IMPROVEMENT IN COGNITIVE FUNCTION BY SUPPLEMENT CONTAINED PLASMALOGEN FOR HEALTHY JAPANESE
— A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY —

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Abstract

Objective: The objective of this study was to investigate the effectiveness of daily ingestion of two soft capsules, which contain plasmalogen.
Methods: A randomized, placebo-controlled, double-blind study was conducted to evaluate the cognitive function. The study used MMSE (Mini Mental State Exam), U-K test (Uchida-Kraepelin test) and subjective reporting of the cognitive function.
Results: From all of 135 applicants, 54 were eliminated due to not meeting inclusion criteria. Among 81 subjects, 6 were withdrawn (unwell; 2, unexpected business; 3, family bereavement; 1), and 75 subjects completed the study [Test-1 (0.5 mg) sample = 27: M; 9, F; 18, Test-2 (0.25 mg) sample= 23: M; 10, F; 13, Placebo= 25: M; 9, F; 16]. After 12 weeks of ingestion, the study showed significant differences in the results of MMSE. On other hand, the results of U-K test did not show significant differences in any groups. As for the subjective reporting of the cognitive function in form of a questionnaire, intragroup analysis of the test product showed significant differences in more than half of the items, whereas inter-group analysis between the test group and the Placebo showed significant differences in 4 to 14 items. In addition, no adverse effects were observed after the ingestion of the test product.
Conclusion: We found out that the ingestion of the supplement containing plasmalogen for 12 weeks contributed to the improvement of some cognitive functions related to language and situation. In addition, no safety-related matter occurred during the 12-week test period.

Key Words: plasmalogen, cognitive function, MMSE, U-K test

1. INTRODUCTION

Japanese society is now regarded as a "super-aging society" since the ratio of elderly people of aged 65 years or older, to the total population, reached 23% in 2010[1]. Aging not only causes a decline of the motor functionality of the lower back or knees, but also deteriorates the cognitive capacity such as memory. The phenomenon related to the decline of the cognitive capacity such as forgetfulness or memory deterioration is evident in most, as they get older. However, many elderly people tend to fear that they may become demented when their forgetfulness becomes a more serious matter, and this fear eventually makes them worry needlessly about their forgetfulness. According to the releasing of the Ministry of Health, Labour and Welfare, one in every five person of 65 years or get older is estimated to become demented in 2025. To prevent dementia, it is regarded as important to keep the brain in a healthy condition constantly, without giving up the prevention of the forgetfulness and/or the decline of memory skill caused by the aging.

It is said that the memory of information is controlled by hippocampus, which is part of the cerebral limbic system[3]. Plasmalogen, on the other hand, is one of the phosphatides existing intravitally, and it functions as linking fat and protein whilst keeping the function of the cell membrane normal. Plasmalogen counts for about 18% of phosphatide in the human body and it reportedly has antioxidant properties[4]. It was reported in 1999 that the amount of plasmalogen at the frontal lobe and the hippocampus of the brain of an Alzheimer's disease patient is significantly decreased[5]. This finding led to the supposition that plasmalogen had some relationship in the perception skill of humans, and after that a lot of

Table 1 Nutritional content of the test samples per 100 g

Item	Test-1 (0.5 mg)	Test-2 (0.25 mg)	Placebo
Energy	632 kcal	630 kcal	674 kcal
Protein	30.7 g	30.6 g	28.5 g
Lipid	51.8 g	51.1 g	56.7 g
Carbohydrate	10.7 g	11.9 g	12.5 g
Salt equivalent	0.111 g	0.102 g	0.02 g
Vitamin E	25900 mg	25900 mg	−

researchers released study reports that plasmalogen has a beneficial effect on Alzheimer's disease patients and/or the dementia patients. However, there are few studies which focus on the relationship between plasmalogen and the memory skill of a normal (healthy) person.

Therefore, we conducted a randomized, placebo-controlled, double-blind study to verify the effectiveness of plasmalogen for the memory skill and the cognitive capacity of healthy human and its safeness, by having the test subjects ingest the supplement containing plasmalogen.

2. METHODS

2.1. Trial design

A randomized, placebo-controlled, double-blind study was conducted with the aid of a fund from P-Solution Inc. (Tokyo) at JACTA (Tokyo). The study period was 12 weeks, from April 5th to June 29th, 2016. This study was conducted in accordance with the ethical principles of the declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all subjects.

The allocation of the test product to the subjects was carried out by the person in charge of allocation. The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study.

2.2. Subject

Healthy subjects participated in the present study. All of the subjects in this study were public volunteers who had enrolled in the monitor bank of CROee Inc. (Tokyo), recruited from March through April, 2016.

2.2.1. Inclusion criteria

(1) Healthy Japanese males and females aged between 40 and 79 years;

(2) Individuals who have cognitive difficulties but have not been diagnosed with a disease according to the cognitive function questionnaire (**Appendix 1**).

2.2.2. Exclusion criteria

(1) Individuals undergoing treatment of cognitive diseases;

(2) Individuals on medication, including herbal medicines;

(3) Individuals who are pregnant, nursing, or likely to become pregnant during the trial;

(4) Individuals judged to be unsuitable to participate in the trial by the principle investigator.

2.3. Randomization

From all of 135 applicants, 54 were eliminated according to the cognitive function questionnaire results. The inclusion criteria was judged by the principle investigator.

All subjects were sequentially allocated to Group A (n = 26), Group B (n = 28), and Group C (n = 27) using a random number table. In the process of subject assignment, background factors such as gender, age, and U-K test (2.5.2.2) were taken into consideration to avoid biased distribution. Subjects in Group A ingested the placebo, subjects in Group B ingested test sample-2 (0.25 mg of plasmalogen), and subjects in Group C ingested test sample- 1 (0.5 mg of plasmalogen) for 12 weeks.

2.4. Description of test foods and blinding

The test product was prepared by P-Solution Inc. The amount of daily intake was 2 soft capsules (1 soft capsule weighs 270 mg, therefore 2 soft capsules weigh 540 mg). The Placebo does not include plasmalogen. **Table 1** shows the nutritional contents of the samples. All three types of soft capsules were indistinguishable in shape, color, or taste. Soft capsules were managed by the identification symbol. All involved were blinded.

2.5. Experimental procedures

2.5.1. Experimental protocol

Subjects consumed 2 soft capsules of the supplement with hot or cold water every day for 12 weeks. Subjects were instructed as follows: to take the assigned foods as indicated; to maintain their usual lifestyle and habits; to avoid excessive amounts of food, drink, or alcohol; to maintain a daily record of their physical condition and memory loss occurrences during the test period; and to send the diary to the study coordinator.

2.5.2. Outcome

According to the schedule shown in **Table 2**, we measured parameters on efficacy and safety. These assessments were conducted upon pre-intervention and post-intervention.

2.5.2.1. MMSE (Mini Mental State Exam)

For mental state, the Mini Mental State Examination ("MMSE") [6] was used (**Appendix 2**). MMSE tests a number of different mental abilities, including a person's memory, attention, and language. The overall score was evaluated (out of 30).

Table 2　Schedule for the study.

Item \ Term	Screening	Pre trial test	Test period	
			6 w	12 w
Informed consent	●			
Selection and/or allocation	●			
MMSE		●	●	●
U-K test		●	●	●
Subjective reporting		●	●	●
Ingestion of test foods			←——	——→
Log			←——	——→

● : Implementation

↔ : Daily practice during the test period

2.5.2.2. U-K test (Uchida-Kraepelin test)

To calculate ability, the Uchida-Kraepelin test ("U-K test") [7] was used. The U-K test is a serial addition test, which requires takers to perform calculations as fast and accurately as possible within 30 min. This was achieved using pre-printed paper containing 15 lines of random, single-digit, horizontally aligned numbers. For each minute of the test, the subject was instructed to begin a new line regardless of their position on the current line. Each line contained an excess of calculations such that the subjects were not able to finish any line for a particular minute before being prompted to move on to the start of the next line by the examiner's prompt. We evaluated sum up scores in 7 grades, with a higher grade indicating a better result.

2.5.2.3. Subjective reporting

Further, subjective reporting of the cognitive function was observed by a questionnaire as the primary outcome. The questionnaire covered 27 items (**Appendix 3**). Responses to each question were rated on an ordinal scale of 0 to 4, with 2 representing the baseline status, and higher scores indicating better results.

2.5.2.4. Safety

To evaluate the safety of the test foods, adverse events were collected by means of a written questionnaire during the study. Safety was evaluated as a secondary endpoint.

2.6. Data analysis

A full analysis set was adopted in the present study and no sample size design was used. All statistics were expressed as mean ± standard deviation (SD).

We evaluated intragroup comparison of MMSE, U-K test, and subjective reporting by using the paired t-test.

Student's t-test was used for intergroup comparisons of the measured value at week 0, 6, and 12, and changes from the baseline (⊿0-6 w and ⊿0-12 w). One-way analysis of variance was used to compare subject's backgrounds among groups.

Multiplicity according to the occasions was not adjusted. Any subjects with missing values were eliminated from the analysis. Statistical analyses were performed using Statcel 4 (Yanai, 2015), and Excel Tokei 2015 (SSRI). The results were considered significant at a $<5\%$ level in the two-sided test.

3. RESULTS

3.1. Participant demographics

The 81 subjects were randomly assigned to intervention groups and made a start with ingestion. 6 were withdrawn (unwell; 2, unexpected business; 3, family bereavement; 1), and 75 subjects completed the study. Thus, data obtained from 75 subjects (Test-1 group; 27, Test-2 group; 23, Placebo group; 25) were used for efficacy analysis (**Fig. 1**). There were no significant differences in gender ratio, age, and U-K test among groups (**Table 3**).

3.2. MMSE

The result of MMSE is shown in **Fig. 2** and **Table 4**. Significant increases were observed in the intragroup analysis of Test-1 and Test-2 at week 12, respectively. Whereas the intragroup analysis of Placebo increased significantly at week 6. There were no significant differences in the intergroup analysis of Test-1 versus Placebo, or Test-2 versus Placebo.

3.3. U-K test

Fig. 3 and **Table 4** depict the results of the overall evaluation of the U-K test. No significant difference was yielded in the intragroup analysis of Test-1, Test-2, and Placebo. Concerning the intergroup analysis, only the comparison of the measurement grade of week 6 between Test-1 and Placebo tended to differ.

3.4. Subjective reporting

The results of subjective condition assessments are shown in **Table 5**. Regarding the intergroup analysis of changes between Test-1 and Placebo, or Test-2 and Placebo, significant differences were observed in #1 (Test-1, Test-2), #5 (Test-1), #7 (Test-1, Test-2), #8 (Test-1), #12 (Test-1), #13 (Test-1), #17 (Test-1, Test-2), #18 (Test-1), #25 (Test-1), #26 (Test-2), and #27 (Test-1, Test-2) at week 6. Whilst in #1 (Test-1), #2 (Test-1, Test-2), #4 (Test-1, Test-2), #5 (Test-1, Test-2), #8 (Test-1, Test-2), #12 (Test-1), #15 (Test-2), #16

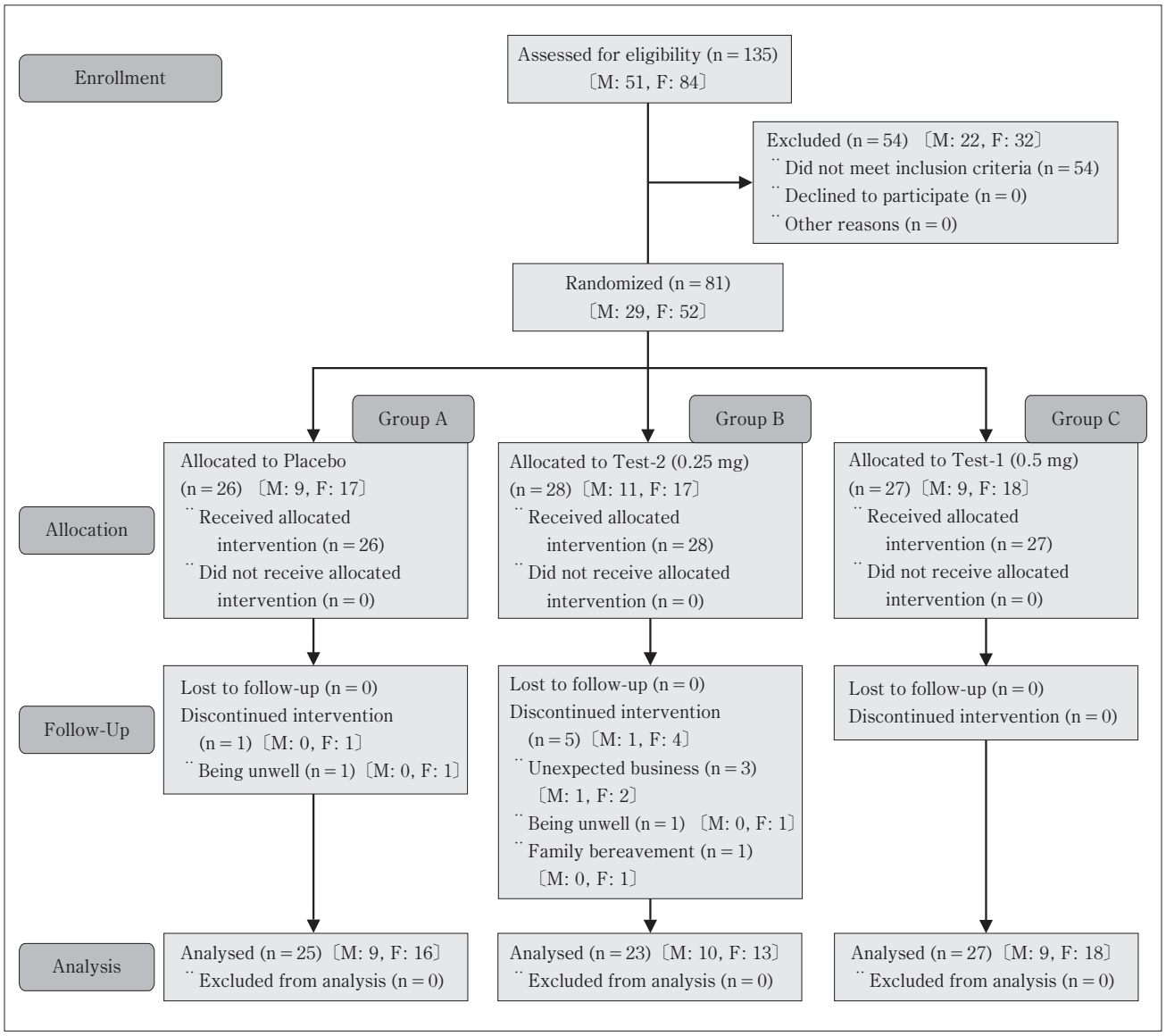

Fig. 1 Flow diagram of subject disposition

Table 3 Subject demographics

Item	Unit	Test-1 (0.5 mg)	Test-2 (0.25 mg)	Placebo	p-value
Subjects	numbers	27	23	25	—
Male: Female*	numbers	9:18	10:13	9:16	0.758
Age*	years	57.0 ± 10.7	58.8 ± 10.5	57.2 ± 9.5	0.795
U-K test*	grade	4.4 ± 1.6	4.0 ± 1.8	4.0 ± 1.5	0.575

mean ± SD

* No significant difference

(Test-2), #17 (Test-2), #20 (Test-2), #21 (Test-2), #23 (Test-2), #24 (Test-1, Test-2), #26 (Test-2), and #27 (Test-1, Test-2) at week 12. In the comparison of Test-1 versus Placebo, 10 items showing a significant difference at week 6 decreased to 6 items at week 12, whereas 4 items showing a significant difference at week 6 expanded to 14 items at week 12 in that of Test-2 versus Placebo.

3.6. Safety

No adverse effects associated with the test product were observed in the course of the reporting.

4. DISCUSSION

We conducted a randomized, placebo-controlled, double-blind study for examining the efficacy of a food

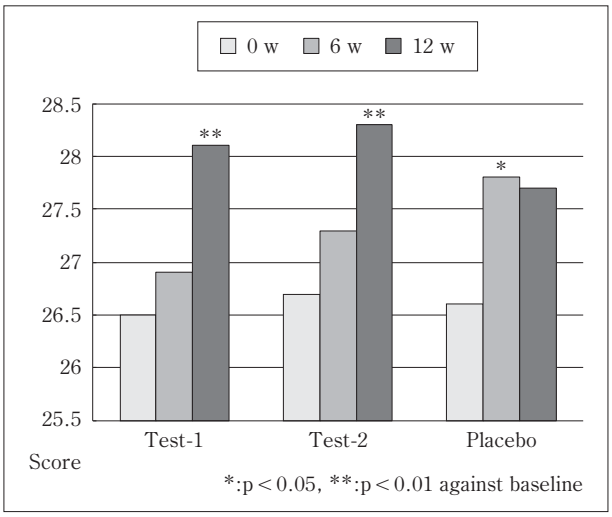

Fig. 2　MMSE

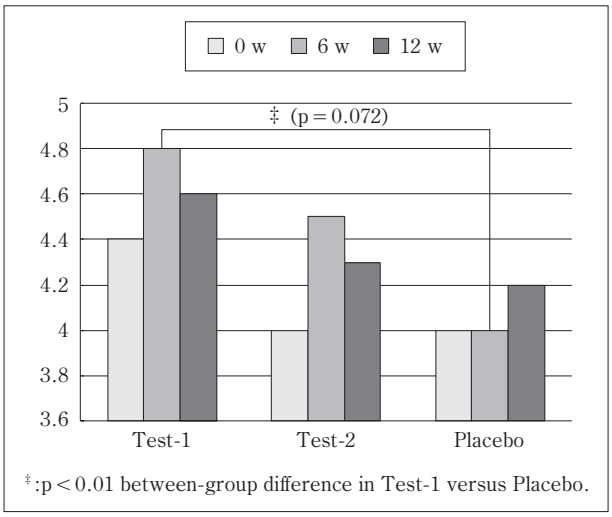

Fig. 3　U-K test

Table 4　Results of test analyses

Item 〈unit〉	Time point	Test-1 (0.5 mg) (n = 27)[1)]	Test-2 (0.25 mg) (n = 23)[1)]	Placebo (n = 25)[1),2)]
MMSE 〈score〉	Baseline	26.5 ± 2.0	26.7 ± 2.2	26.6 ± 2.7
	6-week	26.9 ± 2.3	27.3 ± 1.6	27.8 ± 2.2*
	⊿ 0-6 w	0.4 ± 2.4	0.6 ± 2.5	1.2 ± 2.2
	12-week	28.1 ± 1.8**	28.3 ± 1.7**	27.7 ± 2.5
	⊿ 0-12 w	1.6 ± 2.4	1.7 ± 2.2	1.1 ± 3.5
U-K test 〈grade〉	Baseline	4.4 ± 1.6	4.0 ± 1.8	4.0 ± 1.5
	6-week	4.8 ± 1.3	4.5 ± 1.3	4.0 ± 1.6‡
	⊿ 0-6 w	0.3 ± 1.8	0.4 ± 1.6	0.0 ± 1.6
	12-week	4.6 ± 1.4	4.3 ± 1.6	4.2 ± 1.5
	⊿ 0-12 w	0.1 ± 2.1	0.3 ± 1.5	0.2 ± 1.7

Values are expressed as the mean ± SD.

1) * :p < 0.05, ** :p < 0.01 against baseline.

2) ‡ :p < 0.1 between-group difference in Test-1 versus Placebo.

(supplement) containing plasmalogen. The objective of this study was to verify whether the supplement containing plasmalogen affects the memory skill and the cognitive capacity of a healthy human. The study adapted two types of test method: the test using the test product containing 0.5 mg of plasmalogen (Test 1) and 0.25 mg of plasmalogen (Test 2), and both tests applied placebo-controlled group for comparison.

As the primary outcome, the two test groups both showed significant differences in the results of MMSE after 12-week ingestion, compared to Placebo. The results of U-K test, on the other hand, did not show significant differences in any groups. As for the subjective reporting of the cognitive function in the form of a questionnaire, intragroup analysis of the test product showed significant differences in more than half of the items, whereas inter-group analysis between the test group (Test 2) and the Placebo group showed increases differences in 4 to 14 items. In addition, as the secondary outcome, no adverse effects associated with the test product were observed in the course of the reporting, and the safety of ingesting the test product was established.

Main Findings

In the results of MMSE, significant increases were observed in the intragroup analysis of Test-1 (0.5 mg) and Test-2 (0.25 mg) after 12-week ingestion, respectively. MMSE is the examination which is widely used for testing the cognitive function. Although its main purpose is to screen dementia by measuring the cognitive function, this examination can be also applied to healthy humans who want to verify the decline of their cognitive function[8)]. As for the results of U-K test, on the other hand, no significant difference was observed in the intragroup analysis of Test-1, Test-2, and Placebo. Kraepelin test can evaluate not only one's intelligence or

Table 5 Results of questionnaire analyses (1)

No.	Time point	Scores		
		Test-1（0.5 mg）（n＝27）[1)]	Test-2（0.25 mg）（n＝23）[1)]	Placebo（n＝25）[2)3)]
1	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.4 ± 0.8**	2.2 ± 0.4*	2.0 ± 0.5
	⊿ 0-6 w	0.4 ± 0.8	0.2 ± 0.4	0.0 ± 0.5 [b, e]
	12-week	2.6 ± 0.8**	2.3 ± 0.6*	2.0 ± 0.5
	⊿ 0-12 w	0.6 ± 0.8	0.3 ± 0.6	0.0 ± 0.5 [c]
2	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.2 ± 0.6	2.1 ± 0.5	2.0 ± 0.5
	⊿ 0-6 w	0.2 ± 0.6	0.1 ± 0.5	0.0 ± 0.5
	12-week	2.5 ± 0.8**	2.5 ± 0.5**	2.0 ± 0.7
	⊿ 0-12 w	0.5 ± 0.8	0.5 ± 0.5	0.0 ± 0.7 [b, e]
3	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.2 ± 0.6	2.1 ± 0.3	2.2 ± 0.4*
	⊿ 0-6 w	0.2 ± 0.6	0.1 ± 0.3	0.2 ± 0.4
	12-week	2.4 ± 0.7**	2.3 ± 0.5*	2.4 ± 0.6**
	⊿ 0-12 w	0.4 ± 0.7	0.3 ± 0.5	0.4 ± 0.6 [d]
4	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.2 ± 0.6 [†]	2.3 ± 0.5**	2.0 ± 0.6
	⊿ 0-6 w	0.2 ± 0.6	0.3 ± 0.5	0.0 ± 0.6
	12-week	2.5 ± 0.6**	2.5 ± 0.5**	2.2 ± 0.5 [†]
	⊿ 0-12 w	0.5 ± 0.6	0.5 ± 0.5	0.2 ± 0.5 [b, e]
5	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.4 ± 0.6**	2.4 ± 0.6**	1.8 ± 0.6 [†]
	⊿ 0-6 w	0.4 ± 0.6	0.4 ± 0.6 [##]	− 0.2 ± 0.6 [c]
	12-week	2.6 ± 0.6**	2.5 ± 0.6**	1.8 ± 0.6 [†]
	⊿ 0-12 w	0.6 ± 0.6	0.5 ± 0.6	− 0.2 ± 0.6 [c, f]
6	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.4 ± 0.7**	2.2 ± 0.5 [†]	2.3 ± 0.5 **
	⊿ 0-6 w	0.4 ± 0.7	0.2 ± 0.5	0.3 ± 0.5
	12-week	2.4 ± 0.8**	2.3 ± 0.4*	2.1 ± 0.4
	⊿ 0-12 w	0.4 ± 0.8	0.3 ± 0.4	0.1 ± 0.4 [a]
7	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.3 ± 0.8*	2.2 ± 0.4*	2.0 ± 0.0
	⊿ 0-6 w	0.3 ± 0.8	0.2 ± 0.4	0.0 ± 0.0 [b, e]
	12-week	2.4 ± 0.8**	2.2 ± 0.5 [†]	2.2 ± 0.4*
	⊿ 0-12 w	0.4 ± 0.8	0.2 ± 0.5	0.2 ± 0.4
8	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.4 ± 0.8**	2.1 ± 0.5	2.0 ± 0.2
	⊿ 0-6 w	0.4 ± 0.8	0.1 ± 0.5	0.0 ± 0.2 [b]
	12-week	2.5 ± 0.8**	2.3 ± 0.5*	2.0 ± 0.0
	⊿ 0-12 w	0.5 ± 0.8	0.3 ± 0.5	0.0 ± 0.0 [c, e]
9	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.1 ± 0.8	2.1 ± 0.3	2.0 ± 0.5
	⊿ 0-6 w	0.1 ± 0.8	0.1 ± 0.3	0.0 ± 0.5
	12-week	2.4 ± 0.7*	2.4 ± 0.5**	2.1 ± 0.4
	⊿ 0-12 w	0.4 ± 0.7	0.4 ± 0.5	0.1 ± 0.4 [d]

Scores are expressed as the mean ± SD.

1) [†]:$p < 0.1$, *:$p < 0.05$, **:$p < 0.01$ against baseline.

2) [b]:$p < 0.05$, [c]:$p < 0.01$ between-group differences in Test-1 versus Placebo.

3) [d]:$p < 0.1$, [e]:$p < 0.05$, [f]:$p < 0.01$ between-group differences in Test-2 versus Placebo.

Table 5　Results of questionnaire analyses (2)

No.	Time point	Scores		
		Test-1（0.5 mg）（n = 27）[1]	Test-2（0.25 mg）（n = 23）[1]	Placebo（n = 25）[2)3)]
10	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.3 ± 0.6*	2.2 ± 0.4*	2.0 ± 0.2
	⊿ 0-6 w	0.3 ± 0.6	0.2 ± 0.4	0.0 ± 0.2 [a, d]
	12-week	2.4 ± 0.7**	2.4 ± 0.6**	2.2 ± 0.4*
	⊿ 0-12 w	0.4 ± 0.7	0.4 ± 0.6	0.2 ± 0.4
11	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	1.9 ± 0.6	2.2 ± 0.4*	2.0 ± 0.4
	⊿ 0-6 w	− 0.1 ± 0.6	0.2 ± 0.4	0.0 ± 0.4 [d]
	12-week	2.1 ± 0.4	2.3 ± 0.5*	2.1 ± 0.3 [†]
	⊿ 0-12 w	0.1 ± 0.4	0.3 ± 0.5	0.1 ± 0.3
12	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.3 ± 0.6**	2.3 ± 0.4*	2.0 ± 0.4
	⊿ 0-6 w	0.3 ± 0.6	0.3 ± 0.4	0.0 ± 0.4 [b, d]
	12-week	2.4 ± 0.5**	2.3 ± 0.5*	2.0 ± 0.4
	⊿ 0-12 w	0.4 ± 0.5	0.3 ± 0.5	0.0 ± 0.4 [c, d]
13	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.3 ± 0.5*	2.2 ± 0.4*	2.0 ± 0.3
	⊿ 0-6 w	0.3 ± 0.5	0.2 ± 0.4	0.0 ± 0.3 [b, d]
	12-week	2.2 ± 0.5*	2.2 ± 0.4*	2.0 ± 0.5
	⊿ 0-12 w	0.2 ± 0.5	0.2 ± 0.4	0.0 ± 0.5
14	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.1 ± 0.6	2.3 ± 0.6**	2.0 ± 0.5
	⊿ 0-6 w	0.1 ± 0.6	0.3 ± 0.6	0.0 ± 0.5 [d]
	12-week	2.2 ± 0.6 [†]	2.4 ± 0.6**	2.2 ± 0.4*
	⊿ 0-12 w	0.2 ± 0.6	0.4 ± 0.6	0.2 ± 0.4
15	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.1 ± 0.7	2.3 ± 0.5**	2.1 ± 0.6
	⊿ 0-6 w	0.1 ± 0.7	0.3 ± 0.5	0.1 ± 0.6 [d]
	12-week	2.2 ± 0.6	2.4 ± 0.5**	2.0 ± 0.5
	⊿ 0-12 w	0.2 ± 0.6	0.4 ± 0.5	0.0 ± 0.5 [f]
16	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.3 ± 0.6**	2.3 ± 0.5**	2.1 ± 0.3 [†]
	⊿ 0-6 w	0.3 ± 0.6	0.3 ± 0.5	0.1 ± 0.3
	12-week	2.4 ± 0.8*	2.7 ± 0.6**	2.1 ± 0.3
	⊿ 0-12 w	0.4 ± 0.8	0.7 ± 0.6	0.1 ± 0.3 [a, f]
17	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.3 ± 0.7*	2.3 ± 0.5**	1.9 ± 0.5
	⊿ 0-6 w	0.3 ± 0.7	0.3 ± 0.5	− 0.1 ± 0.5 [b, f]
	12-week	2.4 ± 0.9*	2.4 ± 0.5**	2.1 ± 0.4
	⊿ 0-12 w	0.4 ± 0.9	0.4 ± 0.5	0.1 ± 0.4 [e]
18	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.3 ± 0.5**	2.0 ± 0.4	2.0 ± 0.3
	⊿ 0-6 w	0.3 ± 0.5	0.0 ± 0.4	0.0 ± 0.3 [b]
	12-week	2.4 ± 0.8*	2.1 ± 0.4	2.2 ± 0.4*
	⊿ 0-12 w	0.4 ± 0.8	0.1 ± 0.4	0.2 ± 0.4

Scores are expressed as the mean ± SD.

1）[†]:$p < 0.1$, *:$p < 0.05$, **:$p < 0.01$ against baseline.

2）[a]:$p < 0.1$, [b]:$p < 0.05$, [c]:$p < 0.01$ between-group differences in Test-1 versus Placebo.

3）[d]:$p < 0.1$, [e]:$p < 0.05$, [f]:$p < 0.01$ between-group differences in Test-2 versus Placebo.

Table 5 Results of questionnaire analyses (3)

No.	Time point	Scores		
		Test-1（0.5 mg）（n = 27）[1)]	Test-2（0.25 mg）（n = 23）[1)]	Placebo（n = 25）[2)3)]
19	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.2 ± 0.6	2.1 ± 0.3	2.0 ± 0.5
	⊿ 0-6 w	0.2 ± 0.6	0.1 ± 0.3	0.0 ± 0.5
	12-week	2.4 ± 0.6	2.3 ± 0.6**	2.2 ± 0.5 †
	⊿ 0-12 w	0.4 ± 0.6	0.3 ± 0.6	0.2 ± 0.5
20	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.3 ± 0.7 †	2.1 ± 0.5	2.1 ± 0.3
	⊿ 0-6 w	0.3 ± 0.7	0.1 ± 0.5	0.1 ± 0.3
	12-week	2.3 ± 0.7*	2.4 ± 0.5**	2.0 ± 0.4
	⊿ 0-12 w	0.3 ± 0.7	0.4 ± 0.5	0.0 ± 0.4 [a, f]
21	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.3 ± 0.7*	2.3 ± 0.4*	2.1 ± 0.5
	⊿ 0-6 w	0.3 ± 0.7	0.3 ± 0.4	0.1 ± 0.5
	12-week	2.3 ± 0.7*	2.6 ± 0.7**	2.1 ± 0.4
	⊿ 0-12 w	0.3 ± 0.7	0.6 ± 0.7	0.1 ± 0.4 [f]
22	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.2 ± 0.6 †	2.2 ± 0.5 †	2.0 ± 0.4
	⊿ 0-6 w	0.2 ± 0.6	0.2 ± 0.5	0.0 ± 0.4
	12-week	2.4 ± 0.7**	2.2 ± 0.4*	2.1 ± 0.3
	⊿ 0-12 w	0.4 ± 0.7	0.2 ± 0.4	0.1 ± 0.3
23	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.2 ± 0.6 †	2.1 ± 0.3 †	2.0 ± 0.5
	⊿ 0-6 w	0.2 ± 0.6	0.1 ± 0.3	0.0 ± 0.5
	12-week	2.3 ± 0.8 †	2.7 ± 0.7**	2.2 ± 0.4*
	⊿ 0-12 w	0.3 ± 0.8	0.7 ± 0.7	0.2 ± 0.4 [f]
24	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.2 ± 0.8	2.1 ± 0.3 †	1.9 ± 0.4
	⊿ 0-6 w	0.2 ± 0.8	0.1 ± 0.3	− 0.1 ± 0.4 [d]
	12-week	2.3 ± 0.8*	2.5 ± 0.7**	1.8 ± 0.5
	⊿ 0-12 w	0.3 ± 0.8	0.5 ± 0.7	− 0.2 ± 0.5 [b, f]
25	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.4 ± 0.7**	2.2 ± 0.4*	2.1 ± 0.3
	⊿ 0-6 w	0.4 ± 0.7	0.2 ± 0.4	0.1 ± 0.3 [b]
	12-week	2.4 ± 0.7*	2.4 ± 0.5**	2.2 ± 0.4*
	⊿ 0-12 w	0.4 ± 0.7	0.4 ± 0.5	0.2 ± 0.4 [d]
26	Baseline	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	6-week	2.2 ± 0.7	2.3 ± 0.6*	2.0 ± 0.2
	⊿ 0-6 w	0.2 ± 0.7	0.3 ± 0.6	0.0 ± 0.2 [e]
	12-week	2.3 ± 0.8	2.6 ± 0.7**	2.1 ± 0.3
	⊿ 0-12 w	0.3 ± 0.8	0.6 ± 0.7	0.1 ± 0.3 [f]
27	Baseline	52.0 ± 0.0	52.0 ± 0.0	52.0 ± 0.0
	6-week	58.6 ± 12.0**	57.5 ± 6.5**	52.7 ± 4.3
	⊿ 0-6 w	6.6 ± 12.0	5.5 ± 6.5	0.7 ± 4.3 [b, f]
	12-week	61.7 ± 15.8**	62.0 ± 7.9**	54.4 ± 3.7**
	⊿ 0-12 w	9.7 ± 15.8	10.0 ± 7.9	2.4 ± 3.7 [b, f]

Scores are expressed as the mean ± SD.

1) †: $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$ against baseline.

2) [a]: $p < 0.1$, [b]: $p < 0.05$ between-group differences in Test-1 versus Placebo.

3) [d]: $p < 0.1$, [e]: $p < 0.05$, [f]: $p < 0.01$ between-group differences in Test-2 versus Placebo.

working property but also one's mental health, by doing a simple addition continuously[9]. Regarding the subjective reporting of the cognitive function using a questionnaire, the intragroup analysis of both Test-1 (0.5 mg) and Test-2 (0,25 mg) showed significant differences in more than half of the items. Also in the intergroup analysis, Test-1 group showed significant improvement in 10 items after 6-week ingestion, while Test-2 group showed significant improvement in 14 items after 12-week ingestion, compared to Placebo. In addition, in the total score there was significant difference in all the groups (including Placebo), compared to the baseline. The questionnaire items about the cognitive function were all prepared by the test doctor in charge of this study, and these items were designed to judge the decline of cognitive function or life function. This questionnaire is also based on the knowledge of Tokyo Metropolitan Institute of Gerontology[10]. In this questionnaire, significant difference was mainly found in the items related to living functions such as language, position or situation. There were also a lot of items showing significant difference in the Placebo. Based on the above, regarding the ingestion of the test product even though in the Kraepelin test, which adopts the calculation-task as its evaluation instrument, we could not observe any significant difference, in the other tests we could observe the improvements of some cognitive functions related to the memory of information.

Plasmalogens contained in the test product of this study are a unique subclass of glycerophospholipids characterized by the presence of a vinyl ether bond at the sn-1 position of the glycerol backbone, and they are found in high concentration in cellular membranes of many mammalian tissues. On the other hand, the human brain consists of three parts: the brain stem controlling the basic function for living, the limbic system with functions such as a primitive instinct, an emotion and memory, and the cerebral cortex that takes care of advanced activities. The hippocampus, which controls memory, is located in the cerebral limbic system. New information is once filed by the hippocampus (short-term storage). Then, the information is stored in the cerebral cortex if the hippocampus recognizes it as necessary (long-term storage). The hippocampus is very fragile and delicate, and once it becomes dysfunctional, we cannot remember something new. Also, the damage of the brain caused by insufficient oxygen starts from that of the hippocampus. In addition, it is believed that diseases such as posttraumatic stress disorder or Alzheimer dementia are triggered as a result of the disorder of the hippocampus[11)12)]. The decline of cognitive function is thought to be induced by the damage of the hippocampus by oxidant stress or the accumulation of amyloid β-protein[13)].

Several reports indicate that there is a paucity of plasmalogen in the brain of patients with Alzheimer's disease[14)15)], and this supports the hypothesis that plasmalogen is related to the cognitive function. It is reported that plasmalogen inhibits neuronal cell death (apoptosis) by enhancing the activation of protein kinase such as Akt (protein kinase B) or ERK (Extracellular signal-Regulated Kinase)[16)]. Another report indicates that plasmalogen has the promotive effect of neurogenesis, the anti-neuroinflammatory effect and anti-amyloidogenic effect of amyloid β[17)]. It is reported that the oral administration of plasmalogen increases the level of erythrocyte ethanolamine plasmalogen[18)]. Based on these discussions, it can be speculated that the oral administration of plasmalogen contributes to the improvement of the cognitive function, which is caused by the increase of the level of erythrocyte ethanolamine plasmalogen, the activation of protein kinase, the inhibition of production/accumulation of amyloid β and the promotion of neurogenesis of hippocampus[19)]. On the other hand, the abilities required for the Kraepelin test involve not only the short-term memory controlled by hippocampus but also the complex functions such as concentration or calculation, which require the involvement of other parts of brain[20)]. The results of the questionnaire conducted in this study tended not to show significant improvement in the items other than those of language, position or situation, which require functions other than memory. Therefore, it can be said that the oral administration of plasmalogen is effective for the improvement of the cognitive function as explained above, while it does not contribute to the rather complex functions the other parts of brain mutually involve.

Secondary Findings

In this study, adverse events were collected by means of a written questionnaire during the study, and no abnormal change caused by the test product was observed during the ingesting period. During the test period six test subjects discontinued the test. The reasons of discontinuance were personal ones such as illness (catching a cold) or work, and it has nothing to do with the ingestion of the test product. These results indicated the safety of the ingestion of the test product for the 12-week test period.

General Information

In the present Japanese aging society, the number of both outpatients and inpatients with dementia is increasing. Also, facts such as the increase in the number of care-receivers, the falling birth rate and the trend toward nuclear families all trigger an increase of the caregiver burden, and they eventually lessen the quality of a caregiver's life remarkably. These phenomena are now regarded as a serious social problem[21)]. The symptom of dementia gets worse and worse with the lapse of time since it is a progressive brain disorder[22)23)]. Therefore, it is critical to consider how to prevent dementia. The plasmalogen used in this study restrains the accumulation of amyloid β which is believed as the causative agent of dementia[24)], and this fact supports the hypothesis that it

is also effective for the prevention of dementia. Also, Researchers have well established that a variety of cognitive abilities including memory, processing speed, and problem solving, decline with increasing age [25]. Therefore it is reasonable to consider that the improvement of the cognitive function significantly relates to the improvement of quality of life (QOL) [26]. Based upon the above discussion, it is contemplated that the ingestion of the food of supplement type (which is easily prepared) contributes to allowing the elderly to become more active in life.

Limitations

In this study we verified the cognitive function of human. Since the cognitive function is a feature of the brain, it can be influenced by the consciousness or the feelings of the test subject. Therefore it is undeniable that the result has some sort of bias caused by a placebo effect [27]. We observed significant improvement in some items of the questionnaire conducted by the Placebo group, and this result may be a result of placebo effects. Therefore, for the analysis of the test results it is recommended to evaluate not only the subjective reporting such as a questionnaire, but also some objective indicators as well. In addition, it is necessary to gather the data from a larger number of test subjects, and compare the actual effect and the placebo effect based on it.

5. CONCLUSION

In conclusion, we found out that the ingestion of the supplement containing plasmalogen for 12 weeks contributed to the improvement of some cognitive functions related to language and situation. In addition, no safety-related matter occurred during the 12-week test period.

CONFLICT OF INTEREST

All parts of this study were funded by P-Solution Inc. Hitoshi Nagata is a principal. All authors state that the study was conducted in the absence of any other relationships that could be interpreted as a conflict of interest.

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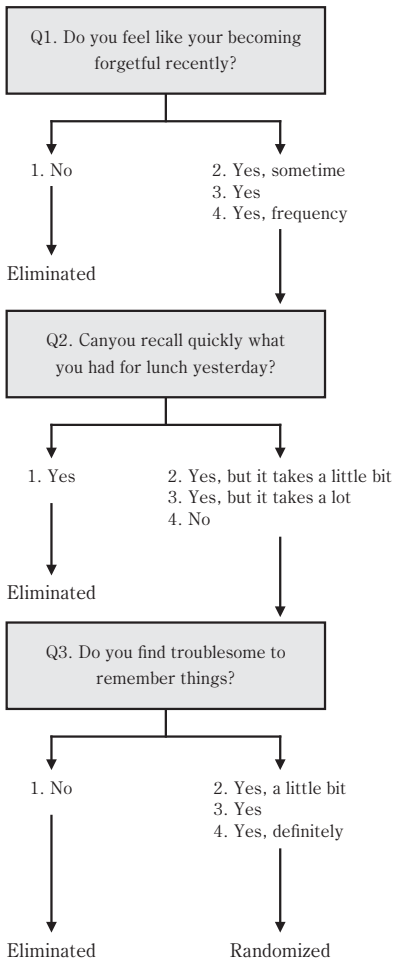
Appendix 1. **Questionnaire for screening**



Appendix 2. **MMSE**

The Mini-Mental State Exam

Patient :_____ Examiner :_____ Date :_____

<Maximum><Score>

Orientation

5 ( ) Whatis the (year) (season) (date) (day) (month)?
5 ( ) Where are we (state) (country) (town) (hospital) (floor)?

Registration

3 ( ) Name 3 objects: 1 second to say each. Then ask the patient all 3 after you have said them. Give 1 point for each correct answer. Then repeat them until he/she learns all 3. Count trials and record. Trials_____

Attention and Calculation

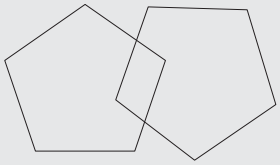
5 ( ) Serial 7's. 1 point for each correct answer. Stop after 5 answers. Alternatively spell "world" backward.

Recall

3 ( ) Ask for the 3 objects repeated above. Give 1 point for each correct answer.

Language

2 ( ) Name a pencil and watch.
1 ( ) Repeat the following "No ifs, ands, or buts"
3 ( ) Follow a 3-stagecommand:
"Take a paper in your hand, fold it in half, and put it on the floor."
1 ( ) Read and obey the following: CLOSE YOUR EYES
1 ( ) Write a sentence.
1 ( ) Copy the design shown.



Total score :_____

Interpretation: 24-30; No cognitive impairment, 18-23; Mild cognitive impairment, 0-17; Severe cognitive impairment

Appendix 3. **Questionnaire of subjective reporting**

Scale of 0 to 4, with 2 representing the baseline status, with higher scores indicating a better result.

Question #1: Do you know the date?
Question #2: Can you get to a place where you have been before?
Question #3: Can you remember your address and phone number?
Question #4: Can you remember the place where you leave things?
Question #5: If an item is not in its usual place, can you find it easily?
Question #6: Can you manage the use of your TV, washing machine, remote, etc.?
Question #7: Can you select cloths according to situation?
Question #8: When buying something, can you pay the correct amount in cash?
Question #9: Despite being healthy, do you feel lack of motivation for activities?
Question #10: Can you fully understand the contents of a book or TV program?
Question #11: Do you write letters?
Question #12: Can you remember what you talked about with someone a few days prior?
Question #13: Do you find it troublesome recalling a conversation a few days ago without someone's help?
Question #14: Whilst having a conversation, do you forget things you want to say?
Question #15: Whilst having a conversation, do you forget easy vocabulary?
Question #16: Can you recognize the face of people you know?
Question #17: Can you remember the name of people you know?
Question #18: Can you recall where those people live and work?
Question #19: Do you become forgetful even regarding recent things?
Question #20: During a short time, do you repeat things you say or ask?
Question #21: Do you often misplace, or forget to leave things behind?
Question #22: Do you miss or become late for appointments due to problems of recalling details?
Question #23: Do you often make mistakes on calculations?
Question #24: Do you get lost in familiar places?
Question #25: Do you find yourself forgetting to turn off the tap or gas after using it?
Question #26: Can you easily urinate? Do you urinate frequently? Sometimes do you experience leakage?
Question #27: Overall