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ORIGINAL ARTICLE

# Serum docosahexaenoic and eicosapentaenoic acid and risk of cognitive decline over 10 years among elderly Japanese

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**BACKGROUND/OBJECTIVES:** To clarify the association of serum docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) levels with cognitive decline over 10 years.

**SUBJECTS/METHODS:** This study was part of the National Institute for Longevity Sciences - Longitudinal Study of Aging, and was conducted with 232 male and 198 female Japanese community-dwelling subjects aged 60–79 years in the second wave (2000–2002). Cognitive function was assessed with the Mini-Mental State Examination (MMSE) in both the second and seventh (2010–2012) waves. Fasting venous blood samples were collected in the morning, and serum DHA and EPA levels were measured. Multiple logistic regression analysis was performed among participants with an MMSE score $\geqslant 24$ in the second wave ($n = 430$) to estimate the odds ratio (OR) and 95% confidence interval (CI) for MMSE score $\leqslant 23$ or MMSE score decline $\geqslant 4$ 10 years later. These estimates were based on baseline tertiles of serum DHA or EPA levels, and controlled for age, sex, education, MMSE score at baseline, alcohol consumption, current smoking, body mass index and disease history.

**RESULTS:** Fifteen (3.5%) subjects whose MMSE score was $\leqslant 23$ and 36 (8.3%) subjects whose MMSE score declined to $\geqslant 4$ showed cognitive decline. Multivariate-adjusted OR (95% CI) for the lowest through highest tertiles of serum DHA to MMSE score $\leqslant 23$ or decline $\geqslant 4$ were 1.00 (reference), 0.11 (0.02–0.58) and 0.17 (0.04–0.74), or 1.00 (reference), 0.22 (0.08–0.61) and 0.31 (0.12–0.75), respectively ($P$ for trend $= 0.01$ or 0.04). Serum EPA was not associated with cognitive decline.

**CONCLUSIONS:** The study gives some indication that a moderately high level of serum DHA might prevent cognitive decline among community-dwelling elderly Japanese individuals.

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**Keywords:** DHA; EPA; serum; cognition; Japanese; elderly

## INTRODUCTION

An estimated two million people in Japan suffer from dementia and this number will likely increase as the population ages.[1] The essential n-3 polyunsaturated fatty acids (PUFA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) that constitute the predominant long-chain PUFAs of membrane phospholipids in mammalian brains and neural tissues, are crucial for maintenance of brain function.[2]

Fish consumption, particularly fatty fish, and intake of marine n-3 PUFA, DHA and EPA are thought to play a protective role against age-related cognitive decline.[3] However, results of cross-sectional and longitudinal studies examining the association between fish or n-3 PUFA intake and cognitive performance have been inconsistent, with some studies showing that high intake of n-3 PUFA was associated with better cognitive performance[4–6] and other studies showing no association.[3,5] One possible reason for these inconsistent results is the limited ability of dietary assessments to quantify blood levels of fatty acids (FA). Blood FA biomarkers can be measured to indicate differences in their delayed response to short- and long-term dietary intakes.[7,8] Studies using n-3 series PUFA in the blood have shown that higher concentrations of DHA in erythrocyte membranes,[9] DHA in plasma phosphatidylcholine[10] and plasma EPA[11] are associated with a lower risk of cognitive decline or Alzheimer's disease. Recently, lower red blood cell EPA and DHA levels were reported to be correlated with smaller brain volumes in elderly subjects without clinical dementia.[12] Furthermore, it has been proposed that FAs in the blood are associated with cognitive function.[13] However, other studies focusing on dementia not only reported no difference in DHA in plasma cholesterol esters and phospholipids,[14] but also reported significantly higher DHA in plasma phospholipids[15] or cholesteryl esters.[16,17] Hence, results of studies examining the association between blood FA and cognitive performance have been inconsistent.

Mean DHA and EPA intake/serum DHA/EPA levels among Caucasian subjects are substantially lower than those of Japanese subjects.[18–21] The effect of serum DHA/EPA levels on cognitive function may vary among Japanese subjects, and the association between serum DHA and EPA levels and cognitive decline among Japanese subjects remains unclear. In addition, studies that examined the effectiveness of serum DHA/EPA levels on cognition in Japanese subjects with high serum DHA/EPA levels would explain one of the reasons that DHA/EPA supplementation trials in Caucasians, in whom serum DHA/EPA levels were substantially low, demonstrated essentially no effect from DHA on cognitive impairment.[22,23] We considered that the duration of these intervention studies examining the effectiveness of DHA/EPA on cognitive performance were relatively short, and

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long-term effectiveness of DHA/EPA intake on cognitive performance would be easier to clarify among Japanese subjects because they are naturally exposed to higher DHA/EPA concentrations. No study in Japan and only a few studies among Asians have reported the association between blood FA and cognitive impairment.[24,25]

To clarify the effectiveness of serum DHA and EPA levels on cognitive decline among the Japanese whose DHA and EPA intake/serum DHA/EPA levels are higher than among Caucasians, and who are naturally exposed to high DHA/EPA concentrations, the present longitudinal study was carried out in elderly community-dwelling Japanese subjects and examined the associations of serum DHA and EPA levels with cognitive decline.

## SUBJECTS AND METHODS

### Participants

Data for this survey were collected as part of the National Institute for Longevity Sciences - Longitudinal Study of Aging (NILS-LSA). In this project, the normal aging process has been assessed over time using detailed questionnaires and medical checkups, anthropometric measurements, physical fitness tests and nutritional examinations. Participants in the NILS-LSA included randomly selected age- and sex-stratified individuals from the pool of non-institutionalized residents in the NILS neighborhood areas of Obu City and Higashiura Town in Aichi Prefecture. The first wave of the NILS-LSA was conducted from November 1997 to April 2000 and comprised 2267 participants (1139 men, 1128 women; age range, 40–79 years). Details of the NILS-LSA study have been reported elsewhere.[26]

The second wave of the NILS-LSA was conducted from April 2000 to May 2002 and comprised 2259 participants (1152 men, 1107 women; age range, 40–82 years). Among these participants, 1351 (690 men, 661 women) were also included in the seventh wave of the NILS-LSA, which was conducted from July 2010 to July 2012. The mean (±s.d.) interval between the second and seventh wave for each participant was 10.2 (±0.4) years.

Exclusion criteria were as follows: (1) those who were <60 years in the second wave ($n=868$), as cognitive function tested by the Mini-Mental State Examination (MMSE) was assessed only among participants aged 60 or older; (2) those who had an MMSE score ⩽23 in the second wave ($n=10$); and (3) those who did not complete either the alcohol intake assessments or the self-reported questionnaire ($n=43$). A total of 430 Japanese (232 men, 198 women) who had been between 60 and 79 years in the second wave of the NILS-LSA were available for analysis.

The study protocol was approved by the Committee of Ethics of Human Research of the National Center for Geriatrics and Gerontology (No. 369-2). Written informed consent was obtained from all subjects.

### Blood sampling and serum FA analysis

Upon enrolment in the second wave of the NILS-LSA, venous blood was collected early in the morning after fasting for at least 12 h. Blood samples were centrifuged at 3500 *g* for 15 min. Serum was separated and frozen at −80 °C before analysis for FA content by a single technician. Serum DHA and EPA were measured by gas-liquid chromatography at a clinical laboratory (SRL, Tokyo, Japan). In brief, total lipids in the serum were extracted using the Folch procedure and FAs were then methylated with BF3/methanol. Transesterified FAs were then analyzed using a gas chromatograph (GC-17A; Shimadzu, Kyoto, Japan) with a capillary column (Omegawax 250; Supelco, Bellefonte, PA, USA). The weights of DHA and EPA (g/ml) as FA concentrations were identified by comparison with known standards. Intra- and inter-assay precision and accuracy values (coefficient of variation (CV)) were 2.7 and 6.9 CV% for EPA, and 1.9 and 6.9 CV% for DHA, respectively.

### Assessment of cognitive function

Cognitive function was assessed by the Japanese version of the MMSE through interviews with a trained psychologist or clinical psychotherapist in both the second and seventh waves.[27,28] The MMSE is widely used as a brief screening test for dementia, and scores range from 0 to 30 points, with a higher score indicating better cognitive function. The MMSE includes questions on orientation of time and place, registration, attention and calculation, recall, language and visual construction. We used two different cutoff scores: (1) a decline of at least 4 points in the MMSE score from the second to seventh wave, which has been shown to be meaningful from a clinical point of view,[29–31] and (2) a cutoff score of ⩽23, which is traditionally used to represent 'suggestive cognitive impairment'[27,28] and thus was also used in the main analyses. Among participants in this study with an MMSE ⩾24 in the second wave ($n=430$), (1) 36 (8.3%) who had a decline of at least 4 points in the MMSE score from the second to seventh wave (10 years later), and (2) 15 (3.5%) who had an MMSE score ⩽23 in the seventh wave (10 years later) were classified as showing cognitive decline, respectively. We defined the second wave as baseline, as the MMSE method between the second and seventh wave was consistent, and there were slight modifications of the procedure between the first and second waves.

### Nutritional assessments

Nutritional intakes were assessed using a 3-day dietary record after participation in the second wave survey. The dietary record was completed over three continuous days (both weekend days and 1 weekday),[32] and most subjects completed it at home and returned records within 1 month. Food was weighed separately on a scale (1-kg kitchen scales; Sekisui Jushi, Tokyo, Japan) before being cooked or portion sizes were estimated. Subjects used a disposable camera (27 shots; Fuji Film, Tokyo, Japan) to take photos of meals before and after eating. Dietitians used these photos to complete missing data and telephoned subjects to resolve any discrepancies or obtain further information when necessary. Averages for 3-day food and nutrient intakes were calculated according to the fifth edition of the Standard Tables of Foods Composition in Japan and other sources.[32] Alcohol intake in the previous year was assessed using a food frequency questionnaire; trained dietitians interviewed subjects using this questionnaire.

### Other measurements

Medical history of heart disease, hypertension, hyperlipidemia, diabetes (past and current), education (⩽9, 10–12 or ⩾13 years of school) and smoking status (yes or no) were collected using self-report questionnaires. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Serum triacylglycerol levels were measured using enzymatic methods, and total and high-density lipoprotein-cholesterol levels were measured using the dehydrogenase method and direct method at a clinical laboratory (SRL). These measurements were assessed in the second wave.

### Statistical analysis

All statistical analyses were conducted using statistical analysis system software version 9.1.3 (SAS Institute, Cary, NC, USA). The confounding variables were age (year, continuous), sex, education (⩽9, 10–12, ⩾13 years), MMSE score at baseline (continuous), alcohol consumption (ml/day), current smoking status (yes or no), BMI (kg/m$^2$), history of heart disease, hypertension, hyperlipidemia and/or diabetes (yes or no). Differences in proportions and means of covariates according to the MMSE score in the seventh wave (10 years later) were assessed using the $\chi^2$-test or Fisher's exact probability test (if statistical expectation ⩽5) and independent *t*-test, respectively. Comparisons between baseline dietary intakes according to the MMSE score 10 years later were performed by independent *t*-test.

Multiple logistic regression analysis was performed to estimate the odds ratio (OR) and 95% confidence interval (CI) for (1) a decrease in MMSE score of at least 4 points or (2) an MMSE score ⩽23 in the seventh wave according to tertiles of serum DHA or EPA. The lowest tertile category was used as a reference. The independent variables in the first model were age, sex and education. The second model was further adjusted for MMSE score at baseline, alcohol consumption, current smoking status, BMI, history of heart disease, hypertension, hyperlipidemia and/or diabetes. Trend associations were assessed by assigning dummy variables of −1, 0 and 1 to tertiles of serum DHA or EPA. In the logistic regression analysis, we tested goodness-of-fit (Hosmer–Lemeshow test) using the lackfit option and calculated the generalized $R^2$ (Nagelkerke $R^2$) measure using the $r^2$ option. Age, sex and education-adjusted mean MMSE score according to tertiles of serum DHA or EPA were calculated using the PROC GLM procedure. To eliminate the effects of other confounding variables on MMSE score, a subsequent model included MMSE score at baseline, alcohol consumption, current smoking status, BMI, history of heart disease, hypertension, hyperlipidemia and diabetes as covariates. All reported *P* values are two-sided, and a $P$ value $< 0.05$ was considered significant.

## RESULTS

Baseline characteristics of subjects according to the MMSE score in the seventh wave (10 years later) and subjects excluded from the analyses are shown in Table 1. Fifteen subjects (3.5%) were classified as showing cognitive decline (MMSE score $\leqslant$23). Compared with subjects with an MMSE score $\geqslant$24, those with an MMSE score $\leqslant$23 were significantly less likely to be educated, significantly older and had a significantly higher BMI. Compared with subjects with both an MMSE score $\leqslant$23 and $\geqslant$24, subjects excluded from the analyses were older, more likely to be current smokers, and more likely to have a history of hyperlipidemia and diabetes. Mean serum EPA or DHA among subjects excluded from the analyses was intermediate between subjects with MMSE score $\leqslant$23 and $\geqslant$24.

Table 2 shows baseline dietary intakes of subjects according to MMSE score 10 years later. Compared with subjects with an MMSE score $\geqslant$24, those with an MMSE score $\leqslant$23 ate significantly less fat and vegetables and significantly more fruits and sweets.

Table 3 shows the ORs and 95% CIs for an MMSE score decline of at least 4 points in the seventh wave (10 years later) according to tertiles of serum FAs. In the age-, sex- and education-adjusted model, serum DHA levels were significantly associated with a decreased prevalence of cognitive decline. After further adjustment for other covariates, the association remained statistically significant. The multivariate-adjusted ORs (95% CIs) for the lowest through highest tertiles of serum DHA were 1.00 (reference), 0.22 (0.08–0.61) and 0.31 (0.12–0.75), respectively ($P$ for trend = 0.004, goodness-of-fit Pr > 0.93, $R^2 = 0.22$). Serum EPA was not associated with cognitive decline.

Table 4 shows mean MMSE scores and ORs (95% CIs) for MMSE score $\leqslant$23 in the seventh wave (10 years later) according to tertiles of serum FAs. Mean MMSE scores according to tertiles of serum FAs were not statistically significant. In the age-, sex- and education-adjusted model, serum DHA levels were significantly associated with a decreased prevalence of cognitive decline. After further adjustment for other covariates, the association remained statistically significant; the multivariate-adjusted OR (95% CI) for the lowest through highest tertiles of serum DHA were 1.00 (reference), 0.11 (0.02–0.58) and 0.17 (0.04–0.74), respectively
($P$ for trend = 0.01, goodness-of-fit Pr > 0.85, $R^2 = 0.32$). Serum EPA was not associated with cognitive decline.

## DISCUSSION

This study provides longitudinal evidence that low serum DHA levels were associated with a higher risk of cognitive decline over a 10-year period in community-dwelling Japanese adults aged 60 years and older. This association remained after controlling for baseline MMSE score and other variables. This is the first study to examine the association between serum DHA/EPA levels on cognitive decline among Japanese subjects whose DHA and EPA intake/serum DHA/EPA levels are higher than those seen in Caucasians and in whom ordinary exposure to DHA/EPA concentrations was high.

The ARIC (Atherosclerosis Risk in Communities) and Framingham Study studies, which examined n-3 series PUFA in the blood, showed that higher concentrations of these FAs were associated with a lower risk for cognitive decline.[10,14] Among elderly French subjects, DHA of erythrocyte membranes[9] and plasma EPA[11] have also been shown to be associated with a lower risk for cognitive decline. However, other studies focusing on dementia not only reported no difference in DHA in plasma cholesterol esters and phospholipids,[14] but also reported significantly higher DHA in either plasma phospholipids[15] or cholesteryl esters.[16,17] Hence, the results from studies examining the association between blood FA and cognitive performance have been inconsistent.

However, serum n-3 series PUFA differs markedly in middle-aged Japanese, Japanese-American and Caucasian (American) men.[33] DHA and EPA levels from the blood of Japanese men are

**Table 1.** Baseline characteristics of subjects according to the MMSE score 10 years later and subjects excluded from the analyses in the NILS-LSA study

| | *Subjects available for analyses* (n = *430*) | | | *Subjects excluded from the analyses*[a] (n = *715*)[c] |
|---|---|---|---|---|
| | *MMSE* $\leqslant$*23* | *MMSE* $\geqslant$*24* | P-*value*[b] | |
| Number of subjects | 15 | 415 | | |
| MMSE (mean ± s.d.) | 27.7 ± 1.4 | 28.4 ± 1.4 | 0.04 | 27.5 ± 2.2 |
| Age (mean ± s.d., years) | 70.9 ± 5.9 | 66.4 ± 5.0 | <0.01 | 71.3 ± 5.5 |
| BMI (mean ± s.d., kg/m$^2$) | 24.4 ± 2.7 | 22.8 ± 2.7 | 0.02 | 22.9 ± 3.3 |
| Alcohol (mean ± s.d., ml/day) | 10.2 ± 15.4 | 8.1 ± 13.5 | 0.55 | 7.5 ± 14.7 |
| Female (%) | 46.7 | 46.0 | 0.96 | 50.9 |
| *Education* | | | | |
| $\leqslant$9 years (%) | 66.7 | 31.1 | 0.01 | 47.0 |
| 10–12 years (%) | 6.7 | 15.7 | | 21.8 |
| $\geqslant$13 years (%) | 26.7 | 53.3 | | 31.2 |
| Current smoking status (%) | 6.7 | 15.4 | 0.35 | 17.4 |
| History of hypertension (%) | 53.3 | 31.1 | 0.07 | 42.5 |
| History of hyperlipidemia (%) | 13.3 | 21.5 | 0.45 | 21.9 |
| History of diabetes (%) | 6.7 | 7.2 | 0.93 | 12.8 |
| Triacylglycerol (mean ± s.d., mg/dl) | 85.9 ± 25.7 | 120.9 ± 62.1 | 0.03 | 117.7 ± 66.6 |
| Total cholesterol (mean ± s.d., mg/dl) | 219.1 ± 37.9 | 219.1 ± 33.7 | 0.99 | 216.5 ± 36.1 |
| HDL cholesterol (mean ± s.d., mg/dl) | 61.27 ± 16.1 | 59.9 ± 14.9 | 0.72 | 60.7 ± 15.8 |
| Serum EPA (mean ± s.d., μg/ml) | 74.9 ± 41.1 | 81.5 ± 39.7 | 0.53 | 77.5 ± 40.9 |
| Serum DHA (mean ± s.d.,μg/ml) | 145.0 ± 38.5 | 162.2 ± 45.2 | 0.15 | 157.1 ± 49.4 |

Abbreviations: BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high density lipid; MMSE, Mini Mental State Examination.
[a]Subjects excluded from the analyses included those who were older than 60 years in the second wave and those who did not participate in the seventh wave.
[b]For continuous variables, independent *t*-test was used; for categorical variables, $\chi^2$test or Fisher's exact probability test was used. [c]The number of excluded subjects according to the characteristics listed ranged from 672 to 715.

**Table 2.** Baseline dietary intakes of subjects according to the MMSE score 10 years later in the NILS-LSA study

| | *MMSE⩽23* | *MMSE⩾24* | P-*value*[a] |
|---|---|---|---|
| Number of subjects | 15 | 415 | |
| Energy (mean ± s.d., kcal/day) | 2270.0 ± 371.5 | 2095.9 ± 394.8 | 0.85 |
| Protein (mean ± s.d., energy%) | 14.7 ± 1.5 | 15.7 ± 2.0 | 0.22 |
| Fat (mean ± s.d., energy%) | 21.5 ± 6.0 | 23.5 ± 4.3 | 0.03 |
| Saturated fat (mean ± s.d., g/day) | 16.2 ± 5.2 | 15.4 ± 5.1 | 0.85 |
| Polyunsaturated fat (mean ± s.d., g/day) | 12.2 ± 2.7 | 12.9 ± 3.6 | 0.25 |
| DHA (mean ± s.d., mg/day) | 543.0 ± 250.4 | 590.3 ± 1.4 | 0.07 |
| EPA (mean ± s.d., mg/day) | 302.5 ± 155.6 | 321.3 ± 383.0 | 0.11 |
| Cereals (mean ± s.d., g/day) | 475.0 ± 145.2 | 469.9 ± 139.5 | 0.74 |
| Beans (mean ± s.d., g/day) | 79.2 ± 35.6 | 72.8 ± 49.9 | 0.14 |
| Vegetables (mean ± s.d., g/day) | 283.9 ± 81.3 | 336.0 ± 130.5 | 0.04 |
| Fruits (mean ± s.d., g/day) | 259.8 ± 209.7 | 175.7 ± 129.1 | 0.002 |
| Fish and shellfish (mean ± s.d., g/day) | 113.6 ± 63.5 | 102.2 ± 50.3 | 0.16 |
| Meats (mean ± s.d., g/day) | 40.9 ± 23.8 | 56.7 ± 32.4 | 0.18 |
| Eggs (mean ± s.d., g/day) | 46.9 ± 29.5 | 46.7 ± 25.7 | 0.39 |
| Milk and dairy products (mean ± s.d., g/day) | 213.1 ± 120.5 | 165.6 ± 128.6 | 0.83 |
| Sweets (mean ± s.d., g/day) | 71.7 ± 53.8 | 38.3 ± 38.6 | 0.04 |

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MMSE, Mini Mental State Examination. [a]Independent *t*-test was used.

**Table 3.** ORs and 95% CIs for MMSE scores that declined at least 4 points during 10 years according to tertiles of serum fatty acids

| | *Tertiles of serum fatty acids* | | | |
|---|---|---|---|---|
| | *T1 (low)* | *T2* | *T3 (high)* | *Trend* P[a] |
| EPA (range, μg/ml) | 14.1–59.2 | 59.2< − 90.4 | 90.4< − 31.8 | |
| Number of subjects MMSE score declined ⩾4/⩽3 | 12/129 | 13/129 | 11/136 | |
| Age, sex, and education-adjusted OR (95% CI)[b] | 1.00 (reference) | 1.18 (0.50–2.79) | 0.86 (0.35–2.09) | 0.70 |
| Multiple-adjusted OR (95% CI)[b,c] | 1.00 (reference) | 1.10 (0.44–2.75) | 0.69 (0.27–1.76) | 0.83 |
| DHA (range, μg/ml) | 59.3–138.5 | 138.5< − 175.6 | 175.6< − 354.6 | |
| Number of subjects MMSE score declined ⩾4/⩽3 | 21/118 | 6/138 | 9/138 | |
| Age, sex, and education-adjusted OR (95% CI)[b] | 1.00 (reference) | 0.23 (0.09–0.60) | 0.35 (0.15–0.81) | 0.003 |
| Multiple-adjusted OR (95% CI)[b,c] | 1.00 (reference) | 0.22 (0.08–0.61) | 0.31 (0.12–0.75) | 0.004 |

Abbreviations: CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MMSE, Mini Mental State Examination; OR, odds ratio. [a]On the basis of multiple logistic regression analysis, assigning dummy variables − 1, 0, 1 to tertiles of serum fatty acids. [b]Adjusted ORs and CIs were based on multiple logistic regression analysis. [c]Adjusted for age (year, continuous), sex, education (⩽9, 10–12, ⩾13 years), MMSE score at baseline (continuous), alcohol consumption (ml/day), current smoking status (yes or no), body mass index (kg/m$^2$), and history of heart disease, hypertension, hyperlipidemia and diabetes (yes or no).

**Table 4.** Mean (s.e.) MMSE score and ORs (95% CIs) for MMSE scores ⩽23 10 years later according to tertiles of serum fatty acids

| | *Tertiles of serum fatty acids* | | | | |
|---|---|---|---|---|---|
| | *T1 (low)* | *T2* | *T3 (high)* | *ANCOVA* P | *Trend* P[a] |
| EPA (range, μg/ml) | 14.1–59.2 | 59.2< − 90.4 | 90.4< − 31.8 | | |
| Age, sex and education-adjusted MMSE score[b] | 27.78 (0.15) | 27.53 (0.15) | 27.77 (0.15) | 0.44 | 0.99 |
| Multiple-adjusted MMSE score[b,c] | 27.79 (0.15) | 27.57 (0.15) | 27.72 (0.15) | 0.43 | 0.74 |
| Number of subjects with MMSE ⩽23/MMSE ⩾24 | 4/137 | 9/133 | 2/145 | | |
| Age, sex and education-adjusted OR (95% CI)[d] | 1.00 (reference) | 2.76 (0.78–9.72) | 0.51 (0.08–2.91) | | 0.11 |
| Multiple-adjusted OR (95% CI)[c,d] | 1.00 (reference) | 2.92 (0.74–11.54) | 0.52 (0.08–3.24) | | 0.13 |
| DHA (range, μg/ml) | 59.3–138.5 | 138.5< − 175.6 | 175.6< − 354.6 | | |
| Age, sex and education-adjusted MMSE score[b] | 27.48 (0.15) | 27.89 (0.15) | 27.70 (0.15) | 0.18 | 0.29 |
| Multiple-adjusted MMSE score[b,c] | 27.47 (0.15) | 27.90 (0.15) | 27.68 (0.15) | 0.17 | 0.32 |
| Number of subjects with MMSE ⩽23/MMSE ⩾24 | 10/129 | 2/142 | 3/144 | | |
| Age, sex, and education-adjusted OR (95% CI)[d] | 1.00 (reference) | 0.16 (0.03–0.78) | 0.26 (0.07–0.98) | | 0.02 |
| Multiple-adjusted OR (95% CI)[c,d] | 1.00 (reference) | 0.11 (0.02–0.58) | 0.17 (0.04–0.74) | | 0.01 |

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MMSE, Mini Mental State Examination; OR, odds ratio. [a]On the basis of the general linear model or multiple logistic regression analysis, assigning dummy variables − 1, 0, 1 to tertiles of serum fat [b]Adjusted MMSE scores (mean ± s.e.) were based on the general linear model. [c]Adjusted for age (year, continuous), sex, education (⩽9, 10–12, ⩾13 years), MMSE score at baseline (continuous), alcohol consumption (ml/day), current smoking status (yes or no), body mass index (kg/m$^2$), and history of heart disease, hypertension, hyperlipidemia and diabetes (yes or no). [d]Adjusted ORs and CIs were based on multiple logistic regression analysis.

significantly higher than those from the blood of Caucasian men.[33] Mean (±s.d.) serum EPA and DHA concentrations in our sample of subjects with an MMSE ⩾24 were 81.5 (±39.7) and 162.2 (±45.2) μg/ml, respectively. On the other hand, among cognitively healthy adults aged 70–79 years living in England, these plasma levels were 39.1 (±3.1) and 70.7 (±2.9) μg/ml, respectively.[34]

The biological mechanisms through which serum DHA exerts beneficial effects on cognition can be divided into vascular and non-vascular pathways. In terms of vascular pathways, the beneficial effects of DHA and EPA are well known, including blood pressure reduction[35] and pronounced effects on eicosanoid production[36] and two cardiovascular risk factors that may lead to cognitive decline.[37] In terms of non-vascular pathways, DHA is highly concentrated in membrane phospholipids of brain gray matter, and it has particular effects on membrane properties and cell signaling.[38] The precise mechanism of its effect, however, is unknown, although deficits in DHA could contribute to inflammatory signaling, apoptosis or neuronal dysfunction in the elderly.[39]

In terms of serum DHA levels, the multivariate-adjusted ORs for MMSE score decline of at least 4 points, and MMSE ⩽23 after 10 years were 1.00 (tertile 1, reference), 0.22 (0.08–0.61) and 0.31 (0.12–0.75) ($P$ for trend = 0.004), or 1.00 (tertile 1, reference), 0.11 (0.02–0.58) and 0.17 (0.04–0.74) ($P$ for trend = 0.01), respectively. Statistical significance was confirmed, but a dose–response relationship between serum DHA levels and cognitive decline was not observed. One of the possibilities for this finding is that serum DHA concentrations in our sample were substantially higher than the levels seen in Caucasian subjects,[33] and these higher blood levels of DHA might be above the threshold level to detect any effect on cognitive decline. In most previous studies of Caucasians, the mean DHA blood levels were in the lowest tertile seen in this study.[33,34] In addition, DHA/EPA supplementation trials in Caucasian subjects whose serum DHA/EPA levels were substantially lower demonstrated essentially no benefit of DHA on cognitive impairment.[22,23] One of the reasons these intervention studies failed might be due to the short duration used to examine the effectiveness of DHA/EPA on cognitive performance. In contrast, Japanese subjects, who have a normally high exposure to high DHA/EPA concentrations, might show different findings. No previous studies that we are aware of have examined serum DHA levels and cognitive decline among the people whose serum DHA/EPA levels were high. Hence, we cannot compare our findings with previous studies.[24,25] Our study presents the possibility that low DHA levels formed over time in blood are a risk factor for cognitive decline rather than that high DHA levels are a protective factor against cognitive decline among the population whose ordinary exposure to DHA/EPA concentrations is high.

Although the precise reason that the OR of the highest tertile in serum DHA was higher than that of the second tertile is unknown, we believe that one possible explanation is that the number of cases was too small. In fact, multiple-adjusted MMSE scores after 10 years according to tertiles of serum DHA were 27.47 (tertile 1), 27.90 (tertile 2) and 27.68 (tertile 3) and did not reach statistical significance (ANCOVA $P = 0.17$, $P$ for trend = 0.32) because the number of cases was too small and no differences in MMSE scores could be detected. To address the small number of subjects, we performed subanalyses to examine the relationships between baseline serum DHA concentration and follow-up MMSE score using Pearson's correlation coefficients ($n = 430$). Even after controlling for age at baseline, no significant positive correlations between serum DHA concentrations and MMSE score at follow-up were observed (partial correlation coefficient $r = 0.029$, $P = 0.55$).

Dietary intakes might belie the association between serum DHA/EPA and MMSE score; for example, subjects with an MMSE score <23 might eat less of the traditional Japanese diet that includes high intakes of fish and rice, or eat more of the western diet that includes high intakes of meat and dairy products[40] compared with subjects with MMSE scores ⩾24. Recently, dietary patterns characterized by a high intake of soybeans, vegetables, algae, and milk and dairy products and a low intake of rice were reported to be associated with reduced risk of dementia in the general Japanese population.[41] However, in our study, subjects with an MMSE score ⩽23 had less intake of DHA (543.0 vs 590.3 mg/day, $P = 0.07$), significantly less intake of fat and vegetables and greater intake of fruits and sweets compared with subjects with an MMSE score ⩾24. Fish and shellfish intake between the two groups were not statistically different (113.6 vs 102.2 g/day, in Table 2). To eliminate the effects of dietary intake including sugar, sweets, fruits, fat and vegetables on MMSE decline, we performed multiple logistic regression analysis further adjusted for intakes of sugar, sweets, fruits, fat and vegetables. The association between serum DHA levels and MMSE decline held up even after controlling for these food intakes (data not shown). Hence, no specific dietary pattern or food intake seemed to bias the association between serum DHA/EPA and MMSE score.

Several limitations to the present study warrant consideration. First, we assessed cognitive function only using a general cognitive test, that is, the MMSE. Although the MMSE is widely used as a brief screening test for dementia, it could be affected by demographic variables such as educational level. Among older patients with a college education living in the United States, the MMSE cutoff score of 27 (sensitivity, 0.69; specificity, 0.91) or 28 (sensitivity and specificity, 0.78) has been shown to be better for detecting cognitive dysfunction compared to the value of ⩽23 used in this study (sensitivity, 0.66; specificity, 0.99).[42] Among our Japanese sample, 52% (224/430) had an education level of 13 years or more. Therefore, the MMSE cutoff point of ⩽23 may be inadequate to assess cognitive impairment. On the basis of this limitation, we used the other cutoff score that was (1) a decline of at least 4 points in MMSE score from the second to seventh wave (Table 3) and (2) an MMSE cutoff score of 28 in a subanalysis. The former analysis was consistent with the results when we used the MMSE cutoff point of ⩽23. However, in the latter subanalysis, an MMSE score ⩽27 was seen in 36% of our Japanese sample (118/326) in the seventh wave, although no significant association was observed between serum DHA/EPA levels and cognitive decline (data not shown). Because of the lack of a sufficient number of cases, when the serum DHA levels were divided into quartiles or quintiles, a few categories contained only one case, although there were still statistically significant findings in a few categories (OR of the fourth quartile: 0.21, $P = 0.05$, OR of the third quintile: 0.11, $P = 0.07$, data not shown).

Second, serum FA concentrations were assessed from a single blood sampling. However, Kobayashi *et al.* examined correlations between serum phospholipid FA levels collected twice and FA intake assessed from 7-day weighted dietary records among 87 Japanese men, and reported that a single measurement of serum phospholipids was a useful biomarker of n-3 PUFA.[8] Although that study used serum phospholipids, Ogura *et al.* reported that PUFA levels in plasma and erythrocyte phospholipids were nearly identical among 75 Japanese patients admitted for non-malignant diseases.[43] Third, attrition bias may have affected our results. Compared with included subjects, subjects excluded from the analyses were older, more likely to be current smokers, and more likely to have a history of hyperlipidemia and diabetes. Hence, excluded subjects might have been less healthy than subjects included in the final analysis. However, mean serum EPA or DHA among subjects excluded from the analyses was higher than those among subjects with an MMSE score ⩽23, and our results do not necessarily mean that subjects with lower serum EPA or DHA levels were more likely to drop out during the follow-up period. Fourth, DHA and EPA intake/serum levels among Japanese subjects are substantially higher than those of Caucasian subjects,[18–21] and the tissue n-3/n-6 ratio that would alter

eicosanoid patterns[44] might also differ between these groups. Furthermore, genetic factors, including APOE4, might also modify the metabolism of n-3 PUFA.[45] However, we could not assess the n-3/n-6 ratio or genetic factors in this study.

The main strengths of the present study are as follows: (1) the long average follow-up period of 10 years; (2) the use of an older sample of randomly selected age- and sex-stratified non-institutionalized individuals from the community; and (3) the use of serum FA levels to assess DHA or EPA status. Furthermore, a certain level of serum DHA is modifiable through the consumption of fish or dietary supplements in DHA.[46] Recently, red blood cell levels of DHA plus EPA were reported to be explained by DHA plus EPA intake (25%), heritability (24%) and fish oil supplementation (15%) in the Framingham Heart Study.[47] In our Japanese sample ($n = 430$), Pearson's correlation coefficient between serum DHA and DHA intake assessed by a 3-day dietary record was 0.18 ($P < 0.01$, data not shown). This finding means that serum DHA levels are an adjustable factor to some extent.

In conclusion, the findings of this study give some indication that a moderately high level of serum DHA among the Japanese, whose DHA and EPA intake/serum DHA/EPA levels are higher than among Caucasians, might prevent cognitive decline among elderly, community-dwelling Japanese individuals.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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