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Cognitive Function Improvement with Astaxanthin Intake: A Randomized, Double-Blind, Placebo-Controlled Study

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Astaxanthin, a type of carotenoids, could prevent the reduction of cognitive function by protecting nerve cells. This study aims to assess the enhancement of cognitive function related to the astaxanthin intake. This randomized, double-blind, placebo-controlled study enrolled healthy Japanese subjects experiencing mild forgetfulness during March–September 2018. All participants were randomly allocated into either astaxanthin ingestion (Asx group, n = 22/group) or placebo ingestion (P group, n = 22/group) for 12 weeks. We assessed the cognitive function using Cognitrax and subjective symptoms using the Likert scale (Asx group: 16; P group: 18). The Asx group exhibited significant improvement in change scores in both the composite memory domain on Cognitrax and the subjective symptoms of linguistic remembrance ($P <$ 0.05, respectively). Furthermore, no adverse events were observed. Hence, the astaxanthin intake enhanced the composite memory domain and linguistic remembrance, thereby maintaining and improving the cognitive function (UMIN-CTR: UMIN000031757; Funding: BGG Japan Co., Ltd.)

Keywords: astaxanthin / cognitive function / Cognitrax / composite memory / verbal memory

Introduction

Lately, the number of dementia patients has increased rapidly, and 1 in 5 individual aged ≥65 years will reportedly experience dementia symptoms by 2025 in Japan (Cabinet Office of Japan, 2016). Dementia is caused by various chronic or progressive brain disorders and disrupts developed cognitive function, memory, the ability to think, behavior, and daily life performance (World Health Organization *et al.*, 2012). The leading cause of dementia is a neurodegenerative disease, in which the brain nerve cells die gradually, leading to the development of Alzheimer's dementia (AD), frontotemporal dementia, and Lewy-body dementia (Raz *et al.*, 2016). The amyloid-β (Aβ) protein, which is present in the brain of patients with AD, is gradually deposited over 20 years and progressively impairs nerve cells, leading to the development of AD (Jack *et al.*, 2010). A time lag exists between the onset of AD and the accumulation of Aβ protein, depending on specific strength of nerve cells and the neural network such as the brain reserve capacity and cognitive reserve (Stern, 2006).Thus, it is beneficial to enhance the resistance to neurodegeneration before the appearance of symptoms or even the deposition of the Aβ protein to lower the risk of the development of dementia. Perhaps, easy-to-consume dietary supplements that contain functional ingredients could effectively prevent AD and improve health conditions.

Astaxanthins exhibit various effects such as enhancements in the accommodative function (Nagaki *et al.*, 2002), prevention of arteriosclerosis (Ryu *et al.*, 2012), and an antioxidant effect (Choi *et al.*, 2011). As astaxanthin could cross the blood–brain barrier, brain function decline and brain disease could be affected to a greater degree by astaxanthin's antioxidant properties (Guerin *et al.*, 2003). In the market, astaxanthins are available in two forms—the esterified form derived from *Haematococcus pluvialis* (*H. pluvialis*) and krill, and the free form derived from *Phaffia rhodozyma* (*P. rhodozyma*), *Paracoccus carotinifaciens* (*P. carotinifaciens*), and synthesized products (Sato *et al.*, 2000; Aoi *et al.*, 2018; Hayashi *et al.*, 2018). In a study, with the intake of an equal amount of esterified astaxanthin from

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H. pluvialis, free-form astaxanthin from *P. rhodozyma*, and synthesized free astaxanthin in mice, the group with esterified astaxanthin from *H. pluvialis* exhibited high astaxanthin concentrations in the plasma and liver (Aoi *et al.*, 2018). Hence, differences are present between *H. pluvialis* and *P. carotinifaciens* in the body kinetics, suggesting possible differences in the brain.

Reportedly, foods containing astaxanthin as a single component improve cognitive function in healthy Japanese subjects (Katagiri *et al.*, 2012; Hayashi *et al.*, 2018), and these studies have demonstrated no significant difference between the active and placebo groups. In these studies, Hayashi *et al.* (Hayashi *et al.*, 2018) used astaxanthin derived from *P. carotinifaciens*, and Katagiri *et al.* (Katagiri *et al.*, 2012) used astaxanthin derived from *H. pluvialis.* Both studies have only shown the possibility of astaxanthin improving cognitive function from intra-group comparisons before and after intake. Therefore, evidence regarding the effects on cognitive function of astaxanthin derived from *P. carotinifaciens* and *H. pluvialis* is insufficient. It is crucial to validate the improvement effect of each type of astaxanthin on cognitive function.

Hence, this study aims to investigate the effect of *H. pluvialis*-derived astaxanthin on the cognitive function in healthy adult subjects experiencing mild forgetfulness.

Material and Methods

1. Study design

We conducted a randomized, double-blind, placebo-controlled study at Takara Clinic (Medical Corporation Seishinkai, Tokyo, Japan) between May 28 and September 1, 2018. The study protocol was approved by the independent ethical committee of the Takara Clinic, Medical Corporation Seishinkai (Tokyo, Japan), on March 13, 2018 (approval no. 1803-1802-BJ01-03-TC). This study was conducted in accordance with the Declaration of Helsinki (2013) and the ethical guidelines for medical and health research involving human subjects of Japan and thoroughly considered medical ethics. The protocol has been registered at the University Hospital Medical Information Network Clinical Trials Registry (UMIN000031757).

2. Subjects

In this study, the selection criteria were as follows: (a) experiencing mild forgetfulness in healthy Japanese adult subjects; (b) eligibility to participate in the study by the principal physician; (c) attained a Mini-Mental Status Examination (MMSE) score of ≥24 at screening/before intake; and (d) relatively lower normalized Cognitrax composite memory domain scores at screening/before intake. The exclusion criteria were as follows: (a) medical history of current treatment for malignancy, heart failure, or myocardial infarction; (b) current treatment for cardiac arrhythmia; hepatic, renal, or cerebrovascular disease; rheumatism; diabetes mellitus; hyperlipidemia; hypertension; or other chronic diseases; (c) diagnosis of dementia; (d) diagnosis of mental illnesses, such as major depression, and attention-deficit hyperactivity disorder; (e) daily consumption of medications (including herbal medicines), "foods for specified health uses," "foods with function claims," or other functional food/beverage; (f) daily consumption of food containing DHA, EPA, *Ginkgo biloba* extract, tocotrienol, astaxanthin, GABA, phosphatidylserine, and/or other improved cognitive function food/beverage; (g) an allergic reaction to medications and/or products that contain the study ingredients; (h) pregnant, lactation, or planning to become pregnant; (i) enrolled in other clinical trials within the last 3 months before agreeing to participate in this study; and (j) ineligibility to participate in the study based on the evaluation of the principal physician.

Regularly, all subjects were enrolled through the website (https://www.go106.jp/) operated by ORTHOMEDICO Inc. (Tokyo, Japan) between March 19 and May 12, 2018. The study protocol was comprehensively explained to all subjects. We obtained written informed consent from all subjects before their participation in the study at the ORTHOMEDICO, Inc., office. Notably, no subject was part of the sponsors or funding companies.

3. Sample size determination

The cognitive function was assessed using Cognitrax on the basis of CNS Vital Signs (CNS Vital Signs LLC., Morrisville, the USA) (Gualtieri *et al.*, 2006). The primary outcome was an increment in the score of the composite memory domain. Few studies have evaluated the composite memory domain with the intake of *H. pluvialis*-derived astaxanthin. Thus, per Gualtieri *et al.* (Gualtieri *et al.*, 2006), who used CNS Vital Signs, we calculated the standard deviation (SD) of the primary outcome; SD in the composite memory domain was calculated to be 7.88 in healthy subjects aged ≥20 years, and we hypothetically obtained a similar SD in this study. In addition, the average value of the composite memory domain ≥7.00 points between the two groups was defined as a clinically significant difference to suggest improvement in the cognitive function. Thus, the sample size was evaluated with an assumed α value of 0.05

and (1–β) value of 0.80. Consequently, the sample size was finalized to be 20 subjects per group. Furthermore, we considered 10% of the dropout rate and added two extra subjects to each group (22 subjects/group). As this study compared two groups, 44 subjects were required in this study.

4. Enrollment, randomization, and blinding

Of 121 subjects who signed informed consent, eligible subjects who were considered appropriate for the study and attained an MMSE score of ≥24, who did not experience dementia (Folstein *et al.*, 1975), were selected by the physician. In addition, subjects with relatively lower normalized Cognitrax composite memory scores [normalized score evaluated on the basis of the average of scores corresponding to the subjects' age set at 100 (SD 15)] (CNS Vital Signs LLC., n.d.) before intake were selected as priority subjects for enrollment in this study. An allocation controller equally, but randomly, assigned subjects to either the astaxanthin group (Asx group, n = 22/group) or the placebo group (P group, n = 22/group). The allocation was performed using StatLight #11 Version 2.10 (Yukms Co., Ltd., Kanagawa, Japan), a computerized random-number generator. The allocation method was stratified randomization, and the allocation adjustment factor was defined as the normalized Cognitrax composite memory score, sex, and age of screening. Furthermore, subjects, the physician, the assessor of outcomes, and others who were associated with this study were not aware of group assignments and were not involved in the allocation. Moreover, the allocation controller locked the assignment sheet until the key-opening day (October 23, 2018).

5. Intervention

The test soft capsules included *H. pluvialis*–derived astaxanthin (BGG Japan Co., Ltd., Tokyo, Japan; the total weight content, 160 mg; and astaxanthin, ~9 mg) and safflower oil as placebo (the total weight content, 160 mg). All subjects were asked to consume either 1 astaxanthin capsule or placebo capsule per day before or after breakfast for 12 weeks. Both capsules were declared identical in color, odor, and flavor by the ethics committee.

6. Examination items

Table 1 outlines the schedule for this study. Subjects visited the clinic and underwent examinations before the intake and 8 and 12 weeks after the intake. All subjects abstained from excessive alcohol or exercise from the day before the examination day until the end of the examination. Furthermore, they abstained from eating or drinking anything including the test food, except water, for 6 h before providing blood samples.

(1) Primary outcome: the composite memory domain

Cognitive function was assessed using Cognitrax (Health Solution, Inc., Tokyo, Japan). Cognitrax evaluates various cognitive function domains, such as processing speed, and executive function on the basis of CNS Vital Signs (Gualtieri *et al.*, 2006), evaluating each domain's score from 10 separate tests. The composite memory domain scores were calculated as the sum of the verbal memory (VBM) and visual memory domain (VIM) scores(Gualtieri *et al.*, 2006). At the beginning of the VBM test, 15 words were presented on the screen, one by one, every two seconds. Next, a participant was asked to identify those words nested among 30 words, including new words

Table 1. Schedule of enrollment, intervention, and assessments

TIME POINT	Enrollment	Before intake (Baseline)	Allocation	Intervention period		
				Start intake	8 weeks after intake	12 weeks after intake
ENROLLMENT:						
Eligibility screen	×					
Informed consent	×					
Allocation			×			
INTERVENTIONS:						
Asx group				◆	———	◆
P group				◆	———	◆
ASSESSMENTS:						
Cognitrax		×			×	×
Original questionnaire		×			×	×
Blood analysis		×			×	×
Physical examination		×			×	×
Urinalysis		×			×	×
MMSE		×				
Visual acuity test		×				
Daily record				◆	———	◆
Medical questionnaire		×			×	×

Asx, astaxanthin; P, placebo; MMSE, Mini-Mental State Examination

(immediate memory scores). Furthermore, the participant was again asked to identify the learned 15 words nested among 30 words, including new words after all the tests had been taken (delayed memory scores). In the VIM test, words in the VBM test were replaced with geometric figures, and the procedure remained the same as that of the VBM test. Composite memory score was calculated from the total number of correct answers given in the VBM and VIM tests, and was converted into normalized score. Normalized value was calculated from measured value based on a normal distribution with mean 100 and SD 15; if the measured value of someone is 1SD greater than the average of his/her own age, his/her normalized score is 115. The standard score in domains was assessed as follows: >109 points "Above"; 90–109 points "Average"; 80–89 points "Low Average"; 70–79 points "Low"; <70 points "Very Low" (CNS Vital Signs LLC., n.d.).

(2) Secondary outcomes

The cognitive functions of the participants were evaluated using Cognitrax as follows: neurocognitive index domain; verbal memory domain; visual memory domain; psychomotor speed domain; reaction time domain; complex attention domain; cognitive flexibility domain; processing speed domain; executive function domain; social acuity domain; reasoning domain; working memory domain; sustained attention domain; simple attention domain; and motor speed domain.

In addition, subjective symptoms were assessed using the Likert scale, using the questions as follows: over the past week, have you forgotten things often?; have you been

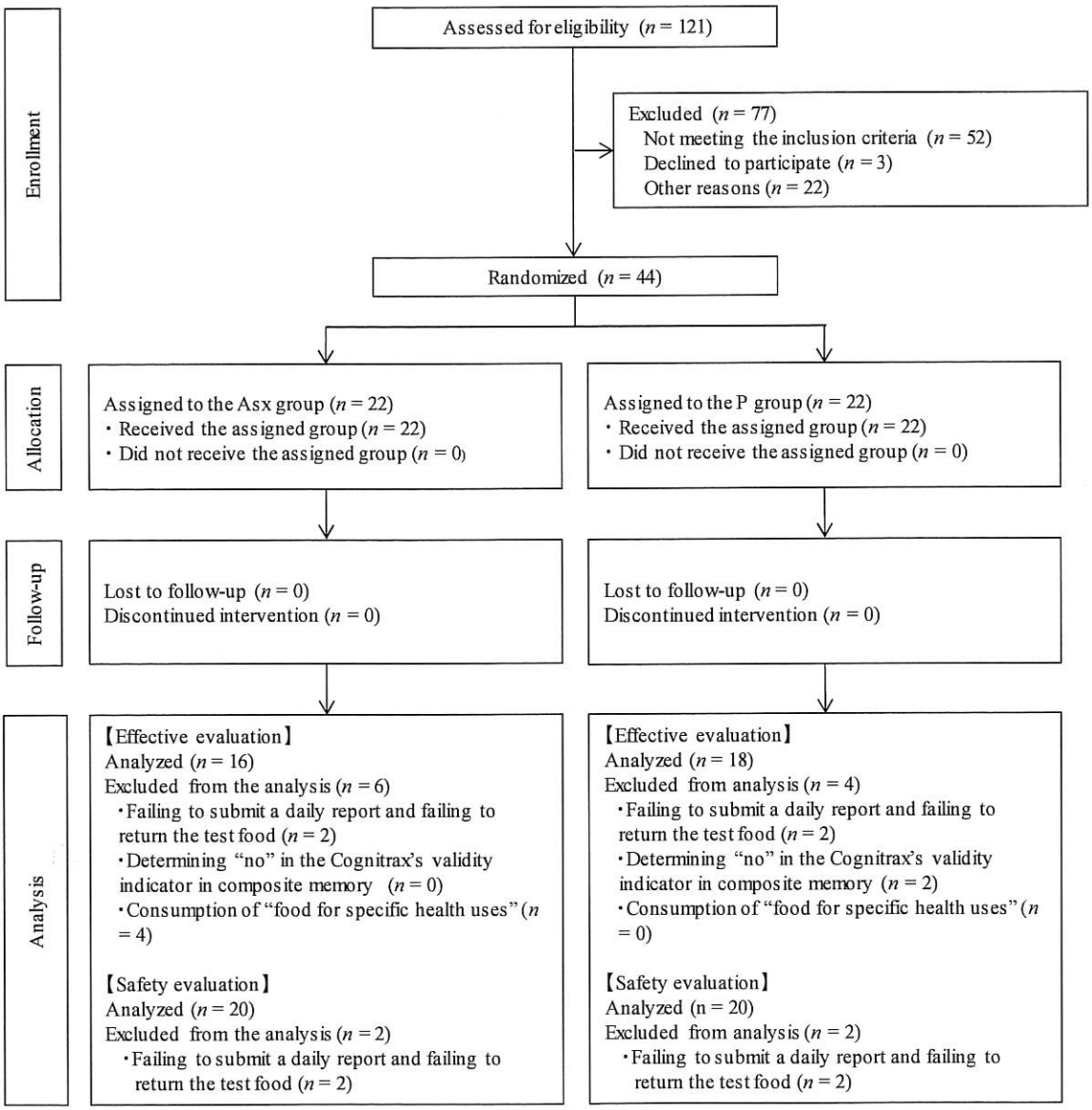

Fig.1. The flowchart of participants in this study

Table 2-1. Subjects' backgroud information (effective analysis subjects only)

Item	Normal range	Unit	Asx group (n = 16)		P group (n = 18)		P Value
			Mean	SD	Mean	SD	
Age	-	years	54.2	6.8	54.6	6.9	0.877
MMSE	-	points	29.1	1.2	29.4	0.8	0.374
IgE (RIST)	≤170	IU/mL	218.6	286.4	176.1	244.1	0.646
Composite Memory	-	points	69.6	11.6	74.6	11.3	0.214

The data are presented as the mean ± standard deviation.
MMSE, Mini-Mental State Examination

Table 2-2. Subjects' backgroud information

Age (years)	Asx group (n = 16)		P group (n = 18)	
	Men (n)	Women (n)	Men (n)	Women (n)
40-49	2	2	2	3
50-59	4	6	5	6
60-69	0	1	1	0
≥70	0	1	0	1

concerned about memory loss during the last week?; is there anytime that you cannot remember a story you heard during the last week?; during the last week have you had trouble remembering people's name or the names of things?; did you leave behind anything over the last week?; did you feel chronically tired during the past week?; were you experiencing eye fatigue during the past week?; did you experience stiff neck or shoulders during the past week?; have you felt depressed for the past week?; did you experience uncomfort in your back over the last week?; was it difficult to get up from the floor or a chair for the last week?; did your knees hurt during crouching or standing up in the last week?; and did your knees hurt while going up and down stairs for the last week?. All these questions were assessed on a scale from 1 (strongly disagree) to 6 (strongly agree).

Furthermore, subjects' blood samples (19 mL) were collected at the Medical Corporation Seishinkai Takara Clinic and tested for the following: brain-derived neurotrophic factor (BDNF); propanoyl lysine (PRL); and pentosidine. In this study, all collected blood samples were entrusted to LSI Medience Corporation (Tokyo, Japan).

(3) Safety evaluation

The safety evaluations were assessed in physical examination, urinalysis, and blood analysis (Tables 4–6). All subjects were asked to fill out a medical questionnaire to understand their health conditions. In addition, subjects were asked to record the daily report such as health conditions, usage of medications, and lifestyles.

7. Statistical analysis

All outcomes were assessed before the intake and 8 and 12 weeks after the intake (three assessment points). Setting before intake as the baseline, each assessment point was subtracted from the baseline and reported as the change in the value (Δ8 and Δ12 weeks). In addition, subjects' background and demographic data were aggregated on the basis of age, MMSE, IgE (RIST), and visual acuity test, and the data of the Asx and P groups were compared using the Welch's t-test.

The cognitive function data in the baseline and changes are represented as mean and SD, which were analyzed using the Welch's t-test. Moreover, subjective symptoms data were analyzed using the Mann–Whitney U-test at the baseline and 8 and 12 weeks after the intake. Physical examination and blood analysis data are presented as mean and SD, which were analyzed at the baseline using the Welch's t-test. Furthermore, we analyzed data at 8 and 12 weeks after the intake using the two-way ANCOVA. When the ANCOVA was used for data analyses, we used the baseline values as covariates. Of note, the between-group comparison was used in the post-hoc analysis. Furthermore, urinalysis data were set to a code where 1 was identified as within the normal range and 0 as outside the normal range. The chi-square test was used for between-group analyses.

All statistical analyses in this study were two-sided, and we set the significance level at 5% with no adjustment for multiple comparisons. The data analyses were performed using Windows SPSS Version 23.0 (IBM Japan, Ltd., Tokyo, Japan).

Results

1. Analysis set

Fig. 1 presents the study flowchart and subject disposition. Of 121 participants, we excluded 77 because of dropout, the medical questionnaire, and the physician's discretion. Thus, 44 eligible subjects were allocated to either the Asx group or the P group (n = 22 each). At the clinical conference after the study, 10 subjects were excluded from the analysis, including 4 for failing to submit a daily report and failing to return the test food, 2 for

Table 3-1. The results of variable in Cognitrax

Domain	Unit	Baseline				Δ8 weeks				Δ12 weeks				*P* Value		
		Asx group (*n* = 16)		P group (*n* = 18)		Asx group (*n* = 16)		P group (*n* = 18)		Asx group (*n* = 16)		P group (*n* = 18)				
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Baseline	Δ8 weeks	Δ12 weeks
Neurocognitive Index	Points	91.3	10.7	91.8	12.4	4.4	8.5	3.3	17.8	8.2	8.5	7.5	11.0	0.907	0.808	0.838
Composite Memory	Points	69.6	11.6	74.6	11.3	14.6	19.5	12.1	20.8	22.3	17.4	9.6	16.2	0.214	0.725	0.035*
Verbal Memory	Points	70.3	14.2	77.4	15.4	11.3	23.9	10.0	22.5	20.9	21.0	12.1	19.0	0.172	0.876	0.211
Visual Memory	Points	81.6	13.5	83.0	14.2	11.7	14.4	8.7	16.5	14.8	19.7	2.3	16.3	0.774	0.572	0.054
Psychomotor Speed	Points	93.5	27.9	99.7	15.1	4.1	7.0	-0.3	18.0	6.3	8.6	4.1	10.8	0.439	0.349	0.514
Reaction Time	Points	103.1	15.2	91.8	15.2	-1.3	17.1	2.2	9.8	-2.9	18.9	7.4	11.4	0.037*	0.488	0.070
Complex Attention	Points	96.3	20.4	99.2	20.4	1.6	20.6	-0.9	31.5	6.1	23.1	5.2	21.1	0.681	0.783	0.906
Cognitive Flexibility	Points	93.7	16.7	93.2	19.8	3.2	13.2	3.6	25.6	9.5	14.4	11.9	16.2	0.934	0.951	0.645
Processing Speed	Points	99.1	11.4	109.1	12.3	9.1	8.8	3.1	13.2	10.2	6.7	6.6	13.3	0.020*	0.125	0.318
Executive Function	Points	95.7	14.9	93.4	19.5	2.4	11.1	3.5	24.4	7.6	12.7	11.4	15.0	0.708	0.861	0.421
Social Acuity	Points	86.8	20.7	69.2	55.3	0.1	29.6	23.2	42.9	2.1	31.2	22.9	50.3	0.221	0.075	0.152
Reasoning	Points	105.3	11.4	102.4	12.1	-8.5	15.3	-2.9	12.2	-11.6	16.3	2.9	12.7	0.482	0.256	0.008*
Working Memory	Points	97.3	19.9	102.0	13.9	-3.3	23.5	5.7	12.2	2.8	19.1	5.9	14.8	0.438	0.182	0.607
Sustained Attention	Points	92.4	16.5	103.0	13.7	4.2	27.5	4.9	12.3	8.9	19.3	4.8	21.4	0.051	0.926	0.561
Simple Attention	Points	99.5	8.4	93.9	24.9	-3.3	18.2	-2.9	25.2	-7.5	29.5	-6.8	62.2	0.383	0.962	0.968
Motor Speed	Points	92.3	33.4	94.6	15.1	-0.3	7.6	-3.2	21.6	1.8	10.6	0.6	11.5	0.802	0.598	0.743

The data are presented as the mean ± standard deviation.
*$P < 0.05$ vs. the P group.

Table 3-2. The effect sizes (Cohen's d) of results of variable in Cognitrax

	Effect size	95%CI
Neurocognitive Index	-0.07	-0.75 to 0.60
Composite Memory	0.78	0.08 to 1.48
Verbal Memory	0.46	-0.23 to 1.14
Visual Memory	0.72	0.02 to 1.42
Psychomotor Speed	0.23	-0.45 to 0.91
Reaction Time	-0.69	-1.39 to 0.00
Complex Attention	0.04	-0.63 to 0.72
Cognitive Flexibility	-0.16	-0.84 to 0.51
Processing Speed	0.35	-0.33 to 1.03
Executive Function	-0.29	-0.96 to 0.39
Social Acuity	-0.51	-1.19 to 0.18
Reasoning	-1.03	-1.75 to -0.31
Working Memory	-0.19	-0.86 to 0.49
Sustained Attention	0.21	-0.47 to 0.88
Simple Attention	-0.01	-0.69 to 0.66
Motor Speed	0.12	-0.56 to 0.79

The data are presented as the effect size (Cohen's d) and confidence interval (CI).

determining "No" in the Cognitrax's validity indicator in the composite memory domain, and 4 for the intake of "foods for specified health uses." Notably, those who were excluded for failing to submit a daily report and failing to return the test food were also excluded from the safety evaluation analysis. The details of subjects excluded from the analysis are presented in Fig. 1. Hence, the per-protocol analysis involved 16 subjects (6 male, 10 female; 54.2 ± 6.8 years) in the Asx group and 18 (8 male, 10 female; 54.6 ± 6.9 years) in the P group. Furthermore, the safety analysis comprised 20 subjects (8 male, 12 female; 54.6 ± 6.6 years) in the Asx group and 20 (8 male, 12 female; 55.4 ± 7.4 years) in the P group.

Table 2-1 and Table 2-2 summarize the demographic characteristics of subjects in the effectiveness of astaxanthin study. No significant differences were observed, and no subject reported any problem while participating in this study.

2. Cognitive function examination

Table 3-1, Table 3-2, and Fig. 2 present the results of the cognitive function examination in the Asx group and P group. Changes in the composite memory domain were 22.3 ± 17.4 points in the Asx group and 9.6 ± 16.2 points in the P group at 12 weeks; the Asx group exhibited a significant increase compared with the P group ($P = 0.035$). However, the Asx group's scores in reasoning were significantly lowered compared with the P group ($P = 0.008$), and the Asx and P groups showed −11.6 ± 16.3 and 2.9 ± 12.7 points at 12 weeks, respectively.

Besides displaying marked differences in the change, the actual measurement values were evaluated. The composite memory scores in the Asx group were 91.9 ± 11.1 points compared with 84.1 ± 17.2 points in the P group after 12 weeks of intake, revealing that the Asx group was prone to a significantly higher score than that in the P group ($P = 0.091$). Furthermore, visual memory domain, which was used for evaluating the composite memory scores, displayed 96.4 ± 13.4 points in the Asx group and 85.3 ± 17.6 points in the P group, resulting in a significantly higher value in the Asx group compared with that in the P group ($P = 0.037$).

3. Subjective symptoms by the Likert scale

Fig. 3 presents the results of the subjective symptom of "during the last week have you had trouble remembering people's name or the names of things?" The change in the

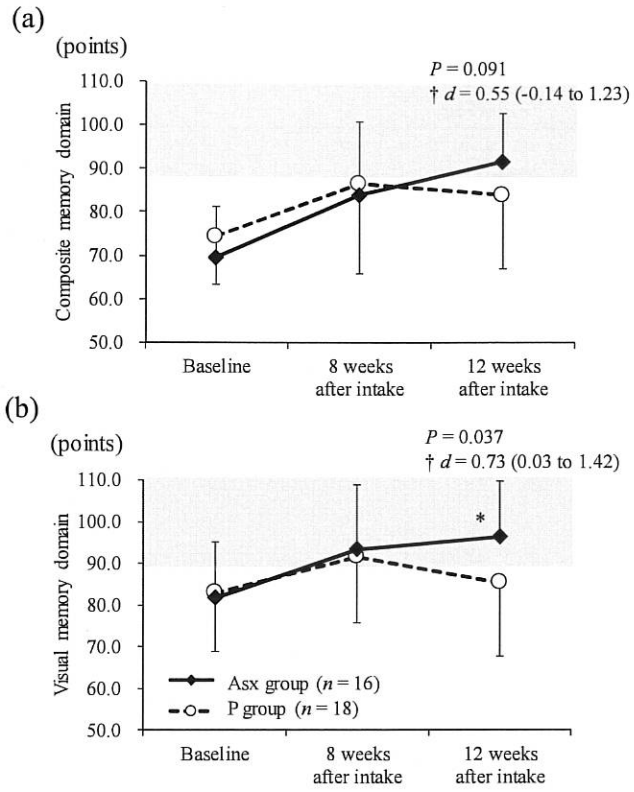

Fig.2. The actual measured values on Cognitrax
The data shows the values baseline to at 12 weeks after intake: (a) the composite memory domain; and (b) the visual memory domain. The data indicates in the astaxanthin group (closed diamond; ◆) and the placebo group (open circle; ○). The gray color indicates in the "Average" range (90–109 points) on Cognitrax.
The data are presented as the mean ± standard error.
*: $P < 0.05$ vs. P group,
†: Effect-size and 95% confidence interval.

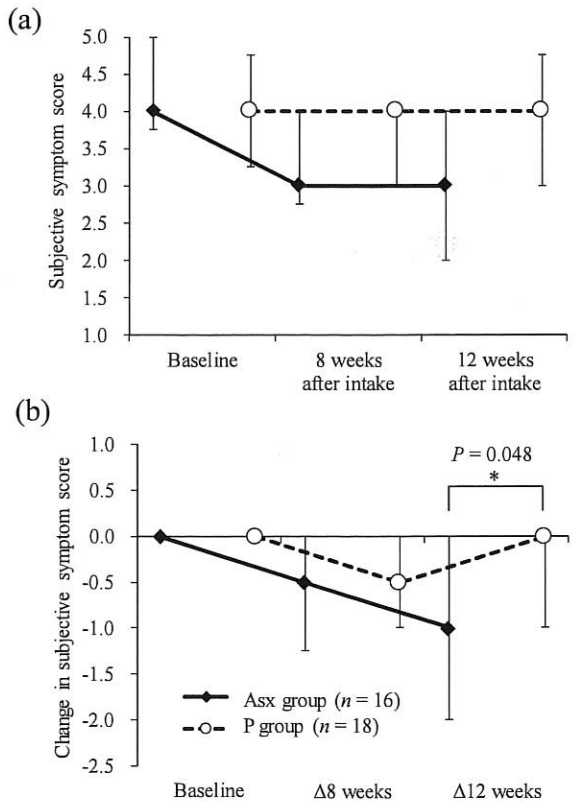

Fig.3. The results of subjective symptom
The data shows (a) measured values and (b) changes of the score of "during the last week have you had trouble remembering people's name or the names of things?" through the intervention period. The data is indicated in the astaxanthin group (closed diamond; ◆) and the placebo group (open circle; ○). Data are presented as median (interquartile range). *: $P < 0.05$ vs. P group.

question displayed a significant decline ($P = 0.048$) after 12 weeks in the Asx group [−1.0 (−2.0 to 0.0)] than that in the P group [0.0 (−1.0 to 0.0)] (Fig. 3b). No significant difference was observed in other question items (data not shown).

4. BDNF, PRL, and pentosidine

No significant differences were observed in both groups (data not shown).

5. Safety evaluation

No adverse events were reported in this study (Tables 4–6).

Discussion

This study investigated the impact of ingesting astaxanthin capsules (9 mg/day of astaxanthin) for 12 weeks on the cognitive function in healthy adult subjects experiencing mild forgetfulness.

The Cognitrax test assesses various cognitive functions, such as memory, attention, processing speed, and others, and evaluates domain scores from multiple combinations of 10 tests based on CNS Vital Signs (Gualtieri *et al.*, 2006). The higher score signifies high-performance function. A study of the cognitive function based on CNS Vital Signs reported that the composite memory scores for peak performance occurs in the 20–29-year age group, after the peak performance decreases with age (Gualtieri *et al.*, 2006). Thus, we considered the enhanced composite memory domain scores as a clinically meaningful improvement in this study. Regarding Cognitrax, the change in the weeks in the Asx group compared with the P group. In addition, the score of Cognitrax was calculated using the average standardized score of 100 points in the same age (SD 15), and the "Average" range was from 90 to 109 points (CNS Vital Signs LLC., n.d.). Based on the actual measured composite memory domain markedly increased after 12

Table 4. The results of the physical examination

Item	Unit	Baseline				8 weeks after intake				12 weeks after intake				*P* value		
		Asx group (n = 20)		P group (n = 20)		Asx group (n = 20)		P group (n = 20)		Asx group (n = 20)		P group (n = 20)				
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Baseline	8 weeks after intake	12 weeks after intake
Height	cm	162.4	8.5	163.3	7.4	—	—	—	—	—	—	—	—	0.713	—	—
Body weight	kg	58.5	12.5	62.1	11.7	58.2	13.2	61.7	11.8	58.2	13.2	61.8	11.9	0.345	0.754	0.768
BMI	kg/m^2	21.9	3.0	23.2	3.9	21.8	3.3	23.1	4.0	21.8	3.3	23.1	4.1	0.244	0.797	0.831
Body fat percentage	%	23.0	5.0	25.4	5.6	23.2	4.8	25.4	5.3	23.3	4.8	25.6	5.1	0.153	0.983	0.724
Systolic blood pressure	mmHg	121.1	15.0	120.1	14.3	122.3	15.1	125.7	18.5	121.1	15.4	125.5	15.5	0.831	0.230	0.152
Diastolic blood pressure	mmHg	76.3	13.8	76.2	10.7	77.1	14.1	79.3	12.1	77.0	14.5	78.2	10.9	0.980	0.353	0.603
Pulse rate	bpm	75.6	11.2	71.9	12.0	75.6	14.3	72.3	11.2	72.1	13.2	70.1	8.5	0.317	0.900	0.621
Body temperature	°C	36.2	0.3	36.1	0.4	36.3	0.4	36.3	0.2	36.4	0.3	36.3	0.3	0.445	0.736	0.299

The data are presented as the mean ± standard deviation.
BMI, body mass index.

Table 5. The results of urinalysis

Item	Assessment point	Asx group (n = 20)		P group (n = 20)		*P* value
		Within the reference range	Outside the reference range	Within the reference range	Outside the reference range	
Protein	Baseline	20	0	20	0	N.A.
	8 weeks after intake	17	3	18	2	1.000
	12 weeks after intake	18	2	19	1	1.000
Glucose	Baseline	20	0	20	0	N.A.
	8 weeks after intake	19	1	20	0	1.000
	12 weeks after intake	20	0	20	0	N.A.
Urobilinogen	Baseline	20	0	20	0	N.A.
	8 weeks after intake	20	0	20	0	N.A.
	12 weeks after intake	20	0	20	0	N.A.
Bilirubin	Baseline	20	0	20	0	N.A.
	8 weeks after intake	20	0	20	0	N.A.
	12 weeks after intake	20	0	20	0	N.A.
pH	Baseline	19	1	20	0	1.000
	8 weeks after intake	20	0	19	1	1.000
	12 weeks after intake	19	1	20	0	1.000
Occult blood	Baseline	18	2	17	3	1.000
	8 weeks after intake	17	3	16	4	1.000
	12 weeks after intake	18	2	18	2	1.000
Ketone bodies	Baseline	20	0	20	0	N.A.
	8 weeks after intake	20	0	19	1	1.000
	12 weeks after intake	20	0	20	0	N.A.

The data are presented as the number of subjects.
BMI, body mass index.

value in the composite memory domain, the baseline value was below "Average" in both groups; however, the value after 12 weeks was in the "Average" range in only the Asx group. An increment from below "Average" to "Average" range suggested a clinically meaningful improvement with the astaxanthin intake.

Although the actual measured value in the visual memory domain, which was used to calculate to the composite memory scores, at the baseline was below "Average," the scores increased to the "Average" range after the astaxanthin intake. Hence, astaxanthin enhanced the visual memory domain, thereby improving the cognitive function. In general, a decrease in episodic memory, which comprises the recollection of personal experiences and spatial memory, leads to forgetfulness (Sandrini *et al.*, 2014). Thus, improvement in general cognitive function may prevent forgetfulness.

The change in the question, "during the last week have you had trouble remembering people's name or the names of things?," as the secondary outcome, revealed a marked decline after 12 weeks in the Asx group. Compared with the actual measured values in both groups, the median values were 4.0 points (slightly agree) in both groups at the baseline. Nevertheless, the median in the Asx group moved to 3.0 points (slightly disagree) at 12 weeks after the intake, resulting in a marked difference between the two groups. Thus, the subjective symptoms related to linguistic whereas the median in the P group remained at 4.0 points,

Table 6. The results of the blood analysis

Item	Reference range	Unit	Baseline				8 weeks after intake				12 weeks after intake				*P* value		
			Asx group (n = 20)		P group (n = 20)		Asx group (n = 20)		P group (n = 20)		Asx group (n = 20)		P group (n = 20)				
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Baseline	8 weeks after intake	12 weeks after intake
Leukocyte count	3300-9000	/μL	5380.0	1143.7	4655.0	1316.5	5210.0	1228.1	5000.0	1259.5	5150.0	1251.7	4720.0	973.7	0.071	0.524	0.847
Erythrocyte count	Male: 430–570 Female: 380–500	$\times 10^4$/μL	453.4	36.0	451.0	35.5	445.3	32.8	449.4	33.6	442.7	36.5	449.3	34.9	0.833	0.221	0.130
Hemoglobin	Male: 13.5–17.5 Female: 11.5–15.0	g/dL	13.7	1.1	14.0	1.2	13.5	1.1	14.0	1.2	13.4	1.2	14.0	1.3	0.573	0.034*	0.070
Hematocrit value	Male: 39.7–52.4 Female: 34.8–45.0	%	43.8	3.3	44.1	3.5	43.4	3.3	44.2	3.4	42.6	3.6	43.5	3.5	0.793	0.239	0.296
Platelet count	14.0-34.0	$\times 10^4$/μL	25.4	5.2	25.0	3.7	25.1	5.1	25.2	4.4	25.9	5.9	24.8	3.7	0.811	0.662	0.330
Mean corpuscular volume (MCV)	85-102	fL	96.7	3.2	97.7	3.9	97.4	3.0	98.5	4.2	96.2	2.8	96.9	4.8	0.380	0.655	0.859
Mean corpuscular hemoglobin (MCH)	28.0-34.0	pg	30.3	1.2	31.0	1.3	30.3	1.1	31.1	1.3	30.3	1.1	31.1	1.5	0.115	0.117	0.399
Mean corpuscular hemoglobin concentration (MCHC)	30.2-35.1	%	31.4	0.7	31.7	0.9	31.1	0.7	31.6	0.7	31.5	0.7	32.1	0.8	0.282	0.059	0.027*
Percentages of neutrophils	40.0-75.0	%	59.2	8.0	53.5	10.3	57.7	7.7	54.7	10.3	57.1	7.0	52.5	9.5	0.058	0.719	0.389
Percentages of lymphocytes	18.0-49.0	%	32.0	7.6	36.3	10.1	34.3	6.7	35.7	9.8	34.5	6.1	37.3	9.6	0.135	0.772	0.784
Percentages of monocytes	2.0-10.0	%	5.3	1.0	5.5	1.3	5.0	1.0	5.9	1.6	5.3	0.9	5.9	1.3	0.460	0.034*	0.107
Percentages of eosinophils	0.0-8.0	%	2.7	1.8	3.8	3.1	2.2	1.5	3.1	2.6	2.4	1.2	3.6	2.7	0.178	0.562	0.264
Percentages of basophils	0.0-2.0	%	0.8	0.7	0.8	0.5	0.8	0.5	0.7	0.4	0.8	0.5	0.8	0.5	0.980	0.454	0.917
AST (GOT)	10-40	U/L	20.3	2.9	21.4	9.4	22.9	7.9	21.3	9.4	22.7	5.0	20.9	7.6	0.619	0.112	0.035*
ALT (GPT)	5-45	U/L	16.6	4.4	17.0	8.9	18.5	9.0	16.1	9.8	20.1	10.0	16.1	9.6	0.858	0.118	0.035*
γ-GT (γ-GTP)	Male: ≤80 Female: ≤30	U/L	32.4	28.3	26.0	14.5	36.6	39.6	25.0	12.7	42.3	45.3	28.2	19.9	0.374	0.272	0.274
ALP	100-325	U/L	190.8	45.0	197.3	38.6	186.9	51.5	184.0	34.0	192.6	46.5	191.7	33.6	0.630	0.171	0.332
LD (LDH)	120-240	U/L	185.8	19.6	181.1	21.9	194.4	21.6	185.7	28.4	194.1	18.2	187.7	33.8	0.479	0.405	0.794
LAP	Male: 45–81 Female: 37–61	U/L	50.0	8.0	49.2	12.8	52.7	10.3	49.2	10.1	54.3	11.3	50.7	13.7	0.814	0.033*	0.116
Total bilirubin	0.2-1.2	mg/dL	0.86	0.21	0.81	0.23	0.92	0.25	0.90	0.21	0.98	0.30	0.92	0.23	0.439	0.868	0.725
Direct bilirubin	0.0-0.2	mg/dL	0.08	0.04	0.06	0.05	0.11	0.03	0.08	0.04	0.09	0.04	0.10	0.04	0.176	0.014*	0.562
Indirect bilirubin	0.2-1.0	mg/dL	0.78	0.21	0.75	0.21	0.81	0.24	0.82	0.19	0.89	0.27	0.82	0.20	0.604	0.548	0.460
Cholinesterase (ChE)	Male: 234–493 Female: 200–452	U/L	323.2	61.3	342.7	56.3	315.4	59.7	328.8	47.0	314.4	62.7	336.1	47.9	0.301	0.669	0.515
Total protein	6.7-8.3	g/dL	7.1	0.3	7.1	0.5	7.0	0.3	6.9	0.4	7.0	0.3	7.0	0.4	0.787	0.247	0.369
Urea nitrogen	8.0-20.0	mg/dL	14.6	4.1	12.3	2.4	14.8	3.8	12.8	3.7	13.9	3.1	12.5	2.4	0.035*	0.814	0.581
Creatinine	Male: 0.61–1.04 Female: 0.47–0.79	mg/dL	0.7	0.2	0.7	0.1	0.71	0.14	0.69	0.13	0.69	0.15	0.69	0.12	0.646	0.901	0.422
Uric acid	Male: 3.8–7.0 Female: 2.5–7.0	mg/dL	4.78	1.19	4.50	1.00	5.2	1.4	4.5	1.1	5.0	1.4	4.6	1.0	0.433	0.035*	0.546
CK	Male: 60–270 Female: 40–150	U/L	108.6	40.6	103.6	44.3	131.7	54.3	111.6	63.9	113.9	44.9	110.4	52.3	0.712	0.301	0.960
Sodium	137-147	mEq/L	142.1	1.8	141.7	1.9	141.7	1.8	140.7	2.3	141.8	1.3	140.6	2.4	0.440	0.176	0.082
Potassium	3.5-5.0	mEq/L	3.9	0.2	4.0	0.3	3.8	0.3	3.8	0.2	3.8	0.3	3.8	0.3	0.453	0.655	0.840
Chloride	98-108	mEq/L	101.7	1.5	101.5	1.9	102.5	1.4	101.4	2.2	101.4	1.6	101.1	2.2	0.714	0.080	0.728
Calcium	8.4-10.4	mg/dL	9.1	0.3	9.0	0.3	9.2	0.3	9.0	0.3	9.1	0.3	8.9	0.3	0.299	0.048*	0.025*
Inorganic phosphorus	2.5-4.5	mg/dL	3.6	0.6	3.4	0.6	3.7	0.5	3.6	0.6	3.8	0.6	3.6	0.4	0.270	0.780	0.364
Serum iron	Male: 50–200 Female: 40–180	μg/dL	98.7	24.3	118.8	33.5	102.2	32.4	113.5	46.4	95.1	26.6	116.3	26.6	0.036*	0.897	0.081
Serum amylase	40-122	U/L	79.1	20.1	87.2	24.3	84.1	27.7	84.6	21.0	76.0	17.0	84.2	19.7	0.258	0.188	0.426
Total cholesterol	120-219	mg/dL	217.7	34.8	230.0	35.6	213.3	38.2	215.4	27.3	212.5	37.0	223.9	33.6	0.274	0.128	0.886
HDL cholesterol	Male: 40–85 Female: 40–95	mg/dL	70.8	17.0	71.0	24.0	68.4	14.3	66.3	17.9	67.9	18.3	70.2	19.7	0.970	0.300	0.420
LDL cholesterol	65-139	mg/dL	129.4	34.4	137.9	26.8	129.0	34.1	129.1	23.0	129.8	36.1	136.3	27.8	0.389	0.089	0.841
Triglyceride	30-149	mg/dL	102.5	72.4	115.5	71.8	94.7	104.4	111.1	73.6	88.1	60.0	103.7	59.8	0.572	0.834	0.527
Glucose	70-109	mg/dL	85.2	9.4	85.3	8.3	88.9	16.0	84.8	5.7	85.8	7.0	87.2	7.0	0.986	0.252	0.492
Hemoglobin A1c	4.6-6.2	%	5.5	0.2	5.4	0.3	5.4	0.2	5.4	0.3	5.4	0.2	5.4	0.4	0.664	0.715	0.220
Glycoalbumin	12.3-16.5	%	13.8	1.1	13.9	1.6	14.0	1.2	14.1	1.9	14.4	1.2	14.4	1.7	0.796	0.729	0.851

The data are represented as the mean ± standard deviation.
*$P < 0.05$ vs. the P group.

remembrance were improving with the astaxanthin ingestion. Furthermore, the composite memory domain scores of Cognitrax were evaluated as the sum of verbal memory and visual memory tests of 10 tests (Gualtieri *et al.*, 2006), and the verbal memory domain reflects the composite memory domain. Subjective cognitive

impairment as self-reported cognitive decrease, which is not detected by objective evaluations, has often been observed in the pre-mild cognitive impairment stage. Also subjective cognitive impairment is predicted to begin more than 20 years prior to the onset of dementia (Reisberg *et al.*, 2008). People with subjective cognitive impairment had begun to exhibit pathological changes in hippocampal gray matter, hippocampal volume, and depression of cerebral metabolism many years prior to the onset of dementia (Reisberg *et al.*, 2008), and cognitive dysfunction may not be unconscious. On the basis of the results, objectives and subjective evaluations were consistent; thus, astaxanthin is considered to affect both objective and subjective cognitive functions. To the best of our knowledge, no clinical studies have investigated the effect of *H. pluvialis*–derived astaxanthin on the subjective evaluation of cognitive function in healthy subjects. Therefore, the results of this study are of critical importance. However, subjective evaluation was set as a secondary outcome, and further verification of subjective symptoms is required.

Reportedly, one of the causes of the cognitive function decline is damage to nerve cells due to oxidative stress in the brain (Nunomura *et al.*, 2006, 2012). The development of AD is attributed to Aβ accumulation, inducing oxidative stress and damaging the surrounding tissues of the hippocampal area (Manczak *et al.*, 2006). Although Aβ is also produced in a healthy brain, it is removed by enzymes such as neprilysin and phagocytic cells. With aging, the removal function declines, and Aβ accumulation increases the risk of developing AD (Iwata *et al.*, 2003; Takata, 2013). Furthermore, Aβ begins to accumulate about 20 years before the AD onset, and nerve cells gradually die, eventually resulting in the AD onset (Jack *et al.*, 2010). Hence, alleviation of oxidative stress in the brain protects nerves cells, preventing the cognitive function decline.

Astaxanthin plays a role against oxidative damages through various mechanisms such as the elimination of singlet oxygen and radicals causing oxidative stress, suppression of lipid peroxidation, and regulation of gene expression associated with oxidative stress (Dose *et al.*, 2016; Galasso *et al.*, 2017). An *in vitro* study reported the neuroprotective effect of astaxanthin against the Aβ toxicity, considering a cause of the antioxidant activity (Chang *et al.*, 2010). Furthermore, some clinical studies have reported improvements in the antioxidant activity (Iwabayashi *et al.*, 2009) and antioxidant state in erythrocytes (Nakagawa *et al.*, 2011) after consuming astaxanthin. Reportedly, astaxanthin could absorb into the blood and cross the blood–brain barrier in rats (Tso *et al.*, 1996) and prevented various brain disorders caused by the reactive oxygen species (Wu *et al.*, 2015; Grimmig *et al.*, 2017; Galasso *et al.*, 2018). Hence, astaxanthin effectively prevents the cognitive function decline.

Regarding the suppression of the cognitive function decline by the neuroprotective activity of astaxanthin, intake of 0.5% of astaxanthin diet generated hippocampal nerves and enhanced abilities of spatial learning and memory in mice (Yook, 2016). In Japanese epidemiological studies, the accumulation of Aβ has been reported to increase with age and rapidly increase from the age of 40 years (Morishima-Kawashima *et al.*, 2000). In this study, the average age of subjects was in the mid-50s and might have been past the beginning of pathological changes resulting in the development of dementia. Hence, astaxanthin revealed improvement in the cognitive function based on increased visual memory domain scores, possibly leading to the prevention of the cognitive function decline by neuroprotective activities.

Despite the improvement in the composite memory domain scores through verbal and visual memory domains, the change in the reasoning domain was markedly decreased on Cognitrax. The Asx group showed a lower score after 12 weeks in the reasoning domain; however, the score was within the "Average" range (CNS Vital Signs LLC., n.d.), suggesting no meaningful changes.

Katagiri *et al.* (Katagiri *et al.*, 2012) did not corroborate with our findings. Katagiri *et al.* (Katagiri *et al.*, 2012) evaluated the 12-week ingestion of *H. pluvialis*–derived astaxanthin in a 6 mg/day (mean age, 51.1 years) group, 12 mg/day (mean age, 51.5 years) group, and a placebo group (mean age, 51.6 years) and demonstrated improvements in the response time, which is a measure of short-term memory and accuracy of delayed recall task; however, they noted no marked differences compared with the placebo group (Katagiri *et al.*, 2012). In this study, although the astaxanthin concentrations (9 mg/day) were lower than those in previous study, composite memory score showed significantly increase after the intake of astaxanthin compared with the placebo group. Moreover, the effect size (Cohen's d) as an impact of *H. pluvialis*–derived astaxanthin on the cognitive function was larger in our study compared with a previous study (Katagiri *et al.*, 2012). In the effect size (Cohen's d) and its confidence interval that was calculated by us from the study by Katagiri *et al.*(Katagiri *et al.*, 2012), the superiority outcomes were observed in the high-dosage group compared with the placebo group at 12

weeks, the largest effect size was 0.44 [CI, −0.07 to 0.95] in Delayed recall (accuracy), and the second effect size was −0.37 [CI, −0.88 to 0.14] in Delayed recall (response time). The effect size (Cohen's d) indicates small at 0.20, medium at 0.50, and large at 0.80 (Cohen, 1992). In the composite memory, the primary outcome of our study, the effect size, and its confidence interval after 12 weeks were 0.55 [CI, −0.14 to 1.23] in measured values and 0.78 [CI, 0.08 to 1.48] in changes values. Although the outcomes were not similar between our study and the study by Katagiri *et al.*(Katagiri *et al.*, 2012), our results showed a larger effect size than in the study by Katagiri *et al.*, when the effect size is considered as an impact of astaxanthin derived from *H. pluvialis* on cognitive function. Therefore, the effect of astaxanthin derived from *H. pluvialis* on cognitive function is also clear from the confidence interval of the effect size, and we succeeded in obtaining more reliable evidence than has been previously achieved.

Despite the improvement in the composite and visual memory domains, no astaxanthin concentrations were measured in the blood; hence, we could not establish whether astaxanthin concentrations increased. An epidemiological study reported that carotenoids, retinol, and tocopherol concentrations in the frontal lobe decrease with age and that these concentrations are critical factors of the AD development (Craft *et al.*, 2004). Thus, by investigating the correlation between the blood concentration of astaxanthin, which is one kind of carotenoid, and the cognitive function, more exciting results might be obtained. Furthermore, further investigation is warranted to enhance the mechanism in the cognitive function by ingesting astaxanthin.

Conclusions

This study established the significant improvement in verbal and composite memory domains of Cognitrax in a placebo-controlled trial of healthy Japanese subjects who were experiencing mild forgetfulness after ingesting astaxanthin (9 mg/day) for 12 weeks. Additionally, the change in the subjective symptom of "during the last week have you had trouble remembering people's name or the names of things?" measured by the Likert scale was displayed significant improvement. This finding of the subjective symptom, which has not been reported in previous studies, is a new knowledge that astaxanthin can improve the subjective symptom of memory impairment. Furthermore, the consumption of astaxanthin capsules was safe under the conditions of this study.

Declaration of interest statement

The sponsor of this study, BGG Japan Co., Ltd., entrusted ORTHOMEDICO Inc., with conducting this study. T. S. and Y.K. are a part of BGG Japan Co., Ltd., and Y. L. is a member of Beijing Gingko-Group Biological Technology Co., Ltd. T. T. (MD), is a part of Medical Corporation Seishinkai, Takara Clinic, and is the Principal Investigator.

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Abbreviations

AD, Alzheimer's disease; Aβ, Amyloid beta; MMSE, Mini-Mental Status Examination; SD, standard deviation; Asx group, astaxanthin group; P group, placebo group; BDNF, brain-derived neurotrophic factor; PRL, propanoyl lysine; ANCOVA, analysis of covariance.

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