantoine T, Dartigues JF, et al. Mapt study: a multidomain approach for preventing alzheimer's disease: design and baseline data. J Prev Alzheimers Dis 2014;1:13–22.
53. AbuMweis S, Jew S, Tayyem R, Agraib L. Eicosapentaenoic acid and docosahexaenoic acid containing supplements modulate risk factors for cardiovascular disease: a meta-analysis of randomised placebo-control human clinical trials. J Hum Nutr Diet 2018;31(1):67–84.
54. Amen DG, Harris WS, Kidd PM, Meysami S, Raji CA. Quantitative erythrocyte omega-3 EPA plus DHA levels are related to higher regional cerebral blood flow on brain SPECT. J Alzheimers Dis 2017;58(4):1189–99.
55. Ammann EM, Pottala JV, 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, Leukotrienes Essent Fatty Acids 2017;121:68–75.
56. Bigornia SJ, Scott TM, Harris WS, Tucker KL. Prospective associations of erythrocyte composition and dietary intake of n-3 and n-6 PUFA with measures of cognitive function. Nutrients 2018;10(9): 1253.
57. Cook RL, O'Dwyer NJ, Donges CE, Parker HM, Cheng HL, Steinbeck KS, et al. Relationship between obesity and cognitive function in young women: the food, mood and mind study. J Obesity 2017; 2017:1.
58. Cook RL, Parker HM, Donges CE, O'Dwyer NJ, Cheng HL, Steinbeck KS, et al. Omega-3 polyunsaturated fatty acids status and cognitive function in young women. Lipids Health Dis 2019;18(1): 194.
59. Coro DG, Hutchinson AD, Dyer KA, Banks S, Koczwara B, Corsini N, et al. 'Food for thought'—the relationship between diet and cognition in breast and colorectal cancer survivors: a feasibility study. Nutrients 2021;14(1):71.
60. Dretsch MN, Johnston D, Bradley RS, MacRae H, Deuster PA, Harris WS. Effects of omega-3 fatty acid supplementation on neurocognitive functioning and mood in deployed U.S. soldiers: a pilot study. Mil Med 2014;179(4):396–403.
61. Erhardt R, Cardoso BR, Meyer BJ, Brownell S, O'Connell S, Mirzaee S, et al. Omega-3 long-chain polyunsaturated fatty acids: are they beneficial for physical and cognitive functioning in older adults? J Nutr Health Aging 2021;25(4):454–61.
62. Johnston DT, Deuster PA, Harris WS, Macrae H, Dretsch MN. Red blood cell omega-3 fatty acid levels and neurocognitive performance in deployed U.S. servicemembers. Nutr Neurosci 2013;16(1): 30–8.
63. Lukaschek K, von Schacky C, Kruse J, Ladwig K-H. Cognitive impairment is associated with a low omega-3 index in the elderly: results from the KORA-Age study. Dement Geriatr Cogn Disord 2016;42(3-4):236–45.
64. Witte AV, Kerti L, Hermannstädter HM, Fiebach JB, Schreiber SJ, Schuchardt JP, et al. Long-chain omega-3 fatty acids improve brain function and structure in older adults. Cereb Cortex N Y N 1991 2014;24:3059–68.