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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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Running title

Erythrocyte ω -3 fatty acids, cognitive outcomes

Abbreviations

ω -3, omega-3; AD, Alzheimer's disease; ADAS-Cog, Alzheimer's Disease Assessment Scale cognitive subscale; ADCS-PACC, Alzheimer Disease Cooperative Study Preclinical Alzheimer Cognitive Composite; ADNI, Alzheimer's Disease Neuroimaging Initiative; ALA, α -linolenic acid; ApoE, apolipoprotein E; BMI, body mass index; CDR, Clinical Dementia Rating; CN, cognitively normal; DHA, docosahexaenoic acid; DSST, Digit Symbol Substitution Test; EDTA, ethylenediamine tetraacetic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; FCSRT, Free and Cued Selective Reminding Test; IDE, insulin-degrading enzyme; JNK, c-Jun N-terminal kinase; MCI, mild cognitive impairment; MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment; PET, positron emission tomography; PUFA, polyunsaturated fatty acids; RBC, red blood cells; RCT, randomized controlled trial; SAS, Statistical Analysis Software; SUVr, standard uptake value ratio; TIV, total intracranial volume; TMT-A, Trail Making Test A; TMT-B, Trail Making Test B.

Abstract

Background: The association between ω -3 polyunsaturated fatty acids and cognition, brain imaging and biomarkers is still not fully established.

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

Methods: 832 Alzheimer's Disease Neuroimaging Initiative 3 participants, mean (SD) age 74.0 (7.9) years, 50.8% female, 55.9% cognitively normal, 32.7% with mild cognitive impairment, 11.4% with Alzheimer's disease (AD) were included. Low ω -3 index (% eicosapentaenoic acid EPA + % docosahexaenoic acid 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 (WMH), PET amyloid- β and Tau) were considered as outcomes in regression models.

Results: Low ω -3 index was not associated with cognition, hippocampal and WMH volume, brain A β and Tau after adjustment for demographics, ApoE ϵ 4, cardiovascular disease, BMI and total intracranial volume in the cross-sectional analysis. In the retrospective analysis, low ω -3 index was associated with greater A β 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 ϵ 4 carriers.

Conclusions: Longitudinally, low ω -3 index was associated with greater A β 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 ϵ 4 carriers.

Alzheimer's Disease Neuroimaging Initiative (ADNI, ClinicalTrials.gov identifier, NCT00106899).

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

Introduction

Omega-3 (ω -3) polyunsaturated fatty acids (PUFA), mainly docosahexaenoic acid (DHA) and eicosapentaenoic acid (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 months (3). In the Multidomain Alzheimer Preventive Trial (MAPT), dementia-free older adults with 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 (A β) 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 A β (7). To our knowledge, only one study investigated the association between serum DHA levels and A β positron-emission tomography (PET) imaging, and reported an inverse correlation with brain A β 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's disease (AD) (11). Interestingly, previous studies also showed that the presence of Apolipoprotein E (ApoE) ϵ 4 allele might modify the relationship between ω -3 PUFA and brain functioning, but results are still conflicting (12–14). Overall, in the literature, most studies were only cross-sectional, 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 ϵ 4 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 hyperintensities – WMH volume, amyloid and Tau PET) among community-dwelling older adults. Sub-group analyses were also conducted according to cognitive status (cognitively normal CN, MCI and AD) and ApoE ϵ 4 genotype. We hypothesized that participants with low erythrocyte ω -3 index would present lower cognitive function, lower hippocampal volume, higher WMH volume, 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 multi-center 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 magnetic resonance imaging (MRI), PET, other biological markers, and clinical and neuropsychological assessment could be combined to measure the progression of MCI and early AD. After three waves (ADNI-1, ADNI-Grand Opportunity – ADNI-GO, and ADNI-2), the ongoing ADNI-3 wave began on August 1, 2016. Older adults aged 55 to 97 years 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; 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 three cognitive status' categories are described elsewhere (16). Study flowchart is presented in **Figure 1**. Among the 849 participants with fatty acids 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.

Isolation of erythrocytes and fatty acids measurement

Fatty acids were measured during the ADNI-3 wave. Red blood cells (RBC) were isolated from the whole blood (10 mL) collected into ethylenediamine tetra-acetic acid (EDTA) tubes, according to the standardized procedures at ADNI sites (ω -3 PUFA measurement is not affected by the presence of anti-coagulant). The blood was centrifuged at 3,000 gravitational force (g) for 15 minutes at 4°C. This results in the formation of a 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, RBC was stored immediately at -80°C in EDTA tubes. Fatty acids analysis was performed at biochemical laboratory of Institut Agro Rennes, France according to previous publication (17). Briefly, lipids were extracted twice from RBC (475 to 525 mg) samples with a mixture of hexane/isopropanol (3:2 v/v), after acidification with 1 mL hydrochloric acid (HCl) 3M (18), containing 5 ppm butylated hydroxytoluene (BHT). Margarine acid (C17:0) was added as internal standard. Total lipid extracts were saponified with 1mL of 0.5M sodium hydroxide (NaOH) in methanol for 30 minutes at 70°C and methylated with 1 mL of boron trifluoride (BF₃) methanol solution (12% w/v in methanol) for 15 min at 70°C. Fatty acid methyl esters (FAME) were extracted twice with pentane. Solvent was removed under nitrogen and FAME were redissolved in 200µL of hexane. Analyses were performed using an Agilent Technologies 7890N gas chromatograph (Bios Analytic, Toulouse, France) fitted with a split injector (10:1) at 250°C (injection volume 1µL) and a bonded silica capillary column (BPX 70, 60m long 250µm inner diameter and 0.25µm film thickness; SGE, Villeneuve-St Georges, France). Helium was used as a carrier gas (constant flow: 1.8 mL/min, 36 cm/sec). The column temperature program started at 170°C, increased by 4°C/min to 250°C and held at 250°C for 2 minutes. 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 amu (3 scan/s). Identification of FAME was based on retention times obtained for FAME

prepared from fatty acid standards and confirmed by comparison of their MS spectra with those of the NIST 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 were expressed as % of total identified fatty acids. ω -3 index was calculated as the sum of %EPA and %DHA, and thus also expressed in % from total fatty acids. 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). Distribution of ω -3 index is shown in **Supplementary Figure 1**.

Cognition, brain imaging and biomarkers

Cognitive, brain imaging and biomarkers 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. They were calculated by dividing each of the component scores by the sample standard deviation 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 four 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, WMS immediate and delayed recall were used to assess memory and category fluency, TMT-A and TMT-B tests were used to assess executive functions.

Secondary outcomes were brain imaging and biomarkers variables and included hippocampal subcortical volume (in left hemisphere, right hemisphere, and total), WMH volume (27), assessed by 3 Tesla (3T) MRI, and brain A β and hyper-phosphorylated 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 (A β +) was defined as ¹⁸F-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 meta-temporal region and in Braak stages 1 to 6 (29) after intravenous bolus injection of [¹⁸F]-radiolabeled AV1451.

Potential confounders

Potential confounders included: age (years), education (years), body mass index (BMI, calculated as body weight in kg divided by height² in m²), ApoE ϵ 4 genotype (carriers, non-carriers), history of cardiovascular disease (yes, no), and total intracranial volume (TIV) (cm³), this last only for WMH and hippocampal volume.

Statistical analysis

Characteristics of the population were presented as mean and standard deviation (SD) for continuous variables and as frequencies and percentages for categorical variables. They were compared according to ω -3 index (1st quartile vs. others) using Student t-test for continuous

variables (with log transformation if necessary) with normal distribution (or non-parametric Kruskal-Wallis test for other quantitative variables) and Pearson's chi-squared test for qualitative variables. In cross-sectional analyses, linear regression models were performed to assess the association between ω -3 index (1st quartile vs. others), cognitive, brain imaging and biomarkers variables and whether the associations differed according to cognitive status (CN, MCI, and AD) or ApoE4 genotype (carriers vs. non-carriers). For this purpose, an interaction in each model was introduced between ω -3 index and cognitive status or ApoE ϵ 4 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 of cognitive, brain imaging and biomarkers variables between participants in the lowest omega-3 index quartile and those in the other 3 quartiles were assessed at 24, 36, 60 and 120 months except for MoCA, WMH volume and amyloid PET (whose maximum retrospective follow-up was 6 years). Analyses were adjusted for potential confounders (age, education, BMI, ApoE ϵ 4 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 cognitively normal and individuals with MCI whether the associations differed according to amyloid status (amyloid positive status, A β ⁺ vs. amyloid negative status, A β ⁻). Analyses were performed using the Statistical Analysis Software (SAS) version 9.4 (Cary, NC, USA), with a significance level established as 5%.

Results

We included 832 individuals (mean (SD) age 74.0 (7.9) years; 50.8% female; 37.7% ApoE ϵ 4 carriers): 465 (55.9%) had a CN status, 272 (32.7%) had MCI and 95 (11.4%) had AD. Mean ω -3 index was 4.85% (SD=1.52). The distribution was as follows: median, 4.68%; interquartile range, 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 to 162 (33.1%) among non-users ($p<0.001$). Participants in the lowest ω -3 index quartile ($\leq 3.70\%$) 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 ϵ 4 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 A β and tau accumulation in unadjusted models or after adjustment for age, education, ApoE ϵ 4 genotype, history of cardiovascular disease, BMI and TIV (the last one only for WMH and hippocampal volume) (**Table 1**). Interactions were found between low ω -3 index and ApoE ϵ 4 genotype (**Table 2**). Among the subgroup of ApoE ϵ 4 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 (**Supplementary Table 1**), with significant better cognitive, brain imaging and biomarkers conditions among the subgroup of AD if ω -3 index was low (**Supplementary 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 A β load (SUVR) and tau accumulation after adjustment but was significantly associated with lower right hippocampal volume (β = -85.64, SE=43.03, p =0.047). Interactions were found between low ω -3 index, ApoE ϵ 4 genotype and amyloid status but not cognitive status. Among ApoE ϵ 4 carriers only, low ω -3 index was significantly associated with lower right hippocampal volume after adjustment (β = -212.50, SE=73.39, p =0.004) and with higher Tau accumulation in several regions. Among A β + 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: β =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 A β accumulation over time compared to those in the 3 other quartiles (adjusted β = 0.02, 95% CI [0.01, 0.03]; p =0.003 between M-60 and M0 months) (**Table 4** and **Figure 2**). No significant differences were observed between the two 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 **Supplementary Figure 2**). In both groups, cognitive performances declined between M-60 and M0 months (lowest ω -3 index quartile: unadjusted β = -1.25, 95% CI [-2.12, -0.38]; 3 highest ω -3 index quartiles: unadjusted β = -0.08, 95% CI [-0.59, 0.42]). Compared to those in the 3 highest ω -3 index quartiles, individuals in the lowest ω -3 index quartile had a significant greater cognitive decline (adjusted β = -1.18, 95% CI [-2.16, -0.19]; p =0.019). Total ADAS-Cog cognitive trajectories were also different between the two ω -3 index groups, with low ω -3 index unexpectedly associated with lower cognitive decline across certain time periods (**Table 4** and **Supplementary Figure 2**). In our retrospective longitudinal analysis, cognitive, brain imaging and biomarkers trajectories between participants in the 2 ω -3 index groups did not statistically differ according to ApoE ϵ 4

genotype (**Supplementary 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 to those with a CN status (**Supplementary Table 4** and **Supplementary 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 to those in the 3 highest quartiles had a significant lower cognitive decline in our retrospective analysis (**Supplementary Table 4** and **Supplementary 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 A β 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 omega-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, cognitive or amyloid status. However, unlike non-carriers, ApoE $\epsilon 4$ 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, PET A β 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 A β accumulation over time. Although no differences were observed on the composite cognitive scores, individuals with 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 omega-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 A β and tau accumulation. Only one cross-sectional study with 61 older adults reported an inverse correlation between serum DHA levels 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 omega-3 fatty acids and Tau PET are needed to assess this important relationship.

Studies investigating the relationship between ω -3 PUFA, brain morphology and volumes have also provided mixed findings (10). Higher erythrocyte ω -3 index was correlated with larger total normal brain volume and hippocampal volume in females after menopause from the Women's Health Initiative Memory Study (31). However, no relationships were observed between erythrocyte omega-3 index and total hippocampal volume among dementia-free Framingham Study participants (5). Although accumulating evidence has reported a beneficial role of ω -3 PUFA 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 PUFA 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 ϵ 4 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 ϵ 4 non carriers (35–38), whereas others showed benefits among ApoE ϵ 4 carriers only (13). In the Three-City (3C) Study, greater plasma DHA and EPA were associated with lower cognitive decline in ApoE ϵ 4 carriers (39). Similar findings were observed in secondary analyses from the Rush Memory and Aging Project, with ApoE ϵ 4 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) was only protective towards incident AD in ApoE ϵ 4 carriers. In cognitively unimpaired ApoE ϵ 4 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 red blood cell DHA quintile was 49% lower

compared with the lowest quintile and that ApoE ϵ 4 carriers may benefit more from higher DHA levels than non-carriers (43). There is a biological rationale for a vulnerability of ApoE ϵ 4 carriers to lower ω -3 PUFA status, especially in early AD, since several mechanisms linked ApoE ϵ 4 with reduced brain DHA metabolism (13). It has been suggested that ApoE ϵ 4 carriers β -oxidize DHA at greater rates than non-carriers (44), and are more likely to exhibit dysfunctions in blood-brain barrier (45), which can impair the DHA delivery to the brain (44,46).

In spite of 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 PUFA 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 omega-3 index on cognitive health, including on A β accumulation and confirmed the greater vulnerability of ApoE4 ϵ 4 carriers to lower ω -3 PUFA status. We also observed a trend towards higher tau accumulation among A β + participants with low omega-3 index, which was not confirmed in the retrospective longitudinal analysis. Finally, low omega-3 index was overall mostly associated with lower global cognitive performances 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 PUFA 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 A β and tau accumulation) and their association with ω -3 PUFA status among a large sample of older adults with different cognitive and ApoE ϵ 4 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 to plasma or serum measurements (since fatty acids are stable in erythrocyte membranes over its lifespan, i.e. up to 3 months) (3,49), and known to reflect fatty acids 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 sub-group analyses according to cognitive status included few individuals with AD in the longitudinal analysis, therefore, the results should be taken with caution. Erythrocyte fatty acids assessment was only performed once at baseline during ADNI-3 wave, on defreeze 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 omega-3 index, supporting the reliability of our erythrocyte fatty acids assessment. However, although our

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

Conclusions

Our 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 A β 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 ϵ 4 carriers in the cross-sectional analysis. Our findings are consistent with the vulnerability of ApoE ϵ 4 carriers to lower ω -3 PUFA status reported in the literature and reinforce the group of evidence supporting the potential use of ω -3 PUFA for cognitive decline and AD prevention among ApoE ϵ 4 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 greater heterogeneity of participants, especially those with MCI and AD.

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Authors' contributions

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, PSB and BV interpreted data and revised the draft critically for important intellectual content. MW is the Principal Investigator of the Alzheimer's Disease Neuroimaging Initiative, interpreted data and revised the draft critically for important intellectual content. All authors have read and approved the final version of the manuscript.

Conflict of interest statement

SA reports grants from EU programs (H2020, JPND, FP7), Fondation de l'avenir, personal fees from Nestlé, Nestec SA, Sanofi, MSD, and non-financial support from Biogen, Pfizer, grants from France Alzheimer Association, 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 SAB member for Biogen, Alzheon, Green Valey, Norvo Nordisk , Longeveron, but received no personal compensation. He has served as consultant and/or SAB member for Roche, Lilly, Eisai, TauX with personal compensation. The other authors declare no conflict of interest.

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.

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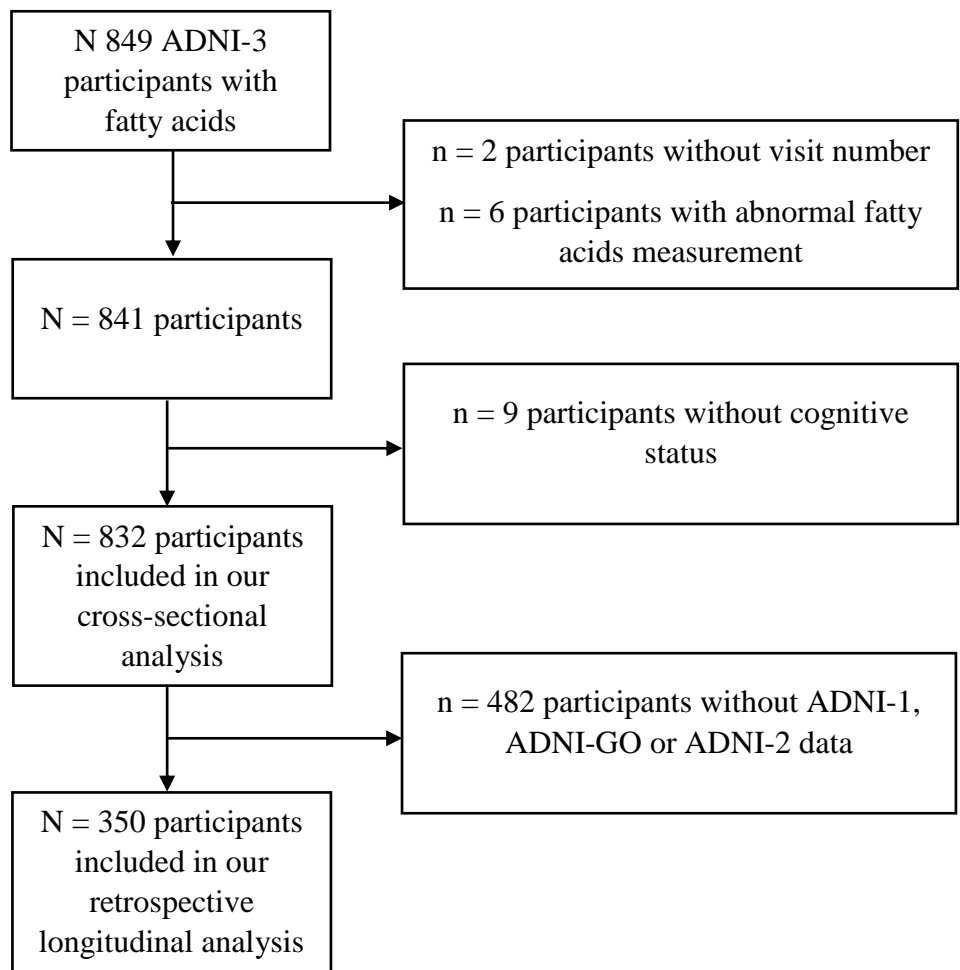
Figure 1. Study flowchart

ADNI: Alzheimer's Disease Neuroimaging Initiative

Figure 2. A β trajectories over time according to the lowest vs. highest ω -3 index quartiles (retrospective longitudinal analysis - whole population)

SUVr, standard uptake value ratio (brain A β load)

Results from mixed linear regression models (mean estimate and 95% CI)



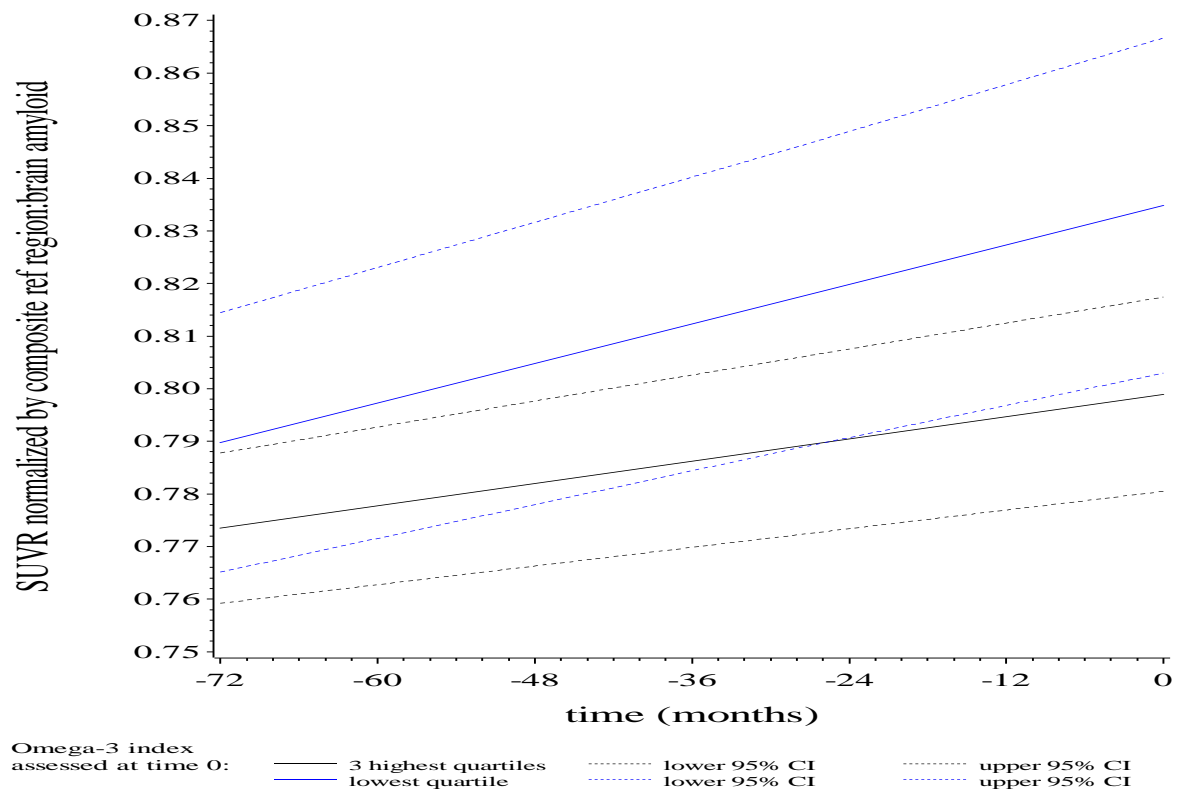


Table 1. Population characteristics, cognitive, brain imaging and biomarkers variables according to ω -3 index status (cross-sectional analysis)

	Total	Lowest ω -3 index quartile ($\leq 3.70\%$) n = 832	3 highest ω -3 index quartiles ($> 3.70\%$) n = 623	unadjusted p-value	adjusted p-value
	mean (SD) ¹	mean (SD) ¹	mean (SD) ¹		
Female sex	423 (50.8%)	109 (52.2%)	314 (50.4%)	0.661 ⁴	-
Age (years)	74.0 (7.9)	73.4 (8.6)	74.3 (7.7)	0.200 ⁵	-
Education (years)	16.5 (2.5)	15.8 (2.4)	16.8 (2.4)	<0.0001⁶	-
Body mass index (kg/m ²) (n = 822)	27.3 (5.2)	27.8 (5.6)	27.1 (5.1)	0.077 ⁵	-
Cognitive status					-
Cognitively normal	465 (55.9%)	117 (56.0%)	348 (55.9%)	0.682 ⁴	
Mild cognitive impairment	272 (32.7%)	65 (31.1%)	207 (33.2%)		
Alzheimer's disease	95 (11.4%)	27 (12.9%)	68 (10.9%)		
ApoE ϵ 4 genotype carriers (n = 794)	299 (37.7%)	69 (34.2%)	230 (38.9%)	0.235 ⁴	
History of cardiovascular disease (n=820)	573 (69.9%)	141 (68.5%)	432 (70.4%)	0.605 ⁴	-

Cognitive tests

Composite cognitive score ² (n=801)	0.00 (3.31)	-0.13 (3.29)	0.04 (3.31)	0.534 ⁵	0.882
MMSE score (n = 828)	27.8 (3.0)	27.9 (2.9)	27.8 (3.1)	0.907 ⁶	0.350
ADAS-Cog delayed recall (n=831)	3.9 (2.6)	3.8 (2.5)	3.9 (2.6)	0.987 ⁶	0.552
Total ADAS-Cog (n=823) ³	17.2 (8.6)	17.5 (8.7)	17.1 (8.6)	0.465 ⁵	0.968
Wechsler Memory Scale-delayed recall (n = 826)	10.5 (5.6)	10.0 (5.5)	10.7 (5.6)	0.109 ⁵	0.293
Wechsler Memory Scale-immediate recall (n = 828)	12.1 (5.2)	11.8 (5.0)	12.2 (5.2)	0.349 ⁵	0.949
Category Fluency test (words) (n = 829)	19.4 (6.1)	18.8 (5.4)	19.6 (6.3)	0.089 ⁵	0.574
TMT-A (seconds) (n = 825) ³	37.4 (21.2)	38.1 (21.1)	37.2 (21.3)	0.351 ⁵	0.513
TMT-B (seconds) (n = 808) ³	97.4 (62.9)	98.5 (63.5)	97.0 (62.8)	0.714 ⁵	0.768
MoCA total score (n = 810)	24.2 (4.5)	24.0 (4.3)	24.2 (4.5)	0.508 ⁵	0.819
CDR sum of boxes (n = 829)	1.15 (2.05)	1.18 (2.09)	1.15 (2.04)	0.759 ⁶	0.995
CDR score > 0 (n=829)	362 (43.7)	93 (25.7)	269 (74.3)	0.780 ⁴	0.892

Brain imaging and biomarkers variables

Total hippocampal volume (mm ³) (n=651)	7168.8 (1090.8)	7203.7 (1034.1)	7156.9 (1110.1)	0.634 ⁵	0.699
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Left hippocampal subcortical volume (mm ³)	3525.2 (546.5)	3547.3 (515.7)	3517.7 (556.8)	0.548 ⁵	0.504
Right hippocampal subcortical volume (mm ³)	3643.5 (571.2)	3656.4 (546.8)	3639.2 (579.7)	0.739 ⁵	0.933
White matter hyperintensities volume (cm ³) (n=773) ³	5.6 (10.6)	5.1 (11.4)	5.7 (10.4)	0.294 ⁵	0.422
Cortical SUVR (n=472) ³	1.18 (0.24)	1.18 (0.24)	1.18 (0.24)	0.896 ⁵	0.561
Amyloid status (n=472)					
A β positive (SUVR > 1.11)	218 (46.2%)	57 (44.2%)	161 (46.9%)	0.593 ⁴	0.837
A β negative (SUVR \leq 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 ⁵	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 ⁵	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 ⁵	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 ⁵	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 ⁵	0.806

A β , amyloid- β ; ADAS, Alzheimer's disease assessment scale; ApoE, apolipoprotein E; CDR, Clinical Dementia Rating; MMSE, Mini Mental State

Examination; MoCA, Montreal Cognitive Assessment; TMT, Trail Making Test; SD, standard deviation; SUVR, standard uptake value ratio normalized by

whole cerebellum; ¹except otherwise specified; ²The modified composite score included the delayed word recall from the Alzheimer's Disease Assessment

Scale - Cognitive Subscale (ADAS-Cog), the Trail Making Test B (TMT-B), the MMSE total score and the Wechsler Memory Scale delayed recall; ω -3 index

defined as erythrocyte % docosahexaenoic acid (DHA) + % eicosapentaenoic acid (EPA); p-values based on Student's T-test ⁵, Kruskal-Wallis test ⁶ or Chi-square test ⁴, after log transformation when needed; Significant p-values are presented in bold, and marginally significant p-values are presented in italics; ³variables with log transformation; Analyses adjusted for age, education, ApoE ε4 genotype, history of cardiovascular disease, body mass index 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.

Table 2. Interactions between ω -3 index (lowest vs. 3 highest quartiles) and ApoE ϵ 4 genotype (carriers vs. non-carriers) on cognitive, brain imaging and biomarkers variables among community-dwelling older adults (cross-sectional analysis)

	n	ApoE ε4 non-carrier	ω-3 index x		
			ApoE ε4 carrier		
			β	SE	p-value
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 ¹	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 (seconds) ¹	787	Ref.	0.02	0.07	0.769
TMT-B (seconds) ¹	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) ¹	736	Ref.	0.15	0.28	0.591
Cortical SUVR ¹	459	Ref.	0.02	0.04	0.608
Tau 18F-AV1451 meta-temporal region ¹	660	Ref.	0.10	0.04	0.010
Tau 18F-AV1451 Braak stage 1 ¹	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 ¹	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 ¹	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.	<i>-1.47</i>	<i>0.84</i>	<i>0.081</i>
Category Fluency test (words)	771	Ref.	-0.47	0.97	0.630
TMT-A (seconds) ¹	767	Ref.	0.04	0.07	0.560
TMT-B (seconds) ¹	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) ¹	718	Ref.	0.18	0.25	0.478
Cortical SUVR ¹	454	Ref.	0.01	0.04	0.783
Tau ¹⁸ F-AV1451 meta-temporal region ¹	648	Ref.	0.09	0.04	0.023
Tau ¹⁸ F-AV1451 Braak stage 1 ¹	648	Ref.	0.11	0.05	0.023
Tau ¹⁸ F-AV1451 Braak stage 2	648	Ref.	<i>0.09</i>	<i>0.05</i>	<i>0.060</i>
Tau ¹⁸ F-AV1451 Braak stages 3 and 4 ¹	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
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ADAS, Alzheimer's disease assessment scale; CDR, Clinical Dementia Rating; MoCA, Montreal Cognitive Assessment; MMSE, Mini Mental State Examination; TMT, Trail Making Test; SE, standard error; SUV_r, standard uptake value ratio normalized by whole cerebellum. The modified composite score included the delayed word recall from the Alzheimer's Disease Assessment Scale - Cognitive Subscale (ADAS-Cog), the Trail Making Test B (TMT-B), the MMSE total score and the Wechsler Memory Scale delayed recall; ω-3 index defined as erythrocyte % docosahexaenoic acid (DHA) + % eicosapentaenoic acid (EPA). Model 2 was adjusted for age, education, history of cardiovascular disease, body mass index and total intracranial volume (this last only for white matter hyperintensities and hippocampal volume); ¹Variable with log transformation; Significant p-values are presented in bold, and marginally significant p-values are presented in italics. Results from linear regression models.

Table 3. Associations between ω -3 index (lowest vs. 3 highest quartiles) and cognitive and brain biomarkers variables among community-dwelling older adults ApoE ϵ 4 carriers and non-carriers (within-group estimates according to the interaction between ω -3 index and ApoE ϵ 4 status presented in Table 2) (cross-sectional analysis)

	ApoE ϵ 4 non-carriers			ApoE ϵ 4 carriers		
	β	SE	p-value	β	SE	p-value
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 ¹	-0.03	0.02	0.294	0.08	0.03	0.014
Tau 18F-AV1451 Braak stage 1 ¹	-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 ¹	-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 ¹	-0.03	0.02	0.192	<i>0.06</i>	<i>0.03</i>	<i>0.066</i>
Tau 18F-AV1451 Braak stage 1 ¹	-0.04	0.03	0.242	<i>0.08</i>	<i>0.04</i>	<i>0.052</i>
Tau 18F-AV1451 Braak stage 2	-0.02	0.03	0.448	<i>0.06</i>	<i>0.04</i>	<i>0.078</i>
Tau 18F-AV1451 Braak stages 3 and 4 ¹	-0.03	0.02	0.198	<i>0.05</i>	<i>0.03</i>	<i>0.061</i>
Tau 18F-AV1451 Braak stages 5 and 6	-0.04	0.04	0.303	<i>0.09</i>	<i>0.05</i>	<i>0.076</i>

SE, standard error; ω -3 index defined as erythrocyte % docosahexaenoic acid (DHA) + % eicosapentaenoic acid (EPA). Model 2 was adjusted for age, education, history of cardiovascular disease, body mass index and total intracranial volume (this last only for white matter hyperintensities and hippocampal volume); ¹Variable with log transformation; Significant p-values are presented in bold, and marginally significant p-values are presented in italics. Results from linear regression models.

Table 4. Differences in retrospective change of cognitive, brain imaging and biomarkers variables between the lowest ω -3 index quartile and the 3 highest quartiles (longitudinal analysis)

Outcome	Time	Lowest ω -3 index quartile			3 highest ω -3 index quartiles			Lowest vs highest ω -3 index quartiles		
		unadjusted						adjusted ¹		
		N	β	95% CI	N	β	95% CI	β	95% CI	p-value
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
<u>Total ADAS-Cog²</u>	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
	Whatever time									0.644
TMT-A (seconds) ²	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
	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
TMT-B (seconds) ²	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
	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
MoCA total score	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
	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
CDR sum of boxes	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
	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
White matter hyperintensities (cm3) ²	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
	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
Cortical SUVR (brain A β load)	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

¹Adjustments: age (years), Education participant (years), apoE4 (yes vs no), History of cardiovascular disease (yes vs no), BMI (Kg/m2) + Intra-Cranial Volume (ICV only for WMH) and their interaction with time; ADAS, Alzheimer's disease assessment scale; CDR, Clinical Dementia Rating; MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment; TMT, Trail Making Test; SUVR, standard uptake value ratio. Results from mixed linear regression models. ²variable with log transformation;

Table 5. Studies exploring erythrocyte omega-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). / Omega-3 index correlated to the feeling subsection of the WebNeuro ($r = 0.25$, $p = 0.01$).
Ammann et al., 2017 (55)	6,706 women ≥ 65 years, 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 omega-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)	1,032 subjects (45–75 years)	Prospective observational study	Verbal fluency, executive function, memory, global cognitive function. / Erythrocyte omega-3 index and omega-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 years) participants of the MAPT Study	Secondary exploratory analysis of a RCT with	Memory, lexical fluency, attention, executive function. / Participants receiving placebo in the lowest quartile of baseline omega-3 index ($\leq 4.83\%$) underwent more 3-year cognitive decline than the other

		omega-3 supplementation	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 years)	Cross-sectional	Impulsivity, attention, information processing, memory and executive function. / Adjustment for omega-3 index weakened the interaction effect between BMI and cognitive domains.
Cook et al., 2019 (58)	299 healthy women (18-35 years)	Cross-sectional	Impulsivity, attention, information processing, memory and executive function. / Participants in the low omega-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 (\pm 10.2) years	Cross-sectional	Visual memory, learning, visuospatial memory, executive function, attention, short-term visual memory, sustained attention, executive function, working memory. / Omega-3 index was not associated with cognitive outcomes.
Dretsch et al., 2014 (60)	78 American service members deployed to Iraq (18-55 years)	RCT with omega-3 supplementation	Reaction time, processing speed, cognitive flexibility, executive functioning, verbal memory, working memory. / Changes in omega-3 index were not associated with changes in cognitive outcomes.

Erhardt et al., 2021 (61)	142 Australian community-dwelling older adults (60-85 years)	Cross-sectional	Memory, language, executive function, visuospatial skills. / Erythrocyte omega-3 index was not associated with cognitive performance.
Johnston et al., 2013 (62)	78 American service members deployed to Iraq (20-54 years)	Cross-sectional	Processing speed, complex attention, reaction time, cognitive flexibility, and executive function. / The omega-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 years)	Cross-sectional	Cognitive status. / In model adjusted by sex and age, subjects with a low omega-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)	1,575 individuals aged 67 ± 9 years	Cross-sectional	Visual memory, executive function, abstract thinking. / Participants with lower omega-3 index (Q1 vs Q2-4) 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) ¹	1,279 older adults from the Three-City (3C) Study	Prospective observational study	Risk of dementia, global cognition, memory. / Higher levels of plasma omega-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 years)	RCT with omega-3 supplementation	Executive function, memory performance, sensorimotor speed, attention. / Changes in omega-3-index correlated positively with improvements in executive functions.

BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HR, hazard ratio; MAPT, Multidomain Alzheimer Preventive Trial; OR, odds ratio; RCT, randomized controlled trial; SD, standard deviation. ¹This study analyzed omega-3 index in plasma, not in erythrocytes.

