

Effects of Food Supplementation with Fish Oil on Cognitive Function in Middle-Aged and Older Adults

—A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study

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Abstract

Background: To delay the onset of dementia with age, healthy adults must take preventive measures before cognitive decline occurs. Cognitive decline leads to a decrease in the quality of life (QOL). Fish oil also improves cognitive functions. Therefore, we examined the effects of fish oil intake on the cognitive function and QOL of middle-aged and older adults. **Methods:** A randomized, double-blind, placebo-controlled, parallel-group study was conducted to evaluate the effects of fish oil on cognitive function and QOL in middle-aged and older participants. One hundred and ten participants were enrolled and randomly divided into active and placebo food groups. Participants consumed either food containing fish oil or food without fish oil for 12 weeks. As the main outcome, cognitive function was assessed before and after ingestion using the Wechsler Memory Scale-Revised and the Stroop test. The secondary outcome, QOL, was also examined using SF-36. **Results:** The active food group showed statistical significance in the “Logical Memory I” and “Stroop interference rate” compared to the placebo food group ($P < 0.05$). Furthermore, the active food group showed statistically significant differences in “Mental Component Summary” compared to the placebo food group ($P < 0.05$). No adverse events attributable to the study food were observed during the study period. **Conclusions:** Overall, fish oil improved cognitive function in middle-aged and older adults.

Keywords

Fish Oil, Docosahexaenoic Acids, Cognitive Function

1. Introduction

Cognitive function is divided into multiple domains, including memory, attention, language, and executive function. Among these, specific functions have been reported to decline with aging. Specifically, declines in function have been confirmed with age in areas such as episodic memory—which retains memories of specific events—and aspects of attention like selective attention and divided attention, which involve complex attentional tasks [1]. Aging is a risk factor for dementia, and more than 55 million people worldwide are currently affected with nearly 10 million new cases annually [2]. Mild cognitive impairment (MCI) is a prodromal stage of dementia, with an annual conversion rate of approximately 10%, and up to 44% of patients may revert to normal [3]. Therefore, mitigating cognitive decline to prevent the transition from normal to MCI, or from MCI to dementia, is important.

Furthermore, cognitive decline has been suggested to lead to a decrease in quality of life (QOL) through mental symptoms such as depressed mood [4]. Therefore, suppressing cognitive decline is linked to maintaining QOL and is considered extremely important for maintaining and promoting the health of middle-aged and older adults.

Fish oil is rich in n-3 polyunsaturated fatty acid (n-3 PUFA) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). n-3 PUFA has been reported to reduce cardiovascular risk, lower triglycerides, and attenuate cognitive decline in MCI and increasing their dietary intake is recommended in several countries [5]. Multiple systematic reviews have confirmed significant improvements in cognitive function following n-3 PUFA supplementation, particularly in healthy older adults and those with MCI [6] [7]. In Japanese middle-aged adults, the intake of fish oil-containing foods improves cognitive function [8].

Therefore, we conducted a randomized, double-blind, placebo-controlled, parallel-group study to evaluate the effects of a newly developed fish oil-containing food on the cognitive function and QOL of middle-aged and older adults whose cognition ranged from normal to MCI.

2. Materials and Methods

2.1. Study Subjects and Setting

The target sample size for this study was calculated based on the Stroop test results from a report on the effects of fish oil-containing foods on cognitive function in middle-aged Japanese individuals [8], using a significance level of 0.05 and a power of 0.8. After accounting for dropouts and discontinuations, the sample size was set to 55 participants per group (110 participants).

Participants were recruited as paid volunteers, and the investigator enrolled those who met the inclusion criteria and did not violate the exclusion criteria. To target adults with cognition ranging from normal to MCI and excluding dementia, the Mini-Mental State Examination—Japanese version (MMSE-J) of the Dementia Screening Test was administered during screening; a score of 23 or below was defined as the cut-off value for suspicion of dementia [9].

Before enrollment, all candidates received a sufficient explanation of the study and provided written informed consent.

The inclusion criteria were as follows: 1) males and females aged 60 - 79 years; 2) subjects whose MMSE-J score was 24 or more on screening tests; 3) awareness of forgetfulness or awareness of memory decline and/or judgment decline; and 4) subjects who can make self-judgments and voluntarily give written informed consent.

The exclusion criteria were as follows: 1) subjects who have been determined by a physician to have dementia; 2) those who are taking or have taken drugs related to dementia; 3) those who have a history of and/or contract serious diabetes and/or cerebrovascular disease; 4) those who have contract serious diseases (e.g., liver disease, kidney disease, heart disease); 5) those taking supplements that may improve cognitive function; 6) those who have a history and/or a surgical history of digestive disease affecting digestion and absorption; 7) those who are under treatment for or have a history of alcoholism; 8) those who have declared with food allergies; 9) those who can't stop drinking from a day before each measurement; 10) those who have alcohol intake more than approximately 20 g/day of pure alcohol equivalent and a habit of drinking not less than 4 days a week; 11) those with extremely irregular eating habits and rhythm of life; 12) those suffering from depression or other psychiatric disorders; 13) those who have donated over 200 mL of blood and/or blood components within the last one month prior to the current study or over 400 mL of blood and/or blood components within the last three months prior to the current study; 14) those who are planning to participate and/or had participated in other clinical studies within the last one month prior to the current study; 15) those who are judged as unsuitable for the current study by the investigator for other reasons.

This study was reviewed and approved by the “Ethical Committee of the Kobuna Orthopedics Clinic” (Chairman: Toshio Kawada; approval date: January 30, 2025). The study was conducted in accordance with the “Declaration of Helsinki (amended October 2013)” and “Ethical Guidelines for Medical and Biological Research Involving Human Subjects” (partially amended on March 27, 2023), and under the supervision of physicians at the Shinagawa Season Terrace Health Care Clinic (Tokyo, Japan).

The research plan for this study was registered in the clinical trial registration system operated by the University Hospital Medical Information Network Research Center with registration ID UMIN000057070 (trial registration name: A Study on the Effect of Test Food on Cognitive Function: A Randomized, Double-

blind, Placebo-controlled, Parallel-group Study).

2.2. Research Methods

This study was conducted as a randomized, double-blind, placebo-controlled, parallel-group study (allocation ratio: 1:1) for 13 weeks, consisting of a pre-observation period (1 week) and an intake period (12 weeks), with no methodological changes after study entry. The statistical analyst used computer-generated random numbers to allocate the subjects using a block randomization method (block size of 4) with age, sex, and MMSE-J results as adjustment factors. The two allocated groups were assigned to the active food group and the placebo food group by the study food allocation manager, who was not directly involved in the study. Furthermore, the study food allocation manager prepared and sealed a table with the allocation results (key codes) and kept it in a sealed container until the key code was disclosed after the analysis subjects were determined, thereby ensuring the blinding of persons other than the study food allocation manager. Study foods were dispensed in plain aluminum sachets to ensure blinding of participants and providers.

Throughout the study, participants were instructed to avoid using medicines or health foods that may affect cognitive function, maintain their pre-study lifestyle, avoid excessive alcohol intake, and refrain from participating in other studies. Other precautions included abstaining from alcohol from the day before all tests, going to bed early, and avoiding late nights before tests, and abstaining from smoking from waking until test completion on test days. The use of medications required permission from the principal or sub-investigator, except during emergencies.

2.3. Intake of Study Food

During the intake period, participants consumed the study food daily. The active food was a soft-capsule containing fish oil as the main ingredient, plus starch, γ -aminobutyric acid, Ginkgo biloba extract, elastin, collagen peptide, vitamin E-containing vegetable oil, Piper longum extract, heat-killed lactic acid bacteria powder, edible flaxseed oil, edible olive oil, edible sesame oil, edible fats and oils, green chili fermented extract, algae-derived DHA-containing oil, fermented black ginger powder, glycerin, gelling agent (carrageenan), glycerin fatty acid esters, antioxidants (vitamin E, tea extract), L-theanine, L-tryptophan, and vitamin D. For the placebo food, the fish oil in the active food was replaced with edible olive oil, and adjustments were made so that the placebo food was indistinguishable from the active food in appearance, while the other ingredients were formulated in the same proportions as in the active food formulation. The daily dose of both food types was five capsules of 460 mg each. Participants took five capsules daily with water or lukewarm water.

The caloric and nutrient values per day of intake of the studied foods are shown in **Table 1**. Daily intake of active food included 540 mg of DHA and 100 mg of

EPA.

Table 1. Analysis of nutrient composition values of test food.

	Placebo food (5 capsules)	Active food (5 capsules)
Energy (kcal) ^a	15	15
Protein (g) ^b	0.1	0.1
Fat (g)	1.3	1.3
Ash (g)	0.78	0.73
Sodium (g)	0.01	0.01

a. Calorie conversion factors: protein, 4; fat, 9; carbohydrates, 4. b. Nitrogen protein conversion factor: 6.25.

2.4. Evaluation Items

The primary outcomes were cognitive function, with memory and attention as the endpoints, and QOL was evaluated as an exploratory secondary outcome. These parameters were assessed twice: before and 12 weeks after intake. No changes were made to the outcomes after the start of the study.

Memory was assessed using the Wechsler Memory Scale-Revised (WMS-R) [10]: Logical Memory I and Logical Memory II. Attention was assessed using the New Stroop Test II [11]: Stroop interference rate and reverse-Stroop interference rate. QOL was assessed using the SF-36v2 Japanese version of the with three scores: physical component summary, mental component summary, and role-social component summary, calculated using the three-component scoring method from eight subscales.

The study subjects were given a food diary and a research logbook and asked to complete them daily throughout the intake period, beginning one week prior to the start of intake. The survey items included: 1) the intake of study foods, 2) the presence or absence of physical changes, 3) the presence or absence of defecation, 4) the presence or absence of diarrhea, 5) the presence or absence of changes in living conditions, 6) the presence or absence of changes in exercise, 7) the use of medicines (medicines excluding nutritional drinks, newly designated quasi-drugs, and new-range quasi-drugs), and 8) dietary content (including supplements, health foods, drink products, and alcohol).

2.5. Statistical Analysis

The population analyzed was defined as a Per-Protocol Set (PPS). For each measurement, unpaired t-tests were performed to compare the groups, and the results at the end of the intake were evaluated. All tests were two-tailed, with a significance level of 5%. Statistical analyses were performed using the IBM SPSS Statistics 28. Subject background characteristics are presented as means \pm standard deviations, and other data as means \pm standard error. No additional analyses were conducted.

3. Results

3.1. Participants

A total of 110 subjects (65 men and 45 women) were enrolled and randomized, with no post-randomization dropouts, and all received the allocated interventions (55 per group). None of the subjects discontinued the study, and 110 completed the study. After study completion, 19 participants were excluded from the analysis, leaving 91 (55 men and 36 women) for the PPS analysis. The reasons for rejection were violations of study precautions (active, $n = 9$; placebo, $n = 7$) and marked disturbances in lifestyle or diet during the study period (active, $n = 1$; placebo, $n = 2$). Analyses were performed according to the original allocation.

Table 2. Participant characteristics.

Parameter	Placebo food group ($n = 46$)	Active food group ($n = 45$)
Male/Female	28/18	27/18
Age (years old)	67.4 \pm 5.4	67.0 \pm 4.7
Height (cm)	163.4 \pm 8.7	162.1 \pm 9.1
Body weight (kg)	63.5 \pm 9.7	60.8 \pm 10.8
Body mass index (kg/m ²)	23.7 \pm 2.3	23.0 \pm 2.4
MMSE-J	28.5 \pm 1.6	28.5 \pm 1.1

Values are expressed as means \pm SDs. No significant difference was observed.

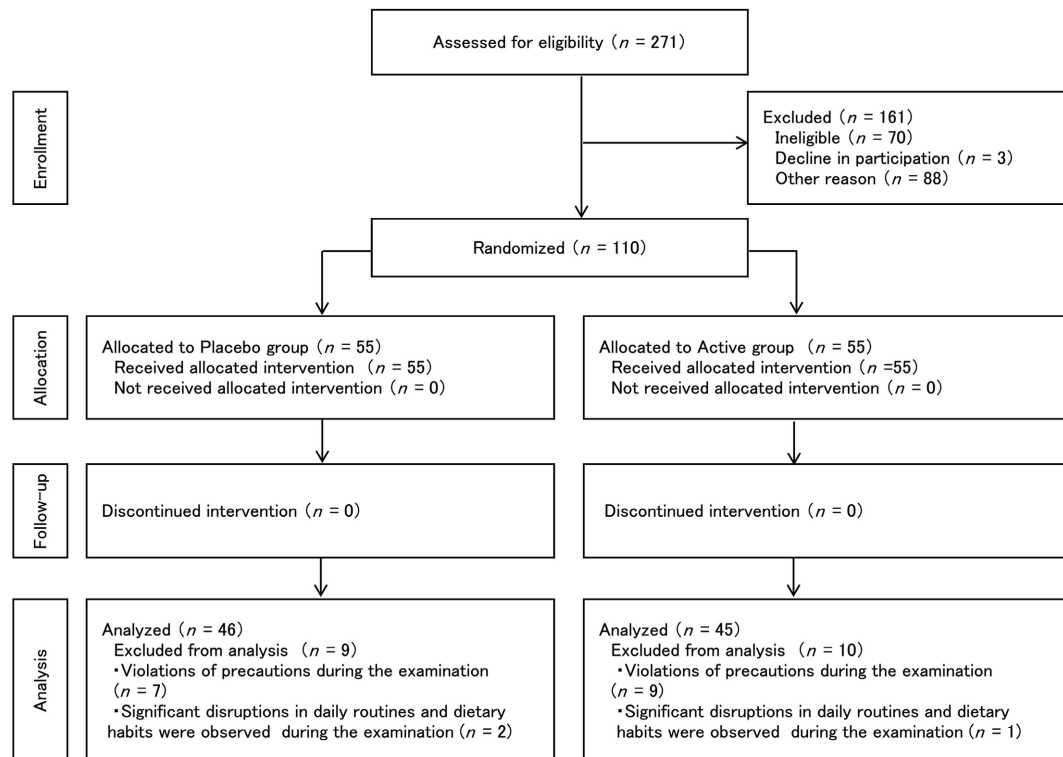


Figure 1. Flow diagram of progress through phases of randomized, double-blind, placebo-controlled, parallel-group study.

The period from recruitment to the end of follow-up was February 2025 to July 2025, and the study was terminated when all participants received follow-up. The backgrounds of the study participants are shown in **Table 2**, and a flowchart illustrating the process from inclusion to analysis is shown in **Figure 1**.

3.2. Analysis Results

The cognitive function results are presented in **Table 3**. Between-group comparisons showed significant differences favoring the active food group in the endline value of the Stroop interference rate (95% CI -10.2 to -0.6 , $P = 0.029$), and in the changes from baseline for Logical Memory I (95% CI 0.12 to 3.83 , $P = 0.037$) and Stroop interference rate (95% CI -8.53 to -0.35 , $P = 0.034$).

Table 3. Cognitive function results.

	Group	Baseline	12 Weeks	Changes from Baseline
Logical memory I (score)	Placebo food ($n = 46$)	22.2 ± 0.9	24.0 ± 0.9	1.9 ± 0.7
	Active food ($n = 45$)	20.7 ± 1.0	24.5 ± 1.1	$3.8 \pm 0.7^*$
Logical memory II (score)	Placebo food ($n = 46$)	18.2 ± 1.1	21.7 ± 0.8	3.5 ± 0.8
	Active food ($n = 45$)	16.9 ± 1.1	21.3 ± 1.3	4.4 ± 0.8
Stroop interference rate (%)	Placebo food ($n = 46$)	16.5 ± 1.5	16.7 ± 1.8	0.2 ± 1.5
	Active food ($n = 45$)	15.5 ± 1.7	$11.3 \pm 1.7^*$	$-4.2 \pm 1.4^*$
Reverse stroop interference rate (%)	Placebo food ($n = 46$)	10.1 ± 1.7	13.4 ± 1.4	3.4 ± 1.8
	Active food ($n = 45$)	11.4 ± 1.5	11.5 ± 1.5	0.1 ± 1.7

Values are expressed as means \pm SEs. *significantly different from the placebo food group ($P < 0.05$).

The QOL results are presented in **Table 4**. The between-group comparison showed a significantly higher endline Mental Component Summary score in the active food group than in the placebo group (95% CI 0.03 to 5.86 , $P = 0.048$).

Table 4. QOL results.

	Group	Baseline	12 Weeks	Changes from Baseline
Physical Component Summary	Placebo food ($n = 46$)	49.4 ± 1.1	50.7 ± 0.8	1.3 ± 1.0
	Active food ($n = 45$)	50.3 ± 0.8	50.0 ± 0.8	-0.3 ± 1.0
Mental Component Summary	Placebo food ($n = 46$)	54.8 ± 1.1	56.0 ± 1.0	1.2 ± 0.6
	Active food ($n = 45$)	57.9 ± 1.2	$59.0 \pm 1.1^*$	1.0 ± 1.0
Role-social Component Summary	Placebo food ($n = 46$)	50.2 ± 1.3	52.7 ± 0.8	2.5 ± 1.3
	Active food ($n = 45$)	52.9 ± 1.0	52.7 ± 1.0	-0.2 ± 1.1

Values are expressed as means \pm SEs. *significantly different from the placebo food group ($P < 0.05$).

3.3. Adverse Events

Adverse events such as colds and dental caries occurred in both groups, and all were assessed by the principal investigator as unrelated to the study foods. No serious adverse events were observed.

4. Discussion

This study investigated the effects of fish oil on cognitive function and associated QOL in middle-aged and older adults with normal to MCI cognitive function. Participants consumed either fish oil-containing foods (active food) or fish oil-free foods (placebo food) for 12 consecutive weeks in a randomized, double-blind, placebo-controlled, parallel-group, comparative trial.

In the evaluation of cognitive function memory, a significant difference was observed in the change from baseline for Logical Memory I, with the active food group showing higher values than the placebo food group. No significant difference was observed in Logical Memory II.

Logical Memory I is a task in which subjects immediately replay a short story read by the inspector, whereas Logical Memory II is a task in which the story is replayed 30 min after being heard [12]. Logical memory is considered an assessment method for episodic memory and is known to decline with age [13]; higher scores indicating a better state. In this test, significant differences were observed in Logical Memory I, suggesting that it is effective for immediate memory within the memory category.

In the evaluation of attention cognitive function, a significant difference was observed in the endline value and the change from baseline for Stroop interference rate, with the active food group showing lower values than the placebo food group. No significant difference was observed in the reverse Stroop interference rate.

The Stroop interference rate measures the ratio of the difference in correct responses between tasks with verbal interference (where the ink color is presented in words of a different color) and tasks without interference (where participants identify the ink color on a sheet of paper). Stroop interference is an indicator of selective attention. This value increased with age [14], with lower values indicating better conditions. However, while no significant difference was observed in the reverse Stroop interference rate, it is considered to reflect different cognitive functions than the Stroop interference rate, and does not decline with age [14]. Therefore, this does not negate the effect of selective attention observed in the Stroop interference rate.

Therefore, the improvement observed in Logical Memory I and Stroop interference rates following the consumption of fish oil-containing foods suggests that they may suppress the decline in cognitive function associated with aging. This suggests that it could be beneficial in halting the transition from a healthy state to MCI, or the progression from MCI to dementia.

In the QOL evaluation, a significant difference was observed in the endline value for the Mental Component Summary, with the active food group showing

higher values than the placebo food group. Higher scores on the Mental Component Summary indicated better condition.

Cognitive decline contributes to a decreased mental QOL [4]. Therefore, the fact that fish oil intake led to improved cognitive function and significantly higher scores on the Mental Component Summary suggests that it may be beneficial in contributing to QOL in middle-aged and older adults.

The mechanism through which fish oil affects cognitive function can be examined based on existing knowledge. DHA, which is abundant in fish oil, enhances synaptic membrane fluidity [15] and activate NMDA receptors [16], suggesting that it contributes to memory maintenance. Furthermore, increased levels of ox-haemoglobin in the prefrontal cortex, which is involved in selective attention, have been confirmed with the intake of fish oil containing DHA [17], suggesting that it activates brain cells in the prefrontal cortex. Therefore, it is believed that DHA in fish oil affects various areas of the brain and enhances cognitive function.

This study also confirmed the safety of fish oil. The results indicated that no adverse events attributable to the consumption of fish oil-containing foods were observed, suggesting that there were no safety concerns associated with the 12-week consumption period.

However, this study had several limitations. While it focused on individuals aged 60 - 79 years, its effects on those under 60 years of age remain unknown. Furthermore, although previous studies have examined the effects of fish oil intake on cognitive function, no study has specifically examined its effects in younger Japanese individuals without cognitive decline. Therefore, a future research challenge would be to conduct studies on the effects of fish oil on cognitive function using a broader range of subjects to confirm these effects. Furthermore, this study did not examine the effects of dietary intake levels of components potentially influencing cognitive function obtained from regular meals.

5. Conclusion

Fish oil-containing foods have been shown to improve cognitive function of specific aspects including memory and attention, in middle-aged and older adults with normal cognitive function or MCI.

Authors' Contributions

Conceptualization, T.K., K.T., G.M., Y.M., A.M., K.K., M.K., H.F., M.T., and C.M.; Methodology, N.M.; Validation, C.F.; Formal analysis, N.M. and C.F.; Investigation, N.M.; Writing-Original Draft Preparation, N.M.; Supervision, K.T. and D.T.; Writing-Review & Editing, T.M. and S.T.; Visualization, N.M. and C.F.; Project Administration, S.T. and T.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

This study was conducted in accordance with the Declaration of Helsinki and approved by The Ethics Committee of Kobuna Orthopedics Clinic (approval date: 30 January 2025; approval number: MK-2501-01).

Informed Consent Statement

Informed consent was obtained from all participants involved in the study.

Data Availability Statement

The data used in this manuscript are not publicly available because of commercial restriction, but are available on reasonable request.

Conflicts of Interest

This study was commissioned by Vitabrid Japan Inc to Toyo Shinyaku Co., Ltd, which then subcontracted it to K.S.O., Inc. The research food for this study was manufactured and provided by Toyo Shinyaku Co., Ltd, who was commissioned by Vitabrid Japan Inc. Eight of the authors (G.M., Y.M., A.M., K.K., M.K., H.F., M.T., and C.M.) are employees of Vitabrid Japan Inc. Six of the authors (N.M., C.F., T.M., S.T., T.K., and K.T.) are employees of and receive their salaries from Toyo Shinyaku Co., Ltd. D.T., a physician affiliated with Shinagawa Season Terrace Health Care Clinic, conducted this study as the principal investigator under contract with K.S.O., Inc.

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