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Effects of dietary supplementation with a combination of fish oil, bilberry extract, and lutein on subjective symptoms of asthenopia in humans

Fuminori KAWABATA and Tomoko TSUJI
Human Life Science R&D Center, Nippon Suisan Kaisha, Ltd., Tokyo 100-8686, Japan

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ABSTRACT

The aim of this study was to determine the effects of dietary supplementation with a combination of fish oil, bilberry extract, and lutein on subjective symptoms of asthenopia in humans by a double-blind, randomized, parallel-group, and placebo-controlled trial. In the Active group, eleven subjects ingested a supplement containing omega-3 fatty acid-rich fish oil (docosahexaenoic acid 783 mg/day, eicosapentaenoic acid 162 mg/day), bilberry extract (anthocyanidin 59 mg/day), and lutein (17.5 mg/day) in soft gel capsule form, every day for 4 weeks. In the Placebo group, nine subjects ingested placebo capsules. Before and after supplementation, subjects completed a questionnaire to determine their asthenopia symptoms and were also assessed for mental fatigue symptom by the visual analog scale (VAS) test. Asthenopia symptoms such as "stiff shoulder, low back pain", "frustration", "dry-eye", and "stuffy head" were improved in the Active group. Furthermore, a score of mental fatigue was improved after 4 weeks of supplementation, and no side effects were observed after the 4-week supplementation and a 2-week washout period in the Active group. These results suggest that dietary supplementation with the combination of omega-3 fatty acid-rich fish oil, bilberry extract, and lutein may safely improve subjective symptoms of asthenopia and mental fatigue in humans.

The number of computer users continues to expand rapidly, and the number of patients who complain of asthenopia symptoms has increased. For such patients to live comfortably, relief of asthenopia symptoms is needed.

Some food ingredients have been reported to be effective contributors to eye health. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are contained in fish oil, are frequently mentioned in this context. DHA is the major polyunsaturated fatty acid found in retinal rod outer segments, and some epidemiologic studies suggest the role of DHA in the prevention of age-related macular degeneration and dry eye syndrome (DES) (3, 10). Intake of EPA has been shown to prevent choroidal neovascularization and related inflammation in mice (5). Moreover, anthocyanins, contained in bilberry, and lutein are also known to be effective ingredients for maintaining eye health. Anthocyanins are potent antioxidants (13) and may enhance the regeneration of rhodopsin (9). Lutein selectively accumulates in the retina and is particularly dense in the macular region (1). Lutein also functions as an antioxidant (7, 14). Since the action site of each ingredient appears to be different, we hypothesized that the intake of a supplement with the combination of these ingredients would be broadly effective for patients of asthenopia induced by many factors. In this study, we examined whether daily intake of a nutritional supplement containing these ingredients was useful in improving asthenopia symptoms in humans by a double-blind, randomized, parallel-group, and placebo-controlled trial. Because it has been reported that asthenopia symptoms are involved in mental fatigue

Address correspondence to: Fuminori Kawabata, PhD
Human Life Science R&D Center, Nippon Suisan Kaisha, Ltd., 2-6-2 Otemachi, Chiyoda-ku, Tokyo 100-8686, Japan
Tel: +81-3-3244-6937, Fax: +81-3-3244-7465
E-mail: f-kawabata@nissui.co.jp

(4), we also examined the effects of these ingredients on patients' psychological status. In addition, we examined the safety of the supplement.

MATERIALS AND METHODS

Experimental supplement. For the Active group, we used a commercially available supplement that contains fish oil, bilberry extract, and lutein in the form of a soft gel capsule (Nippon Suisan Kaisha, Ltd., Tokyo). For the Placebo group, we made a placebo supplement that did not contain these ingredients. The placebo supplement contained mainly middle chain triglycerides as edible oil. Since the placebo supplement was dyed by squid ink pigment, the color of which was similar to bilberry extract color, and neither supplement had a noticeable smell, subjects could not discriminate between the two supplements. We found no reports that middle chain triglycerides or squid ink pigment affects the visual sense. The composition of capsules and the daily dose of functional ingredients of the experimental supplements are shown in Table 1.

Experimental design. This study was a double-blind, randomized, parallel-group, and placebo-controlled trial. The supplementation period was 4 weeks, and the washout period was 2 weeks. Measurements were carried out before and after the 4 weeks of supplementation and after the 2 weeks of washout. Subjects took four capsules at breakfast, lunch, and dinner every day (12 capsules/day). We evaluated changes in asthenopia symptoms by a questionnaire sheet and carried out the visual analog scale (VAS) test to estimate the mental fatigue before and after the supplementation period. Subjects answered these test questions at the same clock time determined by themselves on each of the two test days, to avoid the effects of circadian rhythm. Subjects signed an intake check sheet every day after intake of the experimental supplement. We calculated the intake rate of subjects from their intake check sheets. This study was performed according to the Helsinki Declaration and was approved by the Institutional Review Boards of Kaiyuu Clinic (Tokyo). All experiments were performed under a medical doctor's control.

Subjects. Twenty-two healthy Japanese participated in this study. Final data were calculated from 20 subjects (8 males and 12 females) ranging in age from 20 to 34 years. Basal data of subjects are shown in Table 2. Average usage time of a visual display terminal (VDT) for weekdays was calculated from 3 days' data by questionnaire. In the experimental period, taking of supplements other than the experimental supplements was prohibited. Subjects were instructed to maintain their normal food and exercise habits. Informed consent was obtained from all subjects. All eligible subjects were randomly allocated to the Active or Placebo group. The intergroup differences were minimized in terms of gender and baseline asthenopia symptoms.

Inclusion criteria. Healthy subjects complaining of feeling "mildly tired" or "very tired" on the questionnaire item assessing "eye fatigue" and using a VDT for more than 4 h per day were included.

Exclusion criteria. Subjects participating in other clinical studies, being treated for eye disease, taking drugs and/or supplements, with a severe disorder of the eye, liver, kidney, heart, respiratory apparatus, endocrine function, or metabolism function, with abnormal biochemical parameters in the liver or kidney, pregnant or lactating, with food or drug allergy, and those judged unsuitable by a medical doctor were excluded.

Table 1 *Composition of capsules and daily dose of functional ingredients in the Active and Placebo groups*

		Active	Placebo
Fish oil	mg/capsule	165.5	-
Bilberry extract	mg/capsule	20	-
Lutein rich vegitable oil	mg/capsule	7.5	-
Middle chain triglycerides	mg/capsule	-	193
Squid ink pigment	mg/capsule	-	7
Docosahexaenoic acid	mg/d	783	-
Eicosapentaenoic acid	mg/d	162	-
Anthocyanidin	mg/d	59	-
Lutein	mg/d	17.5	-

Measurement of asthenopia symptoms. We slightly modified Nakamura's asthenopia questionnaire (in Japanese) (12) and determined the changes of asthenopia symptoms before and after supplementation by 11 items related to asthenopia. For example, we evaluated the "eye fatigue" symptom by a 5-grade evaluation (very tired = 5, slightly tired = 4, normal = 3, not tired = 2, and not at all tired = 1) (in Japanese). For each item, the low score indicated good condition and the high score indicated poor condition.

Measurement of mental fatigue. We examined the changes of mental fatigue before and after the supplementation period by a visual analog scale (VAS) test. We used the form written as "I have no fatigue" (0) and "I feel extremely fatigue" (100) on the extreme ends of a 100-mm line.

Physical characteristic measurements, biochemical measurements, and urinary analysis. Physical characteristic measurements, biochemical measurements, and urinary analysis were done three times before and after the supplementation period and 2 weeks after the washout period. Subjects were prohibited from drinking alcohol beginning 2 days before each measurement day. Subjects were also prohibited from taking meals in the 10 h preceding blood and urine sampling. Only drinking water was permitted on each experimental day. Each blood and urinary marker was measured by Mitsubishi Chemical Medience (Tokyo).

Statistical analysis. Data are expressed as the means ± the standard error (SE). The effects of time, treatment, and time × treatment were evaluated by two-way repeated-measured ANOVA. To compare the groups at certain times, we used an unpaired *t*-test. To compare the values with the baseline values in the same group, we used a paired *t*-test or the Wilcoxon signed-ranked test. Statistics were calculated using the StatView software package (Windows Version J 5.0, Abacus Concepts, Berkeley, CA). The significance level was set at $P < 0.05$.

RESULTS

Analyzed subject number and intake rate of supplements

Because one subject found it difficult to continue taking the supplement and we could not remain in contact with another subject, two subjects were dropouts from the experiment and statistical analysis was done using 20 subjects. The average intake rates for the experimental period in the subjects except dropout subjects were 99.2 ± 0.6% and 99.6 ± 0.3% as calculated by the intake check sheet in the Active group and Placebo group, respectively. We judged that the intake rate was adequate to determine the safety and effects of the supplementation on asthenopia, and the intake regimen reflects the ease of ingesting the supplement. Although subjects' ages were statistically different between the two groups (Table 2), we felt there were few effects of age in this study because the differences were very small.

Physical characteristics

Body weight, body mass index, systolic blood pressure, and diastolic blood pressure did not change in either of the two groups during the experiment (Table 3). Although heart rate at 4 weeks after supplementation in the Active group was significantly increased compared to the baseline value, it was within the normal range and was not significantly different between the groups.

Table 2 *Basal data of subjects before supplementation*[1]

		Active (n = 11)	Placebo (n = 9)
Sex			
Female	n	7	5
Male	n	4	4
Age	year	23.3 ± 1.0*	27.1 ± 1.4
Height	cm	163.3 ± 2.7	162.6 ± 3.2
Body weight	kg	57.1 ± 2.6	55.0 ± 2.7
BMI[2]	kg/m^2	21.3 ± 0.6	20.7 ± 0.4
VDT[3] working time	h/day	5.8 ± 0.5	7.5 ± 0.8

[1]Values are the means ± SE. *$P < 0.05$ *vs.* Placebo group (unpaired *t*-test).
[2]BMI, body mass index; [3]VDT, visual display terminal

Table 3 *Changes in physical characteristics, blood biochemical makers, hematological parameters, urinary markers, and serum fatty acids before and after supplementation*[1]

Parameters[2]		Active (n = 11)			Placebo (n = 9)		
		Week 0	Week 4	Week 6	Week 0	Week 4	Week 6
Physical characteristics							
Body weight	kg	57.1 ± 2.6	57.4 ± 2.7	56.6 ± 2.7	55.0 ± 2.7	55.7 ± 2.9	55.3 ± 2.9
BMI	kg/m^2	21.3 ± 0.6	21.4 ± 0.6	21.1 ± 0.6	20.7 ± 0.4	21.0 ± 0.5	20.8 ± 0.4
SBP	mmHg	109 ± 4	111 ± 3	106 ± 2	111 ± 5	108 ± 4	105 ± 5
DBP	mmHg	58.9 ± 1.8	59.7 ± 1.6	57.2 ± 1.6	61.9 ± 2.6	60.0 ± 2.8	57.1 ± 3.3
HR	beats/min	66.2 ± 2.9	72.5 ± 3.0*	70.2 ± 2.8	70.1 ± 2.5	72.6 ± 3.6	76.2 ± 3.6
Blood biochemical markers							
TP	g/dL	7.60 ± 0.15	7.49 ± 0.09	7.51 ± 0.09	7.36 ± 0.19	7.43 ± 0.20	7.40 ± 0.19
Albumin	g/dL	4.82 ± 0.07	4.68 ± 0.06	4.69 ± 0.04	4.66 ± 0.13	4.62 ± 0.15	4.60 ± 0.12
A/G ratio	-	1.76 ± 0.08	1.69 ± 0.05	1.67 ± 0.07*	1.76 ± 0.08	1.66 ± 0.07	1.64 ± 0.04
AST	IU/L	17.7 ± 1.6	15.8 ± 0.7	16.8 ± 0.7	17.0 ± 1.3	17.1 ± 1.9	15.0 ± 1.4
ALT	IU/L	16.9 ± 4.1	13.4 ± 2.0	14.5 ± 2.2	13.1 ± 1.2	13.4 ± 1.5	11.9 ± 0.9
LDH	IU/L	165 ± 6	162 ± 8	157 ± 8	162 ± 8	159 ± 9	154 ± 5
T-Bil	mg/dL	0.81 ± 0.09	0.81 ± 0.06	0.81 ± 0.09	0.86 ± 0.07	0.88 ± 0.11	0.82 ± 0.09
ALP	IU/L	198 ± 18	209 ± 27	202 ± 24	169 ± 11	186 ± 20	184 ± 17
γ-GTP	IU/L	24.6 ± 6.4	21.3 ± 4.6	21.5 ± 5.4	19.2 ± 3.8	17.1 ± 2.3	16.6 ± 2.1
CPK	IU/L	102 ± 11	118 ± 24	114 ± 19	141 ± 32	139 ± 33	97 ± 10
BUN	mg/dL	10.6 ± 0.5	12.8 ± 1.1	10.6 ± 0.5	9.9 ± 0.8	12.4 ± 1.5	11.0 ± 1.1
CRE	mg/dL	0.60 ± 0.04	0.63 ± 0.04	0.65 ± 0.04**	0.68 ± 0.05	0.72 ± 0.05	0.74 ± 0.05
UA	mg/dL	5.10 ± 0.40	5.41 ± 0.42	5.45 ± 0.35	4.44 ± 0.45	4.70 ± 0.50	4.72 ± 0.34
Na	mEq/L	141 ± 0	141 ± 1	141 ± 0	141 ± 0	141 ± 1	141 ± 0
Cl	mEq/L	103 ± 0	104 ± 1	105 ± 1*	103 ± 1	104 ± 1	104 ± 1
K	mEq/L	4.12 ± 0.08	4.09 ± 0.07	4.15 ± 0.06	4.14 ± 0.12	3.98 ± 0.19	4.08 ± 0.11
T-CHO	mg/dL	185 ± 9	180 ± 9	172 ± 9**	191 ± 10	194 ± 6	186 ± 9
LDL-CHO	mg/dL	108 ± 8	103 ± 6	99 ± 8	99 ± 11	99 ± 7	97 ± 8
HDL-CHO	mg/dL	63.4 ± 3.1††	64.4 ± 3.6†	61.9 ± 3.1†	76.2 ± 3.5	79.6 ± 4.3	76.9 ± 4.8
TG	mg/dL	66.9 ± 7.2	70.0 ± 9.1	60.6 ± 5.9	57.8 ± 9.0	61.7 ± 7.7	67.2 ± 10.2
NEFA	mEq/L	0.60 ± 0.06	0.50 ± 0.05	0.63 ± 0.08	0.76 ± 0.06	0.56 ± 0.06*	0.60 ± 0.09
Glu	mg/dL	78.5 ± 1.7	80.0 ± 1.9	78.5 ± 2.2	79.3 ± 2.2	80.1 ± 1.7	82.0 ± 1.3
Hematological parameters							
WBC	$10^3/mm^3$	6.52 ± 0.38†	7.42 ± 0.75	5.94 ± 0.52	5.30 ± 0.31	5.78 ± 0.56	5.48 ± 0.55
RBC	$10^6/mm^3$	4.59 ± 0.13	4.56 ± 0.13	4.59 ± 0.14	4.58 ± 0.12	4.63 ± 0.14	4.63 ± 0.13
Hb	g/dL	13.9 ± 0.4	13.7 ± 0.3	13.9 ± 0.4	13.9 ± 0.5	14.0 ± 0.5	14.0 ± 0.5
Ht	%	43.2 ± 1.0	43.8 ± 1.0	44.7 ± 1.0**	43.1 ± 1.4	44.3 ± 1.4	45.4 ± 1.4
Platelet	$10^4/mm^3$	25.3 ± 1.6	24.8 ± 1.7	26.2 ± 1.9	24.9 ± 2.3	24.8 ± 1.9	25.6 ± 2.6
PT	s	11.7 ± 0.2	11.9 ± 0.1	12.0 ± 0.2	11.6 ± 0.2	11.7 ± 0.2	11.7 ± 0.2
APTT	s	32.0 ± 1.0	35.8 ± 1.2**,†	35.8 ± 1.0**	30.3 ± 0.8	32.5 ± 0.7**	33.1 ± 1.2**
Urinalysis							
pH	-	6.82 ± 0.32	6.00 ± 0.20*	5.73 ± 0.15**	6.56 ± 0.24	6.33 ± 0.27	6.28 ± 0.33
Specific gravity	-	1.0212 ± 0.0018	1.0256 ± 0.0024	1.0242 ± 0.0019	1.0168 ± 0.0032	1.0211 ± 0.0033	1.0207 ± 0.0028
Serum fatty acids							
DGLA	μg/mL	28.6 ± 2.9	21.9 ± 2.1*,††	-	30.0 ± 3.4	30.4 ± 2.0	-
AA	μg/mL	149 ± 10	139 ± 11	-	160 ± 15	156 ± 10	-
EPA	μg/mL	26.4 ± 5.6	27.5 ± 2.9	-	32.2 ± 8.8	27.6 ± 4.5	-
DHA	μg/mL	76.7 ± 8.8	81.6 ± 4.2	-	81.7 ± 8.5	74.0 ± 7.0	-
DHA/AA	-	0.52 ± 0.05	0.61 ± 0.05†	-	0.51 ± 0.04	0.47 ± 0.05	-

[1]Values are the means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* week 0 (paired *t*-test), $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ *vs.* Placebo group (unpaired *t*-test).
[2]BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; TP, total protein; A/G ratio, albumin globulin ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; T-Bil, total bilirubin; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transpeptidase; CPK, creatine phosphokinase; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid; T-CHO, total cholesterol; LDL-CHO, low-density lipoprotein cholesterol; HDL-CHO, high-density lipoprotein cholesterol; TG, triglycerides; NEFA, non-esterified fatty acids; Glu, glucose; WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; Ht, hematocrit; PT, prothrombin time; APTT, activated partial thromboplastin time; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

Blood biochemical markers, hematological parameters, and urinalysis

Although the albumin/globulin ratio, creatinine, Cl, total cholesterol, hematocrit, and urine pH were significantly changed from the baseline values in the Active group, these changes remained within the normal range (Table 3). High-density lipoprotein cholesterol in the Active group at 4 weeks after supplementation was significantly lower than that in the Placebo group. However, the difference was thought to be derived from the baseline difference between groups. Activated partial thromboplastin time (APTT) in the Active group at 4 weeks after supplementation was significantly higher than that in the Placebo group. However, the medical doctor monitoring the patients considered that the change was not of clinical significance, since there was no change in prothrombin time or platelet counts, and since no bleeding tendency was observed.

Serum fatty acids

The serum DHA/arachidonic acid (AA) ratio after supplementation in the Active group was significantly higher than that in the Placebo group (Table 3). The serum dihomo-γ-linolenic acid (DGLA) concentration after supplementation in the Active group was significantly lower than that in the Placebo group. These results suggest that subjects correctly ingested the supplement and DHA was incorporated into circulating blood.

Asthenopia symptoms

Symptoms of "stiff shoulder, low back pain" and "frustration", which are considered to be relevant indicators of asthenopia, were significantly improved in the Active group compared to the Placebo group (Table 4). Although the symptoms of "dry-eye" and "stuffy head" were significantly improved in the Active group compared to the baseline values, there were no changes of these parameters in the Placebo group. The symptoms of "eye fatigue", "bleary eyes", "eye redness", and "eye flicker" were significantly improved in both groups compared to the baseline values. However, there were no significant differences in the changes in these values between the two groups, except for the symptoms of "stiff

Table 4 *Changes of asthenopia symptoms before and after supplementation*[1]

Symptoms	Samples	Week 0	Week 4
Eye-fatigue	Active	4.18 ± 0.19	3.27 ± 0.32*
	Placebo	4.44 ± 0.19	3.67 ± 0.25**
Eye-pain	Active	3.09 ± 0.39	2.45 ± 0.48
	Placebo	3.00 ± 0.43	2.78 ± 0.34
Bleary eyes	Active	3.82 ± 0.28	3.00 ± 0.32*
	Placebo	4.00 ± 0.00	3.22 ± 0.29*
Dry-eye	Active	3.82 ± 0.31	3.09 ± 0.41*
	Placebo	4.00 ± 0.43	3.67 ± 0.47
Eye-redness	Active	3.18 ± 0.28	2.36 ± 0.38*
	Placebo	3.67 ± 0.43	2.78 ± 0.42*
Eye-flicker	Active	3.00 ± 0.27	2.10 ± 0.40*
	Placebo	2.78 ± 0.39	2.22 ± 0.39*
Double vision	Active	2.64 ± 0.38	2.45 ± 0.46
	Placebo	2.50 ± 0.53	2.00 ± 0.45
Stiff shoulder, low back pain	Active †	4.45 ± 0.30	2.91 ± 0.48**
	Placebo †	4.33 ± 0.47	3.78 ± 0.42*
Frustration	Active †	3.18 ± 0.37	2.27 ± 0.35**
	Placebo †	3.33 ± 0.25	3.33 ± 0.18
Stuffy head	Active	3.64 ± 0.35	2.64 ± 0.47*
	Placebo	3.33 ± 0.31	2.89 ± 0.21
Headache	Active	3.09 ± 0.36	2.36 ± 0.45
	Placebo	3.33 ± 0.31	3.00 ± 0.35
Deepness of sleep	Active	2.82 ± 0.31	2.64 ± 0.32
	Placebo	3.56 ± 0.26	3.11 ± 0.28

[1]Values are the means ± SE. Active group (n = 11), Placebo group (n = 9). *$P < 0.05$, **$P < 0.01$ *vs.* week 0 (Wilcoxon signed-ranked test). †There was a significant difference between groups by two-way repeated-measured ANOVA ($P < 0.05$).

shoulder, low back pain" and "frustration".

Mental fatigue

The VAS score of mental fatigue after the supplementation in the Active group was significantly improved from the baseline value (Fig. 1), while the score in the Placebo group was not changed. The score after supplementation in the Active group tended to be lower than that in the Placebo group ($P = 0.078$ by unpaired *t*-test).

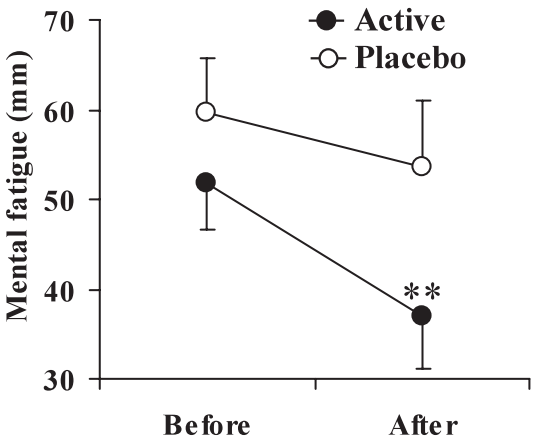

Fig. 1 Changes in the mental fatigue score before and after supplementation with the mixture of fish oil, bilberry extract, and lutein capsules (n = 11) or placebo capsules (n = 9) for 4 weeks. Each value is the mean ± SE. $^{**}P < 0.01$ *vs.* baseline value by paired *t*-test.

DISCUSSION

The results of this study suggest that asthenopia symptoms such as "stiff shoulder, low back pain" and "frustration" were improved safely by repeated supplementation with a mixture of fish oil, bilberry extract, and lutein. The subjective symptoms of "dry-eye" and "stuffy head" were also thought to be improved by the supplement. These results suggest that this supplementation may be useful for improving some of the subjective symptoms related to asthenopia. Since subjective symptoms such as "eye fatigue", "bleary eyes", "eye redness", and "eye flicker" were improved in both the Active and Placebo groups, we could not evaluate the efficacy of the supplement on these symptoms.

Since Mori *et al.* reported that DHA supplementation enhances blood flow of forearm microcirculation (11), the "stiff shoulder, low back pain" symptom may have been improved by DHA-induced blood flow improvement in the present study. Furthermore, it has been reported that DHA/EPA administration improves mood states such as vigor, anger, anxiety, fatigue, depression, and confusion (6). Thus, the improvements of the "frustration" symptom and the mental fatigue measured by VAS test may have been induced by DHA/EPA. Since it has not been reported whether DHA/EPA improves "stuffy head" symptom, we didn't judge whether the improvement of the "stuffy head" symptom observed in this study was induced by DHA/EPA. Kajimoto *et al.* reported that improvement degrees of asthenopia by anthocyanidin were correlated with improvement degrees of mental fatigue (4). The supplementation used in the present study may have improved asthenopia symptoms by relieving mental fatigue. Further studies are needed to reveal the correlation of asthenopia with mental fatigue and mood states.

The intake of 62.5 mg/day of anthocyanidin for 4 weeks was reported to improve asthenopia symptoms such as "eye fatigue", "bleary eyes", "eye-flicker", "stiff shoulder", "frustration", and "stuffy head" and mental fatigue (4). Because the dose of anthocyanidin in the present study was 59 mg/day, the improvement of asthenopia symptoms observed in this study may have been partly induced by anthocyanidin. However, the "dry-eye" symptom was not affected by anthocyanidin in the report (4). Thus, it is possible that the ingredients other than anthocyanidin affected the "dry-eye" symptom. Because Miljanović *et al.* reported that higher dietary intake of omega-3 fatty acids is associated with a decreased incidence of DES in women (10), the effect of the mixture supplementation in this study on the "dry-eye" symptom may have depended on the DHA/EPA contained in the supplement. It is necessary to determine whether supplementation of DHA/EPA improves DES in humans.

Macular pigmentation density was increased by lutein supplementation (12 mg/day) for 4 months in humans (2). Since the supplementation of lutein in this study was 17.5 mg/day for one month, the improvement of asthenopia symptoms by the mixture supplementation in this study may have been partly induced by lutein-induced enhancement of macular pigmentation. Although it is likely that lutein supplementation improves visual acuity and contrast sensitivity *via* the augmentation of macular pigment density (8), it is unknown whether the possible improvements of visual acuity and contrast sensitivity by lutein are involved in the improvements of asthenopia symptoms observed in the present study.

In this study, we newly made the mixture supplement containing three ingredients. Since these action mechanisms for ocular tissue appear to be different,

each ingredient may have been able to complement limitations of other ingredients. Indeed, the mixture supplement improved asthenopia symptoms broadly.

In summary, our study suggests that dietary supplementation with a combination of omega-3 fatty acid-rich fish oil, bilberry extract, and lutein may safely improve subjective symptoms of asthenopia derived from a variety of different factors and reduce mental fatigue in humans.

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