沖縄県地域在住80歳以上住民における血清ω-3不飽和脂肪酸濃度と認知機能の関係について

学位論文

Associations between serum omega-3 fatty acid levels and cognitive functions among community-dwelling octogenarians in Okinawa, Japan: The KOCOA study

琉球大学大学院医学研究科　医科学専攻

西平　淳子

Associations between serum omega-3 fatty acid levels and cognitive functions among community-dwelling octogenarians in Okinawa, Japan: The KOCOA study

Junko Nishihira, MD[1)], Takashi Tokashiki, MD, PhD[1)], Yasushi Higashiuesato, MD, PhD[2)], Donald Craig Willcox, MHSc, PhD[3) 4)], Nora Mattek, MPH[5)], Lynne Shinto, ND, MPH[5)], Yusuke Ohya, MD, PhD[1)], Hiroko H. Dodge, PhD[5) 6)]

1) Dept. of Cardiovascular Medicine, Nephrology and Neurology, University of the Ryukyus, Okinawa, Japan
2) Clinical Laboratories, University of the Ryukyus Hospital、Okinawa, Japan
3) Department of Human Welfare, Okinawa International University, Okinawa, Japan
4) Department of Geriatric Medicine, John A. Burns School of Medicine, University of Hawaii, Honolulu, USA
5) Dept. of Neurology and Layton Aging & Alzheimer's Disease Center, Oregon Health & Science University, Portland, OR, USA
6) Dept. of Neurology and Michigan Alzheimer's Disease Center, University of Michigan, Ann Arbor, MI, USA

Junko Nishihira, M.D.

Department of Cardiovascular Medicine, Nephrology and Neurology

University of the Ryukyus School of Medicine

207 Uehara, Nishihara-cho, Okinawa 903-0215, Japan

Telephone: +81-98-895-1150; Fax: +81-98-895-1416; Email; jonko100jp@yahoo.co.jp

Corresponding author:

Hiroko H. Dodge, PhD

Director, Biostatistics and Data Core, NIA-Layton Aging and Alzheimer's Disease Center,

Department of Neurology, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, CR131, Portland, OR 97239-3038, USA.

Tel.: +1 503 494-6695; Fax: +1 503-494-7499; Email; hdodge@med.umich.edu

Running title: Relation of Omega-3 with cognitive function

Abstract

Background: Epidemiological studies have found frequent consumption of fatty fish is protective against cognitive decline. However, the association between circulating omega-3 polyunsaturated fatty acid (PUFA) levels and cognitive functions among the oldest old is not well known.

Objective: To examine the association between serum PUFA levels and cognitive function among community-dwelling, non-demented elderly aged over 80 years old.

Methods: The data came from the Keys to Optimal Cognitive Aging (KOCOA) study; an ongoing cohort of relatively healthy volunteers aged over 80 years old, living in Okinawa, Japan. One hundred eighty five participants (mean age 84.1 ± 3.4 years) assessed in 2011 who were free from frank dementia (defined as Clinical Dementia Rating < 1.0) were used for the current cross-sectional study. We examined whether serum omega-3 PUFAs (docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]), arachidonic acid (AA), EPA/AA ratio, DHA/AA ratio and DHA+EPA are associated with (1) age and (2) global cognitive function (Japanese MMSE) and executive function (Verbal Fluency Letters). Data was analyzed univariately by *t*-test and multivariately by cumulative logistic regression models controlling for age, gender, years of education, obesity, hypertension, diabetes, and dyslipidemia.

Results: Serum DHA levels decreased with increasing age ($p = 0.04$). Higher global cognitive function was associated with higher levels of serum EPA ($p = 0.03$) and DHA + EPA ($p = 0.03$) after controlling for confounders.

Conclusions: Higher serum EPA and DHA + EPA levels were independently associated with better scores on global cognitive function among the oldest old, free from dementia. Longitudinal follow-up studies are warranted.

Keywords:

DHA, EPA, PUFA, cognitive function, oldest old, Okinawa, non-demented subjects, KOCOA

INTRODUCTION

According to the recent estimate by the Japanese Ministry of Health, Labour and Welfare, the proportion of those aged 65 years and older in Japan was 24% in 2012 and is projected to increase rapidly in the coming decades, up to 39% by 2050 [1,2]. As populations age, the prevalence of those with cognitive impairment will increase sharply. Identifying therapies that can delay cognitive decline or prevent dementia onset are urgently needed. There is consistent epidemiological evidence showing a decreased risk of dementia among those who consume fish versus those who do not [3-8]. Studies have also shown higher dietary intake of omega-3 polyunsaturated fatty acids (PUFAs), estimated from a food intake questionnaire as well as circulating omega-3 fatty acid levels, are associated with reduced risk for cognitive decline [9,10] suggesting a protective effect of dietary omega-3 fatty acid intake on cognitive decline. In animal studies, omega-3 fatty acid administration has been found to improve learning ability [11,12]. Several studies have also reported that PUFAs have shown promise in reducing cardiovascular disease risk factors including hypertension, hypertriglyceridemia, arrhythmia, inflammation and coronary calcification [13-15], although not all studies necessarily has found these benefits [16,17]. There have been several reports indicating that risk factors of late life cognitive decline and dementia could be different than those found in mid-life. For example, the protective effects of lower triglyceride levels, lower blood pressure, and lower BMI in midlife on cognitive decline appear to be less robust or even reversed in the oldest old populations [18-22]. We previously reported that metabolic syndrome is not

associated with cognitive decline among the oldest old, using a sub-group of the same cohort examined in this study [23].

We investigated the association between PUFAs and cognitive function among healthy volunteers aged 80 years and older, free from frank dementia living in Okinawa, Japan, the southern island prefecture (state) of Japan. The island has one of the highest concentrations of centenarians in the world and is known for healthy aging [24]. We examined global cognitive function as well as executive function based on previous study findings [10,25].

METHODS

Study design and participants

Data came from a cross-sectional study, part of a prospective pilot cohort study of community-dwelling adult aged 80 years and older living in Okinawa, Japan called the Key to Optimal Cognitive Aging Project (KOCOA). A detailed description of the recruitment process has been described elsewhere [26,27]. Briefly, researchers visited 21 senior centers, explained the study protocol, and asked them to participate in the study. A request to join the study was made at the conclusion of each presentation. We recruited community-dwelling adults aged 80 years and older who needed no or partial assistance for all instrumental activities of daily living. The baseline recruitment occurred in 2007 and participants were assessed annually until 2011. Between November 2011 and April 2012, new participants were recruited using the same methods as before. These participants and those followed from the

original cohort were used in the current analyses. Out of 191 participants (60 from the original cohort) who consented to the study and completed a face-to-face interview, four were taking anticholinesterase agents and two had frank dementia defined by Clinical Dementia Rating Scale (CDR) [28] ≥ 1 and thus were excluded from the current study. The remaining 185 participants (CDR = 0 or 0.5) were assessed for their fatty acid levels using serum samples.

This study was approved by the Ethics Committee of the University of the Ryukyus, and the Institutional Review Board at Oregon Health & Science University. Informed consent was obtained from all participants prior to enrollment in the study.

Cognitive Measures

Cognitive function was assessed by a trained interviewer using the Japanese version of the Mini-Mental State Examination (J-MMSE) [29,30]. The J-MMSE is a measure of global cognitive function and ranges from 0 to 30 points, with higher scores representing better cognitive function. It takes approximately 10 minutes to administer and includes questions on orientation to time and place, attention and calculation, recall, language, and visual construction. The Verbal Fluency Letters (VFL) test was used to measure executive function. This test requires the participant to generate as many words as they can beginning with the letter “Ka” (in Japanese) in 60 seconds, and has been validated as an equivalent test to Initial Letter Fluency [31].

Serum fatty acid measurements

Venous blood samples were collected in the morning after an overnight fast. Serum was separated by centrifugation at 3000 r.p.m. for 15 min and stored at -80 °C until analysis. The laboratory analyses were performed by SRL Inc. (Tokyo, Japan), using standard laboratory protocols.

PUFA estimates from food intake questionnaire

In our preliminary analysis, we found that serum DHA levels are negatively associated with age. To shed light on whether this is due to age-associated changes in dietary patterns, we examined the association between the amount of DHA and EPA obtained from food using a brief self-administered diet history questionnaire (BDHQ). The measurement is validated and explained in detail elsewhere [32, 33]. Briefly, the BDHQ is a 4-page fixed-portion questionnaire that asks about the consumption frequency of selected foods to estimate the dietary intake of 58 food and beverage items during the preceding month. The BDHQ consists of five sections: (1) intake frequency of food and nonalcoholic beverage items, (2) daily intake of the Japanese staple rice and miso soup (typical Japanese soup), (3) frequency and amount of alcoholic beverage intake, (4) usual cooking methods, e.g. fried, steamed or broiled in Japanese or Western Style and (5) general dietary behavior e.g. speed of eating and thickness of seasoning. The amount of DHA and EPA consumed from foods is estimated by using the information from these five categories and a validated algorithm.

Potential confounders and covariates

First we assessed the association between age and PUFA levels. Then we examined the association between PUFAs and cognitive functions, controlling for the following confounders: age, hypertension, HbA1c, LDL Cholesterol and obesity. Hypertension was defined by systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or currently taking antihypertensive medication. The HbA1c value was estimated as a Japan Diabetes Society (JDS) method equivalent value (%), which was calculated by the formula: HbA1c (%) = HbA1c (%) (National Glycohemoglobin Standardization Program (NGSP) - 0.4% [34]. High HbA1c was defined by HbA1c level ≥ 6.1% (JDS). High LDL-Cholesterol (LDL-C) was defined by LDL-C level ≥140 mg/dl. Obesity was defined as Body Mass Index ≥ 25 kg/m^2 according to guidelines published by the Japan Society for the Study of Obesity [35].

Statistical Analysis

Associations between nutrient markers (EPA, DHA, AA, EPA/AA, DHA/AA, DHA + EPA) and age were examined by linear regression models. We then categorized J-MMSE and VFL into two groups and used t-tests to compare levels of nutrient markers by dichotomized J-MMSE (J-MMSE < 24 vs. J-MMSE ≥ 24) and dichotomized VFL (VFL < 5 vs. VFL ≥ 5). There is no predefined clinical threshold for VFL. Therefore, we dichotomized below/above the lowest 33% as a cut point (score of 5). To address the skewed distributions, we used a

log-transformation of nutrient markers for the above univariate analyses except DHA which was normally distributed. Our preliminary results showed no association between nutrient markers and VFL. Therefore, subsequent multivariate analyses were conducted only for J-MMSE. Nutrient markers which were significant on univariate analyses were included in the multivariate models. We divided J-MMSE into three groups (Group 1: lowest tertile, Group 2: middle tertile, Group 3: upper tertile). Those in the lowest tertile of J-MMSE score were compared to participants with higher tertiles using cumulative logit models (i.e., the model compares (a) Group 1 vs. 2 and above, and (b) Groups 1 and 2 vs. Group 3 in outcomes). This model generates two intercepts and one coefficient, assuming the effects of independent variables on dependent variables are the same for the comparisons of (a) and (b) above. Our preliminary analysis confirmed that this model assumption was met in our data. We divided each nutrient marker into three groups: lowest 25%, middle 50% and highest 25%, with the lowest 25% group being the reference group and included each marker separately into models controlling for age, gender, years of education, obesity, hypertension, high HbA1c and high LDL-C, with the latter five items as categorical variables. All statistical analyses were performed with JMP® 11 for Windows (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at $p < 0.05$. Additionally, we provided the false discovery rate approximation adjusted p-value in table footnotes as multiple comparison adjusted type I error rates, using equation $\alpha(m + 1)/2m$, where α is 0.05 and m is the number of tests.

RESULTS

Characteristics of the 185 participants are shown in Table 1. Mean age was 84.1 years (standard deviation (SD) = 3.4), and 77% were women. Mean years of education was 7.6 (SD = 2.5) and 63% were cognitively intact (CDR = 0). Mean J-MMSE score was 24.7 (SD = 3.6). The ranges of J-MMSE were 15-23, 24-27 and 28-30 for the 1^{st} 2^{nd}, and 3^{rd} tertiles, respectively. Although we excluded those with CDR $\geq$ 1.0, our cohort is old (aged 80 years and older) with a relatively low education level, thus the lower tertile includes J-MMSE scores of less than 24, the conventional cut point for dementia.

Figure 1 graphically depicts the associations between age and serum PUFAs levels. DHA levels decreased as age increased ($p = 0.04$). Positive univariate associations were found between levels of log EPA ($p = 0.01$), log (EPA/AA) ratio ($p = 0.02$) and log (DHA + EPA) ($p = 0.03$) with dichotomized J-MMSE scores (Table 2). There were no associations between nutrient markers and dichotomized VFL testscores (Table 3).

Table 4 shows the results of cumulative logistic regression analyses with J-MMSE tertile groups as outcomes (lowest J-MMSE group as the reference). After adjustment for cofounders, EPA and DHA + EPA remained significantly associated with J-MMSE scores. The odds of being in a higher J-MMSE tertile group was 2.38 times higher if participants had an EPA value in the highest 25%, compared with those in the lowest 25%. Those with EPA values in the middle 50% had 2.33 times higher odds of being in a higher J-MMSE tertile group compared with those in the lowest EPA 25% tile. Likewise, those with DHA + EPA values in

the highest 25%, and those in the middle 50% of DHA + EPA values were 2.31 times and 2.52 times more likely to have higher J-MMSE scores compared with those in the lowest 25%.

To explore the reason for the negative association between DHA and age, we examined whether the association was due to changes in dietary intake patterns. We examined the amount of DHA obtained from food by using the BDHQ. The estimated mean DHA intake from food was 485.1 ± 380.3 mg per day. Mean EPA was 270.0 ± 246.8 mg per day. We found no association between either DHA and age ($p = 0.49$), or EPA and age ($p = 0.68$), using linear regressions, suggesting that our finding of a negative association between age and DHA is unlikely due to changes in diet (i.e. not due to lower consumption of DHA from food with older age).

Finally fatty fish, the primary source of omega-3 fatty acids in the Japanese diet, is also a good source of vitamin D. Vitamin D has been shown to be associated with cognitive functions [36]. Therefore, as a post hoc anlaysis, we controlled Vitamin D levels estimated from the BDHQ in the cumulative logit models. The mean ± SD (μg/day) was estimated as: 11.3 ± 10.2. The levels were positively but weakly correlated with serum DHA and EPA levels (Pearson's correlation coefficients between Vitamin D and DHA: 0.24 ($p < 0.01$) and log EPA: 0.25 ($p < 0.01$)). Even after adding vitamin D as a control variable, EPA and EPA+DHA maintained its significance in the association with J-MMSE scores. Vitamin D was not associated with J-MMSE in all models, possibly due to a large variability associated with the

estimation from the food intake.

DISCUSSION

The current study, where community-dwelling oldest old volunteers free from frank dementia were examined, has two noteworthy findings. First, serum DHA levels decreased with increasing age among those 80 years old and older, while EPA did not. Second, higher levels of serum EPA were associated with better global cognitive function as measured by J-MMSE.

Associations between PUFAs and age

Kuriki et al. (2002) found that plasma EPA and DHA increased with age among Japanese female dietitians under 66 years old [37]. Otsuka *et al*. also reported that omega-3 PUFAs including α-linoleic acid, EPA, DHA, and docosa-pentaenoic acid increased with age, whereas omega-6 fatty acids including linoleic acid and AA decreased with age among those 40 to 88 years [38]. Authors in both studies suggested changes in diet (older adults may eat more fatty fish and less red meat [39]) as a possible reason for this age trend. The participants in the above two studies were younger with a wider age range than those in our study. Using autopsy data from participants between 33 and 92 years old, the level of in vivo omega-3 PUFAs, particularly DHA, was found to decrease with age [40]. In our study, the consumption of EPA and DHA estimated from a food intake questionnaire was stable regardless of age. EPA is transformed to DHA by a converting enzyme. Reduced ability to

convert the enzyme or to absorb DHA might be reasons for the age-associated decline in DHA levels seen in our study. Alternatively, age-related defects in antioxidant systems may increase lipid peroxidation and thereby decrease levels of omega-3 PUFAs [41]. Further studies are warranted to confirm the underlying mechanisms of the age-associated decline in DHA among the oldest old.

The levels of DHA, EPA and EPA/AA ratio found in our cohort were similar to those found in several other Japanese studies; however our cohort's AA levels were higher in comparison [42, 43]. For example, in Yanagisawa et al's study, the mean level of serum DHA was 123.2 ± 27.0 μg/ml, EPA was 81.9 ± 31.1 μg/ml, AA was 119.8 ± 22.7 μg/ml, and EPA/AA ratio was 0.68 ± 0.22 among those aged 65 and older. In Yagi et al's study, the mean level of serum DHA was 132.5 ± 52.2 μg/ml and EPA was 68.9 ± 40.4 μg/ml. Our mean values were DHA: 129.2 ± 36.3 μg/ml, EPA: 62.5 ± 52.2 μg/ml, AA: 195.1 ± 47.7 μg/ml and EPA/AA ratio: 0.34 ± 0.34 (Table 1). The reason our cohort has higher AA values may be due to the fact that Okinawa had been under the United States occupancy from the end of World War II until 1972 and therefore western food culture has been more prevalent, leading to higher consumption of red meat. As for comparisons with non-Japanese cohorts, using plasma samples, Bowman et al. [44] reported the mean DHA and EPA values were 68.1 ± 17.8 μg/ml and 16.5 ± 10.5 μg/ml, respectively, among community-dwelling elderly aged 80 and older (mean age of 85.7 ± 10) with normal cognition in Portland (Oregon) in the United States. These values are much lower than those shown among our study. The mean EPA value reported in the study by

Bowman et al., is lower than the 25% lowest cut point of EPA (33.5 μg/ml) in our study, with which we found significant association with lower cognitive test scores. Sekikawa et al. [45] examined serum PUFAs values among quadragenarians in Japan and the United States. They reported that mean serum percentage of PUFAs was much higher among the Japanese than in the United States (9.08% versus 3.84%, $p < 0.01$), coinciding with our finding among the octogenarians. The implication of large differences in PUFA values across regions or countries on cognitive declines or dementia incidence would be an interesting future research area. As previously suggested, both inter- and intra- cultural examinations are required to give a full perspective on the association between nutrition and health [26].

The differential effect of DHA and EPA on cognition

We found that higher levels of serum EPA, but not DHA, were associated with better cognitive function as measured by J-MMSE. Studies have shown that omega-3 PUFAs, mainly DHA and EPA, have protective effects with regards to anti-inflammatory properties [46-48], cardiovascular disease [45, 49] and cognitive decline. The Framingham Heart Study showed that higher plasma DHA levels were associated with a 47% reduction in the risk of developing all-cause dementia and a 39% reduction in AD over nine years of follow-up. Their mean cohort age at baseline was about 75 years old. The Framingham Study did not examine EPA and its effects on dementia incidence. The Three-City Study from Bordeaux (France) reported that higher plasma EPA levels, but not DHA levels, were associated with a

lower incidence of dementia of all types (HR = 0.69; 95% CI: 0.48-0.98) [50]. Martins et al. (2009) examined the differential effect of EPA and DHA on depression and cognition through an interventional meta-analysis of 28 randomized controlled trials [51]. They reported that EPA had a larger effect than DHA on depressive status and cognition function. Umegaki et al. found that higher serum triglycerides (TG) were associated with cognitive decline in Japanese non-demented older adults [52], and omega-3 PUFAs, mainly EPA and DHA, were found to reduce serum TG concentrations [53,54]. Harris et al., reported potential TG-lowering mechanisms of EPA and DHA via inhibition of hepatic very-low-density-lipoprotein cholesterol. In a review of omega-3 PUFA supplementation clinical trials to reduce cardiovascular risk, Itakura et al. reported that an EPA/AA ratio > 0.75 was associated with lower major coronary artery events in a Japanese cohort with mean age of 61 years (SD = 9) [55]. EPA may also have neuroprotective effects [56]; prevention of neuronal apoptosis prevents hippocampal dysfunction [57]. EPA was found to be more effective at reducing inflammation in vivo than DHA [58], although both dietary EPA and DHA incorporate into cell membranes. The excessive production of pro-inflammatory eicosanoids derived from AA are competitively compensated for by the anti-inflammatory products of EPA metabolism [59]. Interestingly, we found that the odds of having higher MMSE scores were similar regardless of being in the highest 25% or the middle 50% of EPA or EPA + DHA levels. No dose response relation beyond the lowest 25% threshold suggests that as long as octogenarians keep their levels higher than the 25% tile in EPA and EPA + DHA, they could lower their risk

for worsening cognitive outcomes. The cut off levels for the lowest 25% tile for EPA and EPA + DHA were 37.3 μg/ml and 155.1 μg/ml, respectively.

Vitamin D: a potential confounder

Vitamin D has been found to be associated with cognitive functions. Most recently, Miller et al. reported that those with dementia had a lower level of serum 25-hydroxyvitamin D compared to those with MCI and normal cognition [59]. They also reported that the rates of decline in episodic memory and executive function over time were steeper among Vitamin D-deficient participants than those with adequate status [59]. It is possible the effect of EPA on cognition found in our study might be, at least partly, due to Vitamin D. Therefore, as a post hoc analysis, we included Vitamin D consumption levels estimated from the BDHQ in the multinomial logit models. After controlling for Vitamin D level, serum EPA maintained its significant association with J-MMSE scores. Although vitamin D itself was not associated with the J-MMSE scores, its levels estimated from the food intake questionnnarie in our study had a large variability, making inferences difficult. The examination of their protective effects on cognition among the oldest old is further warranted.

The association of DHA and EPA with executive function

Bowman et al. [10, 43] found significant associations between executive function and DHA as well as EPA. We did not find any associations between EPA, DHA and executive function.

Although the test we used to measure executive function in this study (Word fluency starting with "Ka" (Table 3)) taps the executive domain, it also measures phonetic abilities (language-based executive functions), while Bowman et al. used Trail Making Test B. The difference in tests could explain the discrepancy in findings between our study and theirs.

Limitations of our study include its cross-sectional design and thus our inability to demonstrate a casual relationship between serum omega-3 PUFAs and cognitive function. Also we could not examine the combined effects of PUFAs with other nutrient markers such as trans fat, saturated fats, or vitamins B, C, D and E , which could have synergistic effects on cognition. Moreover participants were healthy volunteers living in Okinawa, which may limit the generalizability of the study results.

In conclusion, we found that higher serum EPA and DHA + EPA levels are associated with better cognitive function among community-dwelling non-demented volunteers aged 80 years and older. Higher intake of seafood may promote better cognitive function, even in the relatively healthy oldest old population. Further research is needed to improve the understanding of the association between circulating EPA, DHA and dementia.

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Conflict of Interest

The authors report no conflicts of interest.

Author Contributions

JN drafted the paper. TT, YH, YO and HHD assisted in the research design. TT and HHD were responsible for obtaining funding. JN performed analyses, JN, TT, NM, DCW and HHD interpreted the results, and performed revisions.

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Table 1. Demographic and clinical characteristics of the cohort (n = 185)

	Variables	Value
Female	n (%)	142 (77%)
Age, yrs	mean ± SD (range)	84.1 ± 3.4 (80-94)
Education, yrs	mean ± SD (range)	7.6 ± 2.5 (0-17)
J-MMSE	1st tertile (score; 15-23), n (%)	71 (38%)
	2nd tertile (score; 24-27), n (%)	61 (33%)
	3rd tertile (score; 28-30), n (%)	53 (29%)
VFL	1st tertile (score; 0–4), n (%)	46 (25%)
	2nd tertile (score; 5–6), n (%)	58 (32%)
	3rd tertile (score; 7–19), n (%)	80 (43%)
CDR	0, n (%)	117 (63%)
	0.5, n (%)	68 (37%)
BMI	mean ± SD (kg/m^2)	24.6 ± 3.4
Obesity	n (%)†	83 (45%)
HbA1c ≥ 6.1%	n (%)‡	34 (18%)
Hypertension	n (%)§	144 (78%)
LDL –C ≥ 140	n (%)	108 (58%)
DHA	mean ± SD (μg/ml)	129.2 ± 36.3
EPA	mean ± SD (μg/ml)	62.5 ± 52.2
AA	mean ± SD (μg/ml)	195.1 ± 47.7

DHA/AA	mean ± SD (μg/ml)		0.69 ± 0.22
EPA/AA	mean ± SD (μg/ml)		0.34 ± 0.34
DHA + EPA	mean ± SD (μg/ml)		191.6 ± 75.2
Dietary intake*	DHA	mean ± SD (mg/day)	485.1 ± 383.0
	EPA	mean ± SD (mg/day)	270.0 ± 246.8
	AA	mean ± SD (mg/day)	151.9 ± 81.2

† Defined as BMI of 25 kg/m^2 or higher

‡HbA1c of 6.1% (Japan Diabetes Society, JDS) was calculated as the National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) -0.4%.

§Defined as systolic blood pressure of ≥ 140 mmHg, diastolic blood pressure of ≥ 90 mmHg, , or the use of antihypertensive medication.

*: Estimated from Brief Self-Administered Diet History questionnaire (BDHQ).

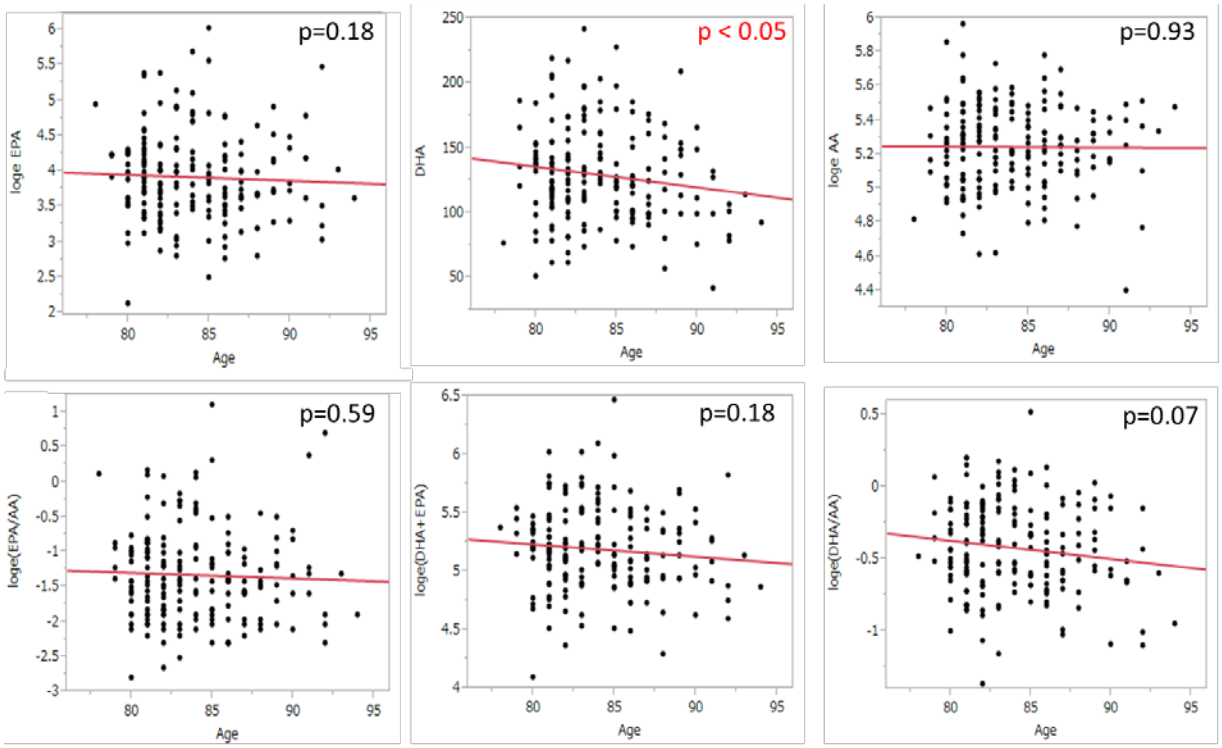

Fig 1. Associations between age and plasma PUFA levels

Table 2. Univariate analyses (*t*-test) of J-MMSE and PUFA levels

Measurement	MMSE < 24 (n = 71)	MMSE ≥ 24 (n = 114)	p-value
DHA	124.64 ± 4.31	132.00 ± 3.40	0.19
log EPA	3.77 ± 0.07	4.00 ± 0.06	0.01**
log AA	5.25 ± 0.03	5.24 ± 3.40	0.81
log (EPA/AA)	- 1.48 ± 0.08	-1.24 ± 0.06	0.02**
log (DHA/AA)	- 0.48 ± 0.04	-0.39 ± 0.03	0.07
log (DHA+EPA)	5.12 ± 0.04	5.23 ± 0.03	0.03*

* $p < 0.05$ ** $p < 0.029$ (multiple comparison adjusted p-value using false discovery rate approximations)

Table 3. Univariate analysis (*t*-test) of Verbal Fluency Letter (VFL) test and PUFAs

Measurement	VFL < 5 (n = 46)	5 ≤ VFL ≤ 19 (n = 138)	p-value
DHA	121.22 ± 5.36	131.83 ± 3.11	0.09
log EPA	3.77 ± 0.09	3.96 ± 0.05	0.77
log AA	5.21 ± 0.04	5.25 ± 0.02	0.34
log (EPA/AA)	- 1.44 ± 0.10	- 1.30 ± 1.64	0.18
log (DHA/AA)	- 0.46 ± 0.05	-0.41 ± 0.03	0.25
log (DHA + EPA)	5.11 ± 0.05	5.22 ± 0.03	0.07

Table 4. Results of cumulative logit models with J-MMSE tertile groups as outcomes

Independent Variables of Interests (PUFAs)		Odds Ratio (OR) of having higher J-MMSE scores [95% Confidence Interval (CI)]
DHA	Low (reference)	1.0
	Middle	1.74 (0.84–3.61)
	High	1.56 (0.67–3.64)
EPA	Low (reference)	1.0
	Middle	2.33 (1.12–4.84)**
	High	2.38 (1.05–5.42)**
EPA/AA	Low (reference)	1.0
	Middle	2.00 (0.96–4.00)
	High	1.94 (0.85–4.41)
DHA + EPA	Low (reference)	1.0
	Middle	2.52 (1.21–5.24)*
	High	2.31 (1.01–5.28)*

* $p < 0.05$; **Significant after adjustment of p-values with false discovery rate approximations are as follows: 0.05(5)/2*4 = 0.031. Low PUFAs; the lowest 25% (Reference

group), Middle PUFAs; middle 50% tile, High PUFAs; the highest 25%. Cumulative logistic regression analyses controlled for age, gender, years of education, hypertension, obesity, HbA1c ≥ 6.1% (JDS) and LDL-C ≥ 140mg/dl.

Younger age, higher education and HbA1c < 6.1% were also significantly associated with higher J-MMSE scores in all models listed above.