Dementia and Geriatric Cognitive Disorders Extra

Research Article

Dement Geriatr Cogn Disord Extra 2025;15:140–151
DOI: 10.1159/000548369

Received: November 13, 2024
Accepted: August 31, 2025
Published online: September 9, 2025

Impact of the Apolipoprotein E ε4 Allele on Cognition and Omega-3 Fatty Acid Levels in the Plasma Membrane of Red Blood Cells in Healthy Elderly Japanese Population

Michio Hashimoto[a, b] Kentaro Matsuzaki[a] Chikashi Matsuda[c] Harumi Wakatsuki[a] Shahdat Hossain[d] Miho Ohno[e] Setsushi Kato[e] Shuzo Ohata[e] Kazuya Yamashita[f] Shuhei Yamaguchi[g] Atsushi Nagai[b] Osamu Shido[a, h]

[a]Department of Environmental Physiology, Faculty of Medicine, Shimane University, Izumo, Japan; [b]Department of Internal Medicine III, Faculty of Medicine, Shimane University, Izumo, Japan; [c]Department of Laboratory Medicine, Shimane University School of Medicine, Izumo, Japan; [d]Department of Biochemistry and Molecular Biology, Jahangirnagar University, Dhaka, Bangladesh; [e]Kato Hospital, Jinjukai Healthcare Corporation, Kawamoto, Japan; [f]Faculty of Nursing and Nutrition, The University of Shimane, Izumo, Japan; [g]Shimane Prefecture Hospital Bureau, Izumo, Japan; [h]Shimane Rehabilitation College, Oku-izumo, Japan

Keywords
Apolipoprotein E ε4 allele · Omega-3 fatty acid · Cognition · Elderly

Abstract
Introduction: The ε4 allele of the apolipoprotein E (APOE4) gene is a well-known risk factor for the onset and development of late-onset Alzheimer's disease (AD). Lipid metabolism also plays a key role in AD. However, data on the association between APOE4, cognitive function, and blood lipid metabolism, particularly fatty acid metabolism, in the healthy elderly Japanese population are lacking. ***Methods:*** We analyzed the baseline data of 506 healthy elderly Japanese individuals (mean age: 73 ± 0.4 years) from Shimane Prefecture, Japan, who participated in six intervention trials conducted between 2008 and 2020. Among them, participants with mild cognitive impairment (MCI) were divided into the following two groups: APOE4 carriers (n = 104) and noncarriers (n = 321). ***Results:*** Compared with the noncarriers, the APOE4 carriers had significantly lower scores in the "recalling five objects" sub-item of Hasegawa's Dementia Scale-Revised and longer total times in the Cognitive Assessment for Dementia, iPad version. The ratio of docosahexaenoic acid (DHA)-to-arachidonic acid was significantly decreased, and the erythrocyte eicosapentaenoic acid (EPA) and DHA levels tended to be reduced in APOE4 carriers. ***Conclusion:*** These findings suggest a possible association between the APOE4 allele and reduced erythrocyte EPA and DHA levels, even in healthy elderly Japanese individuals with high ω-3 fatty acid intake. Such alterations in lipid metabolism may be linked to cognitive vulnerability in older adults and individuals with MCI.

© 2025 The Author(s).
Published by S. Karger AG, Basel

karger@karger.com
www.karger.com/dee

© 2025 The Author(s).
Published by S. Karger AG, Basel

This article is licensed under the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC) (https://karger.com/Services/OpenAccessLicense). Usage and distribution for commercial purposes requires written permission.

Correspondence to:
Michio Hashimoto, michio1@med.shimane-u.ac.jp

Introduction

Apolipoprotein E (APOE), which is the most abundant apolipoprotein in the brain, plays a crucial role in the transport and metabolism of cholesterol and other lipids essential for optimal neuronal function and membrane integrity. Given the importance of membrane lipid composition in maintaining the health and functionality of neuronal cells, APOE is recognized as a critical protein in brain functions [1]. The gene encoding APOE has also been reported to be strongly linked to the pathology of late-onset Alzheimer's disease (LOAD) [2]. Among its three common alleles, i.e., ε2, ε3, and ε4, the ε4 allele (APOE4) is identified as a significant genetic risk factor for the development of LOAD. Epidemiological studies have demonstrated that individuals carrying the APOE4 allele are at an increased risk of developing the disease, with those having the two ε4 alleles (homozygous APOE4/4) facing an approximately 10-fold higher risk as compared to noncarriers [3, 4]. In addition to increasing the risk of LOAD, the APOE4 allele affects lipid transport and metabolism in ways that may disrupt fatty acid distribution in the brain [5, 6]. This distribution may impair the uptake and distribution of omega-3 polyunsaturated fatty acids (ω-3 PUFAs), particularly docosahexaenoic acid (DHA), which plays a key role in maintaining brain health [7, 8]. Evidence from both human and animal studies supports this notion. For example, Yassine et al. [9] reported reduced brain DHA uptake in cognitively normal APOE4 carriers. Similarly, Vandal et al. [10] demonstrated impaired DHA transport to the brain in APOE4 transgenic mice, suggesting reduced central DHA availability. In line with these findings, Tomaszewski et al. [11] observed a smaller increase in plasma DHA levels following supplementation in APOE4 carriers compared to in noncarriers, indicating altered peripheral DHA metabolism. Despite these findings, studies exploring the relationship between APOE4 and blood fatty acid profiles in cognitively healthy older adults – particularly in Japanese populations – remain limited. Further research in broader populations is warranted to better understand this association.

Numerous epidemiological and observational studies have emphasized the essential role of ω-3 PUFAs in promoting neurological development and cardiovascular health [12–16] and in preserving cognitive function throughout life. Among the ω-3 PUFAs, DHA is particularly important, as it is the predominant ω-3 fatty acid in the human brain. Given the brain's limited ability to synthesize DHA endogenously, adequate levels must be obtained through dietary sources [14–16]. Reduced serum DHA levels are reported to be associated with cognitive decline in both AD patients and elderly individuals with mild cognitive impairment (MCI) [9, 16, 17], suggesting that DHA intake could potentially support brain health and improve the cognitive performance in these populations.

Despite the promising link between DHA and cognitive health, results from numerous randomized controlled trials (RCTs) evaluating the impact of DHA or ω-3 PUFA supplementation have been inconsistent [18, 19]. One reason for this inconsistency may be the failure of many studies to access the baseline blood ω-3 PUFA level, a critical factor in assessing the true impact of supplementation [20]. Measuring the blood ω-3 PUFA levels is essential because they serve as biomarkers of long-term dietary intake and fatty acid status. Particularly, red blood cell plasma membrane (RBC-PM) fatty acids are considered a more reliable indicator of long-term fatty acid consumption, reflecting its dietary intake over the previous 120 days [21]. Contrarily, the serum and plasma fatty acids fluctuate more readily in response to short-term dietary changes.

In the present study, we focused on the RBC-PM fatty acid composition, which provides a more accurate reflection of tissue fatty acid metabolism, to explore the relationship between the APOE4 genotype, cognitive function, and lipid profiles in healthy elderly Japanese individuals. Understanding these interactions is crucial, as APOE4 carriers have an increased genetic risk for LOAD, and their response to DHA supplementation may differ from noncarriers. By examining the RBC-PM fatty acid levels in relation to cognitive function and lipid metabolism, we also discussed the potential for DHA supplementation as a strategy to mitigate cognitive decline, particularly in APOE4 carriers who may be more vulnerable to deficiencies in ω-3 PUFA metabolism and associated neurodegenerative risks.

Methods

Study Design and Data Collection

In this study, we analyzed baseline cross-sectional data from six human intervention trials conducted between 2008 and 2020 in Shimane Prefecture [22–27]. All trials were approved by the Ethics Committee of the Shimane University School of Medicine (Approval No. 1027, 1028, 1889, 1940, 2899, 3194, 3497) and Jinjukai Kato Hospital (approval numbers: 2008-2, 2012-009). Participants taking psychotropic drugs (e.g., antidepressants, anxiolytics, antipsychotics) were excluded. In addition, individuals who regularly consumed nutritional supplements that known to affect cognitive

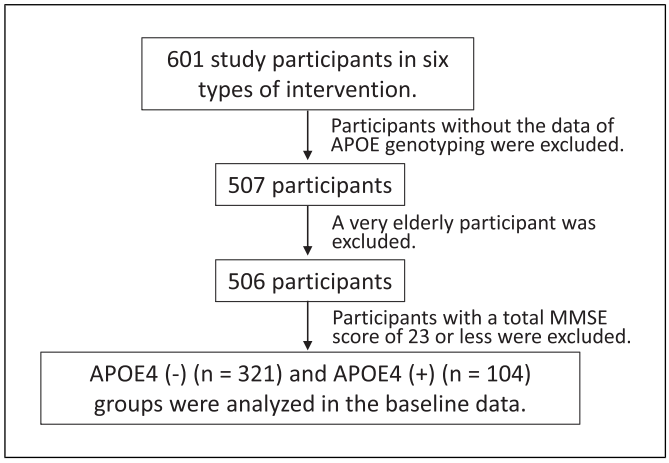


Fig. 1. Flow diagram of analytical data selection.

function or lipid metabolism (e.g., omega-3 fatty acids, DHA, eicosapentaenoic acid [EPA], or vitamin D) were also excluded. Occasional or general supplement use, such as multivitamins, was not considered exclusionary. Out of the 601 elderly participants initially enrolled, 103 individuals were excluded due to missing APOE genotyping and/or cognitive function data, leaving 507 participants. The mean age of these participants was 73 ± 0.4 years. None of the 507 participants showed any signs of medical disorders, including cardiac, hepatic, renal, respiratory, endocrine, metabolic, or hematological diseases, diabetes mellitus.

Previous research has demonstrated that the effects of DHA supplementation may vary depending on APOE genotype, particularly in individuals with normal cognition, MCI, and AD. Notably, Quinn et al. [28] reported that individuals with dementia who carry APOE4 allele may exhibit limited responsiveness to DHA supplementation. These findings suggest that depending on APOE genotypes, DHA supplementation could be beneficial for elderly individuals with MCI. Accordingly, we analyzed healthy elderly participants who had a baseline Mini-Mental State Examination (MMSE) [29] total score of 24 or higher, in line with guidelines set by the Japan Gerontology Society [30–32]. One participant aged over 100 years was excluded. The exclusion criteria and methods used for participant selection are outlined in Figure 1.

Plasma Biochemical Analysis

Biochemical analyses included the assessment of aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, albumin, total cholesterol, triglycerides, blood urea nitrogen, creatinine, blood sugar, and high- and low-density lipoprotein cholesterol levels. These measurements were performed using an automatic analyzer (BiOLis 24; Tokyo Boeki Medical System, Tokyo, Japan). Hemoglobin A1c levels were measured using a commercially available kit (TFB Inc., Tokyo, Japan).

Serum and Erythrocyte Membrane Fatty Acid Measurements

The fatty acid profiles in the serum and RBC-PM were determined by direct transmethylation, as previously described [33, 34].

Evaluation of Cognitive Functions

The cognitive functions of the study participants were evaluated using the following cognitive assessment tools: Hasegawa's Dementia Scale-Revised (HDS-R) [35], MMSE [29], Frontal Assessment Battery (FAB) [36], and Cognitive Assessment for Dementia iPad version (CADi) [37].

APOE Genotyping

APOE genotyping was conducted as previously described [22, 23]. Single-nucleotide polymorphism (SNP) genotyping for APOE was performed using real-time polymerase chain reaction with the 7900HT system (Applied Biosystems, Foster City, CA). The TaqMan SNP genotyping assay was used to target rs429358 and rs7412, with identification numbers G_3084793-20 and C_904973-10, respectively (Applied Biosystems).

Statistical Analysis

Data normality was assessed using the Shapiro-Wilk test. The data were considered to follow a normal distribution when $\alpha = 0.05$ and $p > 0.05$. Group comparisons were performed using either the independent t test or Mann-Whitney U test, depending on data distribution. Comparisons between groups by APOE genotypes were analyzed using one-way analysis of covariance (ANCOVA). The analysis was adjusted for age and sex, as these are well-established confounders in studies of cognitive function [38]. Additionally, LDL-C was included as a covariate due to previous reports, suggesting its association with both omega-3 fatty acid metabolism and cognitive decline [39]. BMI was also adjusted for, as it has been linked to lipid metabolism and may influence fatty acid composition and cognitive function [40, 41]. These adjustments aimed to control for potential confounding effects on the associations being examined in this study. Although educational attainment is often considered as a covariate in studies of cognitive function, information on

Table 1. Genotype and allele frequency of the APOE gene of study participants in Shimane, Japan

	n	%
Genotype		
E2/E2	1	0.2
E2/E3	39	7.7
E2/E4	8	1.6
E3/E3	343	67.7
E3/E4	107	21.1
E4/E4	9	1.8
Alleles		
E2	49	4.8
E3	832	82.1
E4	133	13.1

APOE, apolipoprotein E; E2, ε2 allele; E3, ε3 allele; E4, ε4 allele.

participants' education levels was only available for a subset of the cohort and was therefore insufficient to be included as a covariate in the present analyses. Due to the exploratory nature of the study, no formal correction for multiple comparisons was applied. All statistical analyses were conducted using SPSS software version 26.0 for Windows (IBM Corp., Armonk, NY, USA). All the statistical tests were two-tailed, and a p value of less than 0.05 was considered statistically significant.

Results

The genotype and allele frequencies of APOE polymorphisms in the healthy elderly participants (n = 507) of this study are presented in Table 1. Consistent with previous reports [42, 43], the E2/E2, E2/E4, and E4/E4 genotypes were relatively rare. Additionally, we observed a high frequency of the APOE3 allele, as commonly found in other studies.

Table 2 shows the anthropometric measurements, blood pressure, blood biochemistry, and cognitive function for each APOE genotype among the healthy elderly participants, regardless of MMSE scores. An elderly individual with the E2/E2 genotype (96 years old, MMSE total score of 9/30) was excluded from the statistical analyses because of markedly advanced age and extremely low cognitive function compared with the rest of the cohort. However, for completeness, these individual's data are shown in Table 2. A significant difference was observed in the serum LDL-C levels (p = 0.039), where the E4/E4 allele showed the highest concentration (129.6 ± 12.3 mg/dL) as compared to the others. Moreover, a trend toward a significant difference in RBC-PM DHA levels (p = 0.093) was observed, where the E4/E4 allele showed the lowest concentration (7.3 ± 0.4 mol%). These findings suggest that the serum LDL-C levels are higher in APOE4 carriers than in noncarriers, whereas the RBC-PM DHA levels might be lower in APOE4 carriers than in their noncarrier counterparts.

Table 3 presents the blood biochemical parameters of healthy elderly individuals with a MMSE total score >23 (n = 425), categorized by the APOE4 carrier status. The analysis revealed that serum LDL-C levels tended to be higher in APOE4 carriers (122.8 ± 2.5 mg/dL) than in the noncarriers, with a p value of 0.088, suggesting a potential association between the APOE4 status and lipid profiles in this population.

Table 4 presents the results of the cognitive assessments conducted in the study with participants having a MMSE total score >23. After adjusting for age, sex, and serum LDL-C levels, the APOE4 carriers had significantly lower scores on the HDS-R sub-item 8, "recalling five objects" (p = 0.033), than the noncarriers (APOE4 [−]: 4.71 ± 0.03 vs. APOE4 [+]: 4.60 ± 0.06). Additionally, the time (second) required to complete the CADi was significantly longer for APOE4 carriers (p = 0.004) (APOE4 [−]: 124.0 ± 3.1 vs. APOE4 [+]: 145.0 ± 8.3). The complete set of sub-item scores for HDS-R, CADi, MMSE, and FAB is provided in online supplementary Table S1 for reference. No significant differences were observed between the groups in the other cognitive test sub-items.

Table 5 presents a comparison of the fatty acid profiles of RBC-PM between the APOE4 carriers and noncarriers with a MMSE total score >23. The ratio of DHA-to-arachidonic acid (AA) was significantly lower in APOE4 carriers (APOE4 [−]: 0.69 ± 0.01 vs. APOE4 [+]: 0.66 ± 0.02) (p = 0.040). Furthermore, the levels of EPA (APOE4 [−]: 2.15 ± 0.06 vs. APOE4 [+]: 1.98 ± 0.09) (p = 0.093)], docosapentaenoic acid (APOE4 [−]: 2.04 ± 0.04 vs. APOE4 [+]: 1.96 ± 0.06) (p = 0.093), and DHA (APOE4 [−]: 7.91 ± 0.09 vs. APOE4 [+]: 7.60 ± 0.16) (p = 0.085) in RBC-PM tended to be lower in the APOE4 carriers than in the noncarriers. However, no significant associations were found between the cognitive scores and serum biochemical parameters or RBC-PM fatty acids in elderly individuals.

Discussion

APOE4 is known to impact the metabolism of DHA both in the peripheral tissues and brain and reduces the transport of DHA from the blood to the brain prior to

Table 2. Participant characteristics in each genotype of APOE in the Shimane study

	E2/E2	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4	*p* value
Anthropometry							
Sex (male/female)	1 (0/1)	39 (17/22)	8 (4/4)	343 (134/209)	107 (50/57)	9 (4/5)	0.669
Age, years	96	73.5±1.3	70.9±2.1	73.3±0.5	73.6±0.8	71.4±1.9	0.868
BMI, kg/m^2	–	23.4±0.5	24.2±1.7	23.1±0.18	23.0±0.4	23.2±1.4	0.797
BP							
Systolic BP, mm Hg	132.0	150.2±3.9	143.4±5.6	145.2±1.2	144.8±2.4	143.4±6.4	0.738
Diastolic BP, mm Hg	70.0	85.1±2.0	83.6±5.2	81.2±0.7	80.8±1.4	81.0±3.2	0.434
Blood biochemistry							
Total cholesterol, mg dL^{-1}	151.0	199.0±5.7	211.1±8.2	206.0±1.9	210.7±3.2	209.4±11.6	0.473
Triglycerides, mg dL^{-1}	109.0	137.5±15.0	138.0±18.7	128.8±4.1	127.2±6.0	102.9±12.5	0.764
BUN, mg dL^{-1}	19.9	15.8±0.9	17.8±14.5	16.6±0.2	16.3±0.5	14.8±1.1	0.540
Creatinine, mg dL^{-1}	0.99	0.81±0.06	0.76±0.05	0.76±0.01	0.75±0.02	0.74±0.07	0.606
Blood sugar, mg dL^{-1}	75.0	123.3±6.7	112.0±14.3	115.5±1.7	111.5±3.0	124.4±14.6	0.353
HDL-C, mg dL^{-1}	44.0	65.8±2.9	67.9±7.1	64.1±1.0	63.6±1.6	61.6±5.1	0.906
LDL-C, mg dL^{-1}	82.0	107.2±4.7	119.1±7.4	119.4±1.7	124.2±2.6	129.6±12.3	0.039
HbA1c (NGSP), %	5.4	5.7±0.1	5.4±0.2	5.7±0.0	5.8±0.1	5.9±0.2	0.324
Cognitive function							
MMSE total score	9.0	26.3±1.1	29.9±0.1	26.5±0.3	26.2±0.6	23.0±2.9	0.166
HDS-R total score	3.0	26.0±1.2	28.3±0.5	25.9±0.4	25.0±0.7	21.6±3.6	0.201
FAB total score	–	16.0±0.3	15.2±0.4	15.1±0.1	15.1±0.2	14.0±0.8	0.113
CADi total score	1.0	7.4±0.6	5.0±0.0	7.2±0.2	7.6±0.3	6.3±1.6	0.519
RBC fatty acids, mol%							
PLA (C16:0)	29.6	25.1±0.5	25.1±1.2	25.4±0.1	25.2±0.3	26.0±0.7	0.828
STA (C18:0)	18.9	17.4±0.2	17.0±0.2	17.4±0.1	17.4±0.1	17.7±0.5	0.875
OLA (C18:1ω-9)	15.3	14.3±0.4	13.8±0.6	14.6±0.1	15.0±0.2	14.8±0.6	0.356
LLA (C18:2ω-6)	9.6	10.5±0.3	9.7±0.8	10.7±0.1	10.9±0.2	10.5±0.8	0.513
ALA (C18:3ω-3)	0.2	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.920
AA (C20:4ω-6)	9.8	11.4±0.3	12.4±0.6	11.7±0.1	11.7±0.2	10.9±0.3	0.526
EPA (C20:5ω-3)	1.5	2.3±0.2	2.2±0.3	2.1±0.1	2.0±0.1	2.2±0.4	0.350
DPA (C22:5ω-3)	1.4	2.2±0.1	2.0±0.2	2.0±0.0	1.9±0.1	2.2±0.3	0.252
C24:0	3.1	4.0±0.1	4.4±0.2	4.0±0.0	4.0±0.1	3.7±0.2	0.278
DHA (C22:6ω-3)	6.2	8.1±0.3	8.8±0.4	7.8±0.1	7.6±0.2	7.3±0.4	0.093
C24:1	4.3	4.1±0.2	4.1±0.3	4.0±0.0	3.8±0.1	4.3±0.3	0.176
ω-6/ω-3	2.1	2.0±0.1	2.0±0.1	2.1±0.0	2.1±0.1	2.1±0.2	0.514
DHA/AA	0.6	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.296
EPA/AA	0.2	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.498

Values are means ± SE. The E2/E2 genotype group consisted of a single 96-year-old female participant with markedly low MMSE scores. Her data are presented for completeness but were excluded from statistical analyses. Because of spinal kyphosis, height, and weight (BMI) could not be measured, and the FAB test was not performed. AA, arachidonic acid; ALA, α-linolenic acid; APOE, apolipoprotein E; BP, blood pressure; BUN, blood urea nitrogen; CADi, Cognitive Assessment for Dementia iPad version; C24:0, lignoceric acid; C24:1, nervonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FAB, Frontal Assessment Battery; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HDS-R, Hasegawa's Dementia Scale-Revised; LDL-C, low-density lipoprotein cholesterol; LLA, linoleic acid; MMSE, Mini-Mental State Examination; OLA, oleic acid; PLA, palmitic acid; RBC, red blood cell; STA, stearic acid.

the onset of dementia [9–11, 28]. However, the beneficial effects of DHA supplementation on cognitive function and dementia are inconsistent across studies. In our study, we examined the association between APOE genotype frequencies and lipid metabolism in healthy elderly Japanese individuals (MMSE> 23). We found that APOE4 carriers, particularly those at risk for MCI in the very early stage of cognitive decline (MMSE> 23, clinically classified as cognitively normal but considered at risk), exhibited a significantly lower DHA-to-AA ratio and tended to have reduced EPA and DHA levels in the RBC-PM. These metabolic alterations were associated

Table 3. Effect of the APOE4 gene ε4 allele on blood biochemistry in study participants with a MMSE total score >23

	APOE4 (–)	APOE4 (+)	*p* value
Anthropometry			
Sex (male/female)	321 (133/188)	104 (53/51)	0.089
Age, years	71.2±0.4	71.1±0.6	0.866
Blood biochemistry			
Total cholesterol, mg dL^{-1}	207.9±2.0	210.9±3.2	0.286
Triglycerides, mg dL^{-1}	133.4±4.4	130.4±6.0	0.817
Blood urea nitrogen, mg dL^{-1}	16.5±0.3	16.2±0.4	0.601
Creatinine, mg dL^{-1}	0.8±0.01	0.8±0.02	0.854
Blood sugar, mg dL^{-1}	113.3±1.6	111.2±3.0	0.531
HDL-C, mg dL^{-1}	66.2±1.0	64.5±1.7	0.452
LDL-C, mg dL^{-1}	118.6±1.8	122.8±2.5	0.088
HbA1c (NGSP), %	5.7±0.03	5.8±0.06	0.449

Values are means ± SE. APOE4, apolipoprotein E gene ε4 allele; E2, ε2 allele; E3, ε3 allele; E4, ε4 allele; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NGSP, national glycohemoglobin standardization program.

Table 4. Effect of the APOE gene ε4 allele on cognitive scores in study participants with a MMSE total score >23

	APOE4 (–)	APOE4 (+)	*p* value
Cognitive index			
MMSE			
Total	28.3±0.1	28.5±0.2	0.302
HDS-R			
Total	28.1±0.1	27.8±0.2	0.152
Sub-item "recalling 5 objects"	4.71±0.03	4.60±0.06	0.033
FAB			
Total	15.3±0.1	15.0±0.2	0.267
CADi			
Total	8.1±0.1	8.3±0.2	0.256
Time required, s	124.0±3.1	145.0±8.3	0.004

Values are means ± SE. APOE4 (–) group: $n = 321$; APOE4 (+) group: $n = 104$. APOE4, apolipoprotein E gene ε4 allele; CADi, Cognitive Assessment for Dementia iPad version; FAB, Frontal Assessment Battery; HDS-R, Hasegawa's Dementia Scale-Revised; MMSE, Mini-Mental State Examination; s, second.

with significantly lower cognitive scores in this group compared with APOE4 noncarriers. Importantly, these associations were observed in an elderly Japanese population, which is typically characterized by a high intake of fish and/or ω-3 fatty acid-enriched foods. This finding suggests that genetic predisposition related to APOE4 allele may override dietary sufficiency, highlighting the importance of considering gene-nutrient interactions in cognitive aging. Consistent with this, similar APOE4-related reductions in DHA metabolism have also been reported in North American cohorts, where habitual fish intake is generally lower than in Japan. For example, Yassine et al. [9] demonstrated reduced brain DHA uptake in APOE4 carriers, and Tomaszewski et al. [11] reported a smaller increase in plasma DHA following supplementation in APOE4 carriers compared to noncarriers. Although direct cross-cohort comparisons are limited, these findings suggest that APOE4-related differences may be more pronounced in populations with lower baseline ω-3 PUFA intake. Thus, the relatively high fish consumption in Japanese elderly may partly attenuate, but not completely eliminate, the APOE4-related differences observed in lipid metabolism and cognition. Future studies directly comparing cohorts

Table 5. Effect of the APOE gene ε4 allele on the red blood cell plasma membrane fatty acid profiles (mol %) in study participants with a MMSE total score >23

	APOE4 (–)	APOE4 (+)	*p* value
Fatty acids, mol %			
PLA (C16:0)	25.3±0.16	25.1±0.26	0.618
STA (C18:0)	17.3±0.08	17.3±0.13	0.788
OLA (C18:1ω-9)	14.6±0.15	14.9±0.23	0.152
LLA (C18:2ω-6)	10.5±0.13	10.8±0.21	0.315
ALA (C18:3ω-3)	0.19±0.01	0.19±0.01	0.219
AA (C20:4ω-6)	11.6±0.11	11.8±0.21	0.567
EPA (C20:5ω-3)	2.15±0.06	1.98±0.09	0.093
DPA (C22:5ω-3)	2.04±0.04	1.96±0.06	0.075
C24:0	4.08±0.04	4.11±0.08	0.950
DHA (C22:6ω-3)	7.91±0.09	7.60±0.16	0.085
C24:1	4.03±0.05	3.89±0.08	0.201
ω-6/ω-3	2.07±0.03	2.15±0.05	0.112
DHA/AA	0.69±0.01	0.66±0.02	0.040
EPA/AA	0.19±0.01	0.18±0.01	0.096

Values are means ± SE. APOE4 (–) group: $n = 321$; APOE4 (+) group: $n = 104$. AA, arachidonic acid; ALA, α-linolenic acid; APOE, apolipoprotein E; APOE4, apolipoprotein E gene ε4 allele; C24:0, lignoceric acid; C24:1, nervonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LLA, linoleic acid; OLA, oleic acid; PLA, palmitic acid; STA, stearic acid.

with different dietary backgrounds are warranted to clarify this issue.

In this study, wherein the distribution of the APOE gene polymorphisms in healthy elderly individuals without dementia in Shimane Prefecture was examined, the APOE3 allele frequency was slightly inconsistent with those of previous reports in the Japanese populations [42] (Table 1). Although the APOE4 allele frequency is less common in Japanese individuals as compared with that in white individuals [42, 43], the frequency observed in this study was similar to that found in white populations and slightly higher than that in previous reports for Japanese individuals. The reasons for this discrepancy in APOE4 frequency among the Japanese population between our study and prior reports cannot be clearly established; it may be related to the sample size or regional differences. Further research is needed to clarify these findings.

APOE is closely associated with circulating LDL and high-density lipoprotein (HDL) and plays a crucial role in lipoprotein metabolism [44, 45]. It also serves as a primary apolipoprotein in lipid transport and metabolism within the central nervous system, influencing various cellular processes. In our study, we found that the APOE4 allele frequency among the participants was 13.1% (Table 1), which is recognized as a significant genetic risk factor for LOAD. APOE4 may lead to increased plasma HDL cholesterol levels in both healthy elderly individuals and patients with dementia [44]. However, while APOE4 is associated with elevated levels of serum total cholesterol and LDL-C, it does not consistently correlate with HDL cholesterol levels [45–47]. This inconsistency in the findings highlights the complexity of the role of APOE in lipid metabolism. Notably, studies specifically examining the relationship between the APOE4 allele and serum lipoproteins in elderly Japanese populations are scarce. In our investigation, we observed a trend indicating that the serum LDL-C levels were higher in APOE4 carriers than in noncarriers (Table 3), suggesting that the genetic factors may influence the lipid profiles in this demographic.

The experiments using postmortem human brains showed that DHA constitutes a significant portion of the total fatty acids (approximately 11%–20%), with concentrations of 30%–40% found specifically in the gray matter of the cerebral cortex. DHA is particularly enriched in the neuronal plasma membranes, especially at synaptic sites, where it plays a crucial role in maintaining membrane fluidity and supporting neurotransmission [14]. However, neurons lack the enzyme delta desaturase, which is essential for the de novo synthesis of DHA, making dietary intake the primary source of this fatty acid [14]. The influence of the APOE4 allele on DHA metabolism has been well documented in the peripheral tissues and brains [5]. In our study, we observed that the APOE4 carriers tend to exhibit lower EPA and DHA levels in their RBC-PM as compared to the noncarriers. Notably, the DHA-to-AA ratio was significantly reduced in the APOE4 carriers (Table 5).

The DHA/AA ratio reflects the balance between anti-inflammatory (DHA) and pro-inflammatory (AA) fatty acids and has been proposed as a sensitive indicator of systemic and neuroinflammatory status [11, 48, 49]. Previous studies have shown that a lower DHA/AA ratio is associated with increased neuroinflammation and accelerated cognitive decline, including in elderly populations [48, 49]. Therefore, the significantly lower DHA/AA ratio observed in APOE4 carriers in our study may have biological significance and could contribute to the reduced cognitive performance seen in this group. Postmortem brain studies have found lower DHA/AA ratios in patients diagnosed with AD [9, 11, 17], supporting the notion that alterations in this ratio may reflect pathological processes related to neurodegeneration. Additionally, the DHA levels in the brain

are declined in both elderly individuals and AD patients. Collectively, these findings suggest that the presence of the APOE4 gene may lead to decreased DHA availability in the brain, which could contribute to cognitive decline in individuals carrying this allele. This highlights the importance of dietary sources of DHA for maintaining cognitive health, particularly in those with a genetic risk for neurodegenerative diseases.

In the present study, the APOE4 carriers exhibited lower cognitive function scores than the noncarriers (Table 4), indicating a potential cognitive decline associated with this variant. Unlike the other APOE isoforms, APOE4 disrupts the blood-brain barrier function, particularly in the hippocampus and broader medial temporal lobe, contributing to cognitive impairment [50]. Older APOE4 mice experience reduced brain uptake of DHA, which limits its accumulation in brain tissues [10]. This suggests that APOE4 carriers with cognitive impairment may face challenges in absorbing DHA from the blood into the brain. Moreover, APOE4 may activate the cerebrovascular endothelial cells and pericytes, leading to the disruption of the integrity of the blood-brain barrier [50]. Additionally, the APOE4 carriers are implicated in AD pathogenesis through various other pathways, including cerebrovascular dysfunction and accelerated accumulation of amyloid-β in the brain [51]. Collectively, these findings provide a possible mechanistic explanation for the association between reduced RBC-PM DHA levels and cognitive decline observed in APOE4 carriers.

DHA plays a crucial role in maintaining membrane fluidity, synaptic plasticity, and neurogenesis, making it essential for cognitive function, inflammation modulation, and clearance of amyloid-β from the brain [14]. Numerous animal studies indicate that long-term DHA supplementation protects against neuronal loss associated with AD and improves cognitive impairment in DHA-deficient AD models [52–55]. Conversely, DHA deficiency in the brains of rats is linked to poorer performance in spatial cognitive tasks, underscoring its importance in cognitive health [14]. These findings suggest that DHA supplementation may improve cognitive decline in healthy elderly individuals experiencing age-related cognitive decline (ARCD) or in patients with MCI before the AD onset. However, human intervention studies have produced conflicting results regarding the benefits of DHA supplementation on cognitive decline in elderly patients with ARCD and MCI. Our previous research indicated that DHA supplementation is beneficial for Japanese elderly with ARCD [22, 24, 56, 57]. Contrarily, most RCTs investigating ω-3 PUFA supplementation have not demonstrated significant benefits for AD or MCI, nor for cognitive decline in general, with the exception of some patients with very early MCI [58–62]. Furthermore, a meta-analysis that systematically reviewed RCTs found no evidence that DHA supplementation improves cognitive decline in the elderly with ARCD [63]. One important factor contributing to these discrepancies appears to be the variation in methodologies used to assess the effectiveness of PUFAs across different studies, which can lead to inconsistent results. This highlights the need for standardized approaches in future research to better understand the role of DHA in cognitive health.

In 2018, the International Society for the Study of Fatty Acids and Lipids (ISSFAL) issued a statement emphasizing the importance of considering fatty acid status when assessing the benefits of ω-3 PUFA supplementation. This includes evaluating the baseline, endpoint, and changes in fatty acid levels, particularly using the Omega-3 Index, which measures the combined levels of EPA and DHA in erythrocytes, to provide an accurate representation of the ω-3 PUFA status in the body. The recommendation highlighted the need to control for variables that might influence the study outcomes related to ω-3 PUFA supplementation and cognitive health. In line with ISSFAL's guidance, Kosti et al. [64] conducted a systematic review and meta-analysis of 1,146 RCTs examining the effects of ω-3 PUFA supplementation on cognitive function in adults with or without MCI as well as a systematic review to eliminate various biases. By applying strict eligibility criteria for articles examining the effects of RBC-PM fatty acids on cognitive function, the meta-analysis found that consuming two or more servings of fish per week was linked to a 30% reduction in the risk of developing AD. This result highlights the potential of dietary DHA, derived from fish, in reducing the risk of cognitive decline and neurodegeneration. These findings reinforce the potential of ω-3 PUFAs, particularly DHA, in preventing ARCD and support previous studies suggesting the protective effects of DHA supplementation in healthy Japanese elderly populations. Taken together, the present study suggests that ω-3 PUFA supplementation, especially with DHA, could potentially serve as a preventive strategy for APOE4 carriers, who are genetically predisposed to AD, by compensating for DHA deficiencies before cognitive decline sets in. Although APOE4-related differences were still observed in this Japanese cohort, where habitual fish intake is relatively high, these differences may be less pronounced compared to reports from Western cohorts. This raises

the possibility that high baseline fish consumption attenuates, but does not completely eliminate, the APOE4-related effects. Further cross-cohort and interventional studies are warranted to validate this interpretation.

The present study has several limitations. First, this cross-sectional study analyzed healthy Japanese elderly individuals who participated in six intervention studies conducted over a 13-year period (2008–2020). The sample group, comprising both APOE4 carriers and noncarriers, was heterogeneous in terms of sex ratios, living environments, and lifestyle habits. This diversity could have influenced the results and introduced confounding factors that cannot be fully controlled. Second, in the present study, the EPA and DHA levels in RBC-PM were not significantly different between the APOE4 carriers and noncarriers. The small sample size limited the power of the study to detect a causal relationship between the APOE4 allele, cognitive function, and RBC-PM DHA levels. A larger sample size would provide more robust evidence and allow for clearer conclusions about these relationships. Third, multiple comparisons were conducted without adjustment for multiple testing. Although a few significant differences were found, the possibility of false positives due to type I error should be considered. Fourth, while our findings indicate that APOE4 carriers exhibited lower RBC DHA levels and reduced cognitive performance, the cross-sectional nature of this study limits our ability to determine the directionality of this relationship. Specifically, it remains unclear whether APOE4 directly leads to lower DHA levels, thereby contributing to cognitive decline, or whether cognitive decline associated with APOE4 alters behavior, dietary patterns, or metabolism in ways that result in reduced DHA levels. Future longitudinal and interventional studies are warranted to clarify the causal pathways linking APOE4, DHA metabolism, and cognitive function. Finally, the small sample size and sample size imbalance between the APOE4 carriers (n = 104) and noncarriers (n = 321) limited the ability to detect significant differences between the APOE4 carriers and noncarriers. Further homogenous studies with a larger sample size are needed to establish a causal relationship between the APOE4 allele and cognition and RBC-PM DHA levels, as well as to clarify the preventive effects of DHA supplementation in the elderly with ARCD and APOE4 carriers before AD onset.

In conclusion, this cross-sectional study in healthy elderly Japanese individuals found that APOE4 carriers tended to have lower DHA levels and DHA-to-AA ratios in RBC-PM, along with modestly lower cognitive scores compared to noncarriers. Although the differences in cognitive performance were not large, the consistently lower levels of ω-3 PUFAs observed in APOE4 carriers suggest a potentially increased vulnerability to ω-3 fatty acid insufficiency, even in a population with high habitual fish consumption. While some studies, including that by Quinn et al. [28], have reported limited benefits of DHA supplementation in APOE4 carriers with dementia, our findings in cognitively healthy older adults may reflect an earlier stage at which ω-3 PUFA intervention could still confer cognitive benefits. These results suggest that DHA supplementation might be more effective as a preventive strategy in APOE4 carriers prior to the onset of significant cognitive decline. Further longitudinal and interventional studies are warranted to clarify these associations.

Acknowledgments

The authors sincerely thank the participants in the six types of intervention studies that contributed to this study and the staff who collected data from the six intervention studies. We also thank Dr. Sheikh Abdullah for the English language review.

Statement of Ethics

This study was approved by the Ethics Committee of the Shimane University School of Medicine (Approval No. 1027, 1028, 1889, 1940, 2899, 3194, 3497) and Jinjukai Kato Hospital (Approval No. 2008-2, 2012-009). All trials were performed according to the principles of the Declaration of Helsinki and Good Clinical Practice. All volunteers provided written informed consent prior to their participation.

Conflict of Interest Statement

The authors have declared that they have no competing interests.

Funding Sources

This work was partly supported by Japan Society for the Promotion of Science KAKENHI (Grant No. 23500955).

Author Contributions

Conceptualization, writing – original draft preparation, project administration, and funding acquisition: M.H.; methodology: M.H., K.M., C.M., M.O., and S.O.; software: M.H., K.M., and H.W.; validation: M.H., K.M., S.H., S.K., S.O., S.Y., and A.N.; investigation: M.H., K.M., S.H., H.W., and K.Y.; formal analysis:

M.H., K.M., H.W., and M.O.; resources: M.H., S.K., K.Y., and A.N.; data curation: K.M., H.W., and M.O.; writing – review and editing: M.H., K.M., S.H., and A.N.; visualization: M.H., K.M., and H.W.; supervision: S.K., S.Y., A.N., K.Y., and O.S. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Solfrizzi V, D'Introno A, Colacicco AM, Capurso C, Todarello O, Pellicani V, et al. Circulating biomarkers of cognitive decline and dementia. Clin Chim Acta. 2006;364(1–2):91–112. https://doi.org/10.1016/j.cca.2005.06.015
2 Mahley RW, Huang Y. Apolipoprotein E. Curr Opin Lipidol. 1999;10(3):207–18.
3 Mahley RW, Rall SC. Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genom Hum G. 2000;1(1):507–37. https://doi.org/10.1146/annurev.genom.1.1.507
4 Troutwine BR, Hamid L, Lysaker CR, Strope TA, Wilkins HM. Apolipoprotein E and alzheimer's disease. Acta Pharm Sin B. 2022;12(2):496–510. https://doi.org/10.1016/j.apsb.2021.10.002
5 Yassine HN, Croteau E, Rawat V, Hibbeln JR, Rapoport SI, Cunnane SC, et al. DHA brain uptake and APOE4 status: a PET study with [1-11C]-DHA. Alz Res Ther. 2017;9(1):23. https://doi.org/10.1186/s13195-017-0250-1
6 Hauser PS, Narayanaswami V, Ryan RO. Apolipoprotein E: from lipid transport to neurobiology. Prog Lipid Res. 2011;50(1):62–74. https://doi.org/10.1016/j.plipres.2010.09.001
7 Meyer BJ, Onyiaodike CC, Brown EA, Jordan F, Murray H, Nibbs RJB, et al. Maternal plasma DHA levels increase prior to 29 days post-LH surge in women undergoing frozen embryo transfer: a prospective, observational study of human pregnancy. J Clin Endocrinol Metab. 2016;101(4):1745–53. https://doi.org/10.1210/jc.2015-3089
8 Makrides M, Gibson RA. Long-chain polyunsaturated fatty acid requirements during pregnancy and lactation. Am J Clin Nutr. 2000;71(1 Suppl l):307S–11S. https://doi.org/10.1093/ajcn/71.1.307S
9 Yassine HN, Braskie MN, Mack WJ, Castor KJ, Fonteh AN, Schneider LS, et al. Association of docosahexaenoic acid supplementation with alzheimer disease stage in apolipoprotein E ε4 carriers: a review. JAMA Neurol. 2017;74(3):339–47. https://doi.org/10.1001/jamaneurol.2016.4899
10 Vandal M, Alata W, Tremblay C, Rioux-Perreault C, Salem N, Calon F, et al. Reduction in DHA transport to the brain of mice expressing human *APOE4* compared to *APOE2*. J Neurochem. 2014;129(3):516–26. https://doi.org/10.1111/jnc.12640
11 Tomaszewski N, He X, Solomon V, Lee M, Mack WJ, Quinn JF, et al. Effect of APOE genotype on plasma docosahexaenoic acid (DHA), eicosapentaenoic acid, arachidonic acid, and hippocampal volume in the Alzheimer's disease cooperative study-sponsored DHA clinical trial. J Alzheimers Dis. 2020;74(3):975–90. https://doi.org/10.3233/JAD-191017
12 Elsevier BV. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-prevenzione trial. Lancet. 1999;354(9177):447–55.
13 Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet. 2007;369(9567):1090–8. https://doi.org/10.1016/S0140-6736(07)60527-3
14 Hashimoto M, Hossain S, Al Mamun A, Matsuzaki K, Arai H. Docosahexaenoic acid: one molecule diverse functions. Crit Rev Biotechnol. 2017;37(5):579–97. https://doi.org/10.1080/07388551.2016.1207153
15 Lacombe RJS, Chouinard-Watkins R, Bazinet RP. Brain docosahexaenoic acid uptake and metabolism. Mol Aspects Med. 2018;64:109–34. https://doi.org/10.1016/j.mam.2017.12.004
16 Chappus-Mccendie H, Chevalier L, Roberge C, Plourde M. Omega-3 PUFA metabolism and brain modifications during aging. Prog Neuropsychopharmacol Biol Psychiatry. 2019;94:109662. https://doi.org/10.1016/j.pnpbp.2019.109662
17 Ebright B, Assante I, Poblete RA, Wang S, Duro MV, Bennett DA, et al. Eicosanoid lipidome activation in post-mortem brain tissues of individuals with APOE4 and Alzheimer's dementia. Alz Res Ther. 2022;14(1):152. https://doi.org/10.1186/s13195-022-01084-7
18 Zhang Y, Chen J, Qiu J, Li Y, Wang J, Jiao J. Intakes of fish and polyunsaturated fatty acids and mild-to-severe cognitive impairment risks: a dose-response meta-analysis of 21 cohort studies. Am J Clin Nutr. 2016;103(2):330–40. https://doi.org/10.3945/ajcn.115.124081
19 Zeng L-F, Cao Y, Liang W-X, Bao W-H, Pan J-K, Wang Q, et al. An exploration of the role of a fish-oriented diet in cognitive decline: a systematic review of the literature. Oncotarget. 2017;8(24):39877–95. https://doi.org/10.18632/oncotarget.16347
20 De Groot RHM, Meyer BJ. ISSFAL official statement number 6: the importance of measuring blood omega-3 long chain polyunsaturated fatty acid levels in research. Prostaglandins Leukot Essent Fatty Acids. 2020;157:102029. https://doi.org/10.1016/j.plefa.2019.102029
21 Arab L. Biomarkers of fat and fatty acid intake. J Nutr. 2003;1 Suppl(3):925S–32S. https://doi.org/10.1093/jn/133.3.925S
22 Hashimoto M, Yamashita K, Kato S, Tamai T, Tanabe Y, Mitarai M, et al. Beneficial effects of daily dietary omega-3 polyunsaturated fatty acid supplementation on age-related cognitive decline in elderly Japanese with very mild dementia: a 2-year randomized, double-blind, placebo-controlled trial. J Aging Res Clin Pract. 2012;1(3):193–201.
23 Hashimoto M, Kato S, Tanabe Y, Katakura M, Mamun AA, Ohno M, et al. Beneficial effects of dietary docosahexaenoic acid intervention on cognitive function and mental health of the oldest elderly in Japanese care facilities and nursing homes. Geriatr Gerontol Int. 2017;17(2):330–7. https://doi.org/10.1111/ggi.12691
24 Ichinose T, Kato M, Matsuzaki K, Tanabe Y, Tachibana N, Morikawa M, et al. Beneficial effects of docosahexaenoic acid-enriched milk beverage intake on cognitive function in healthy elderly Japanese: a 12-month randomized, double-blind, placebo-controlled trial. J Funct Foods. 2020;74:104195. https://doi.org/10.1016/j.jff.2020.104195
25 Hashimoto M, Matsuzaki K, Hossain S, Ito T, Wakatsuki H, Tanabe Y, et al. Perilla seed oil enhances cognitive function and mental health in healthy elderly Japanese individuals by enhancing the biological antioxidant potential. Foods. 2021;10(5):1130. https://doi.org/10.3390/foods10051130
26 Hashimoto M, Matsuzaki K, Maruyama K, Sumiyoshi E, Hossain S, Wakatsuki H, et al. Perilla frutescens seed oil combined with Anredera cordifolia leaf powder attenuates age-related cognitive decline by reducing serum triglyceride and glucose levels in healthy elderly Japanese individuals: a possible supplement for brain health. Food Funct. 2022;13(13):7226–39. https://doi.org/10.1039/d2fo00723a

27 Hashimoto M, Matsuzaki K, Maruyama K, Hossain S, Sumiyoshi E, Wakatsuki H, et al. Perilla seed oil in combination with nobiletin-rich ponkan powder enhances cognitive function in healthy elderly Japanese individuals: a possible supplement for brain health in the elderly. Food Funct. 2022;13(5):2768–81. https://doi.org/10.1039/d1fo03508h
28 Quinn JF, Raman R, Thomas RG, Yurko-Mauro K, Nelson EB, Van Dyck C, et al. Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. JAMA. 2010;304(17):1903–11. https://doi.org/10.1001/jama.2010.1510
29 Folstein MF, Folstein SE, Mchugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189–98. https://doi.org/10.1016/0022-3956(75)90026-6
30 Tsoi KK, Chan JY, Hirai HW, Wong SY, Kwok TC. Cognitive tests to detect dementia: a systematic review and meta-analysis. JAMA Intern Med. 2015;175(9):1450–8. https://doi.org/10.1001/jamainternmed.2015.2152
31 Tariq SH, Tumosa N, Chibnall JT, Perry MH, Morley JE. Comparison of the Saint Louis University mental status examination and the mini-mental state examination for detecting dementia and mild neurocognitive disorder--a pilot study. Am J Geriatr Psychiatry. 2006;14(11):900–10. https://doi.org/10.1097/01.JGP.0000221510.33817.86
32 The Japan Geriatrics Society. Tokyo: assessment of cognitive function and diagnosis of dementia. [cited 2025 Aug 18]. Available from: https://www.jpn-geriat-soc.or.jp/tool/tool_02.html
33 Matsuzaki K, Hossain S, Wakatsuki H, Tanabe Y, Ohno M, Kato S, et al. Perilla seed oil improves bone health by inhibiting bone resorption in healthy Japanese adults: a 12-month, randomized, double-blind, placebo-controlled trial. Phytother Res. 2023;37(6):2230–41. https://doi.org/10.1002/ptr.7728
34 Hashimoto M, Shinozuka K, Gamoh S, Tanabe Y, Hossain MS, Kwon YM, et al. The hypotensive effect of docosahexaenoic acid is associated with the enhanced release of ATP from the caudal artery of aged rats. J Nutr. 1999;129(1):70–6. https://doi.org/10.1093/jn/129.1.70
35 Imai Y, Hasegawa K. The revised Hasegawa's dementia scale (HDS-R)-Evaluation of its usefulness as a screening test for dementia. J Hong Kong Coll Psychiatr. 1994;4:20–4.
36 Dubois B, Slachevsky A, Litvan I, Pillon B. The FAB: a Frontal Assessment Battery at bedside. Neurology. 2000;55(11):1621–6. https://doi.org/10.1212/wnl.55.11.1621
37 Onoda K, Hamano T, Nabika Y, Aoyama A, Takayoshi H, Nakagawa T, et al. Validation of a new mass screening tool for cognitive impairment: cognitive assessment for dementia, iPad version. Clin Interv Aging. 2013;8:353–60. https://doi.org/10.2147/CIA.S42342
38 Kheloui S, Jacmin-Park S, Larocque O, Kerr P, Rossi M, Cartier L, et al. Sex/gender differences in cognitive abilities. Neurosci Biobehav Rev. 2023;152:105333. https://doi.org/10.1016/j.neubiorev.2023.105333
39 Iwagami M, Qizilbash N, Gregson J, Douglas I, Johnson M, Pearce N, et al. Blood cholesterol and risk of dementia in more than 1·8 million people over two decades: a retrospective cohort study. Lancet Healthy Longev. 2021;2(8):e498–e506. https://doi.org/10.1016/S2666-7568(21)00150-1
40 Christian LM, Young AS, Mitchell AM, Belury MA, Gracious BL, Arnold LE, et al. Body weight affects ω-3 polyunsaturated fatty acid (PUFA) accumulation in youth following supplementation in post-hoc analyses of a randomized controlled trial. PLoS One. 2017;12(4):e0173087. https://doi.org/10.1371/journal.pone.0173087
41 Michaud TL, Siahpush M, Farazi PA, Kim J, Yu F, Su D, et al. The Association between body Mass index, and cognitive, functional, and behavioral declines for incident dementia. J Alzheimers Dis. 2018;66(4):1507–17. https://doi.org/10.3233/JAD-180278
42 Arai H, Yamamoto A, Matsuzawa Y, Saito Y, Yamada N, Oikawa S, et al. Polymorphisms of apolipoprotein E and methylenetetrahydrofolate reductase in the Japanese population. J Atheroscler Thromb. 2007;14(4):167–71. https://doi.org/10.5551/jat.e499
43 Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer disease meta analysis consortium. JAMA. 1997;278(16):1349–56.
44 Wehr H, Parnowski T, Puzyński S, Bednarska-Makaruk M, Bisko M, Kotapka-Minc S, et al. Apolipoprotein E genotype and lipid and lipoprotein levels in dementia. Dement Geriatr Cogn. 2000;11(2):70–3. https://doi.org/10.1159/000017217
45 Liu HC, Hong CJ, Wang SJ, Fuh JL, Wang PN, Shyu HY, et al. ApoE genotype in relation to AD and cholesterol: a study of 2,326 Chinese adults. Neurology. 1999;53(5):962–6. https://doi.org/10.1212/wnl.53.5.962
46 Raygani AV, Rahimi Z, Kharazi H, Tavilani H, Pourmotabbed T. Association between apolipoprotein E polymorphism and serum lipid and apolipoprotein levels with Alzheimer's disease. Neurosci Lett. 2006;408(1):68–72. https://doi.org/10.1016/j.neulet.2006.08.048
47 Tao Q-Q, Chen Y, Liu Z-J, Sun Y-M, Yang P, Lu S-J, et al. Associations between apolipoprotein E genotypes and serum levels of glucose, cholesterol, and triglycerides in a cognitively normal aging Han Chinese population. Clin Interv Aging. 2014;9:1063–7. https://doi.org/10.2147/CIA.S62554
48 Leckie RL, Manuck SB, Bhattacharjee N, Muldoon MF, Flory JM, Erickson KI. Omega-3 fatty acids moderate effects of physical activity on cognitive function. Neuropsychologia. 2014;59:103–11. https://doi.org/10.1016/j.neuropsychologia.2014.04.018
49 Colombo J, Jill Shaddy D, Kerling EH, Gustafson KM, Carlson SE. Docosahexaenoic acid (DHA) and arachidonic acid (ARA) balance in developmental outcomes. Prostaglandins Leukot Essent Fatty Acids. 2017;121:52–6. https://doi.org/10.1016/j.plefa.2017.05.005
50 Montagne A, Nation DA, Sagare AP, Barisano G, Sweeney MD, Chakhoyan A, et al. APOE4 leads to blood–brain barrier dysfunction predicting cognitive decline. Nature. 2020;581(7806):71–6. https://doi.org/10.1038/s41586-020-2247-3
51 Duong MT, Nasrallah IM, Wolk DA, Chang CCY, Chang T-Y. Cholesterol, atherosclerosis, and APOE in vascular contributions to cognitive impairment and dementia (VCID): potential mechanisms and therapy. Front Aging Neurosci. 2021;13:647990. https://doi.org/10.3389/fnagi.2021.647990
52 Zhang Y, Wu G, Zhang Y, Wang X, Jin Q, Zhang H. Advances in exogenous docosahexaenoic acid-containing phospholipids: sources, positional isomerism, biological activities, and advantages. Compr Rev Food Sci F. 2020;19(4):1420–48. https://doi.org/10.1111/1541-4337.12543
53 Hashimoto M, Hossain S, Shimada T, Sugioka K, Yamasaki H, Fujii Y, et al. Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. J Neurochem. 2002;81(5):1084–91. https://doi.org/10.1046/j.1471-4159.2002.00905.x
54 Hashimoto M, Tanabe Y, Fujii Y, Kikuta T, Shibata H, Shido O. Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid β–infused rats. J Nutr. 2005;135(3):549–55. https://doi.org/10.1093/jn/135.3.549
55 Lim GP, Calon F, Morihara T, Yang F, Teter B, Ubeda O, et al. A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged alzheimer mouse model. J Neurosci. 2005;25(12):3032–40. https://doi.org/10.1523/JNEUROSCI.4225-04.2005
56 Hashimoto M, Yamashita K, Kato S, Tamai T, Matsumoto I, Tanabe Y, et al. P3-295: beneficial effects of dietary docosahexaenoic acid intervention on cognitive function in elderly people with very mild dementia in Japan. Alzheimer's Demen. 2011;7(4S_Part_17):193–201. https://doi.org/10.1016/j.jalz.2011.05.1737
57 Ichinose T, Matsuzaki K, Kato M, Tanabe Y, Tachibana N, Morikawa M, et al. Intake of docosahexaenoic acid-enriched milk beverage prevents age-related cognitive decline and decreases serum bone resorption marker levels. J Oleo Sci. 2021;70(12):1829–38. https://doi.org/10.5650/jos.ess21195

58 Chiu C-C, Su K-P, Cheng T-C, Liu H-C, Chang C-J, Dewey ME, et al. The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: a preliminary randomized double-blind placebo-controlled study. Prog Neuropsychopharmacol Biol Psychiatry. 2008;32(6):1538–44. https://doi.org/10.1016/j.pnpbp.2008.05.015
59 Rogers PJ, Appleton KM, Kessler D, Peters TJ, Gunnell D, Hayward RC, et al. No effect of *n*-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomised controlled trial. Br J Nutr. 2008;99(2):421–31. https://doi.org/10.1017/S0007114507801097
60 Dangour AD, Lock K, Hayter A, Aikenhead A, Allen E, Uauy R. Nutrition-related health effects of organic foods: a systematic review. Am J Clin Nutr. 2010;92(1):203–10. https://doi.org/10.3945/ajcn.2010.29269
61 Freund-Levi Y, Eriksdotter-Jönhagen M, Cederholm T, Basun H, Faxén-Irving G, Garlind A, et al. ω-3 fatty acid treatment in 174 patients with mild to moderate alzheimer disease: omegad study. Arch Neurol. 2006;63(10):1402. https://doi.org/10.1001/archneur.63.10.1402
62 Yurko-Mauro K, Mccarthy D, Rom D, Nelson EB, Ryan AS, Blackwell A, et al. Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline. Alzheimers Dement. 2010;6(6):456–64. https://doi.org/10.1016/j.jalz.2010.01.013
63 Balachandar R, Soundararajan S, Bagepally BS. Docosahexaenoic acid supplementation in age-related cognitive decline: a systematic review and meta-analysis. Eur J Clin Pharmacol. 2020;76(5):639–48. https://doi.org/10.1007/s00228-020-02843-x
64 Kosti RI, Kasdagli MI, Kyrozis A, Orsini N, Lagiou P, Taiganidou F, et al. Fish intake, n-3 fatty acid body status, and risk of cognitive decline: a systematic review and a dose–response meta-analysis of observational and experimental studies. Nutr Rev. 2022;80(6):1445–58. https://doi.org/10.1093/nutrit/nuab078