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Effect of Multiple Dietary Supplement Containing Lutein, Astaxanthin, Cyanidin-3-glucoside, and DHA on Accommodative Ability

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Abstract:

Objective: The study aimed to verify that ingestion of multiple dietary supplement containing lutein, astaxanthin, cyanidin-3-glucoside and docosahexaenoic acid (DHA) would improve accommodative ability of aged and older subjects who were aware of eye strain on a daily basis.

Methods: A randomized double-blind placebo-controlled parallel group comparison study was conducted for 48 participants aged 45 to 64 years who complained of eye strain. The subjects took multiple dietary supplement containing 10 mg of lutein, 20 mg of bilberry extract and 26.5 mg of black soybean hull extract (a total of 2.3 mg of cyanidin-3-glucoside in both extracts), 4 mg of astaxanthin, and 50 mg of DHA (test supplement) or placebo for four consecutive weeks. Near-point accommodation (NPA) and subjective symptoms were evaluated both before and after four weeks' intake.

Results: The variation of the NPA of both eyes from baseline to 4 weeks' post-intake in the test supplement group was significantly higher than in the placebo group (1.321±0.394 diopter (D) in the test supplement group and 0.108±0.336 D in the placebo group, $p=0.023$). The multiple dietary supplement group showed improvement in the NPA. Regarding subjective symptoms, significant improvement of "stiff shoulders or neck" and "blurred vision" was also found in the test supplement group compared to the placebo group ($p<0.05$). There were no safety concerns in this study.

Conclusion: This study shows that multiple dietary supplement containing lutein, astaxanthin, cyanidin-3-glucoside, and DHA has effect to improve accommodative ability and subjective symptoms related to eye fatigue.

Keywords: Accommodative ability, astaxanthin, bilberry, black soybean, cyanidin-3-glucoside, docosahexaenoic acid, lutein, multiple dietary supplement.

1. INTRODUCTION

Eye diseases associated with aging include presbyopia, cataract, glaucoma, and age-related macular degeneration. Among them, presbyopia is a disease that affects everyone as the age. It is the loss of accommodative ability and makes it difficult to focus on near objects. This reduced accommodative ability is supposedly caused by the loss of lens elasticity, and the loss of ciliary muscles that control lens thickness. People may gradually start realizing it around the age of 45 [1]. Besides, in today's society, with visual display terminal (VDT) operation requiring to look at the display of a computer or the like for long hours, as well as the advancement and spread of compact terminals such as mobile phones and smartphones, much strain is put on accommodative ability of the eye in everyday life. Also, slow or difficult accommodative response may cause asthenopia and

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its related symptoms such as eye strain, stiff shoulders and headache. The development of asthenopia reduces not only visual function but also its related quality of life (QOL).

Therefore, a decrease in asthenopia attributable to reduced accommodative ability helps enhance QOL for people who have such symptoms. However, medical treatment of reduced accommodative ability is limited to some eye-drops, and medical treatment to prevent it, has not been established either. In this context, some studies recently show the effects of food or food ingredients and additives (lutein, astaxanthin, bilberry extract and black soybean hull extract or anthocyanin found in the extracts, and omega-3 fatty acids) to improve asthenopia and eye function [2-14].

Astaxanthin is a red orange carotenoid found in salmon, shrimp, crab and the like, and is known for its high antioxidant potential [12]. Several studies report the effect of astaxanthin to improve accommodative ability as efficacy in the eye [9-11, 15]. It is supposed that the improvement effect on accommodative ability of astaxanthin should be associated with its action to increase blood flow in the retinal capillaries and its inhibition of NF-κB signaling pathway [12].

Other food or food ingredients to improve accommodative ability are bilberry extract [6, 13] and black soybean hull extract [16]. Both are rich in anthocyanin, whose antioxidant power may improve accommodative ability. It is also reported that bilberry extract promotes the resynthesis of rhodopsin other than the improvement effect on accommodative ability [17]. As its functional ingredient, cyanidin-3-glucoside is suggested [18], and it is also highly found in black soybean hull extract.

Multiple dietary supplements containing several functional ingredients as mentioned above in order to improve asthenopia are currently on the market. Also, effects of multiple dietary supplements containing several functional ingredients on subjective symptoms of asthenopia have been verified [13, 19, 20]. On the other hand, there are few dietary supplements and foods designed focusing on the effect on accommodative ability. And their effect on accommodative ability has not been verified either.

It is considered that a target function can be enhanced synergistically and additively by the combination of functional ingredients in multiple dietary supplements. Therefore, the authors decided to prepare dietary supplement to improve accommodative ability by using a lower dose of astaxanthin, which is shown to improve it. Then, the authors prepared multiple dietary supplement of five ingredients containing astaxanthin with other ingredients for synergistic and additive effects; lutein with antioxidant properties, bilberry extract and black soybean hull extract containing cyanidin-3-glucoside, and DHA [21], which is a component of the ciliary body.

Lutein supplementation provides the reduction of the risk of progression to advanced age-related macular degeneration (AMD) from the results of age-related eye disease study part 2 (AREDS2) in recent years [22, 23]. Reactive oxygens produced in crystallin lens denature crystallin proteins that induce cataract and asthenopia [24]. Antioxidative activity of lutein in lens is reported [25] and lutein reduces the risk of cataract [26]. DHA is a component of ciliary body [21] and improves dry eye disease [3]. The reduction of the risk of dry eye disease by a higher dietary intake of omega-3 fatty acid such as DHA, is also reported [27].

The authors had already conducted an open study on the intake of this multiple dietary supplement for 28 days, for men and women aged 45 to 65 years with eye strain, and confirmed improved pupil constriction rate (Matsuoka *et al.*, unpublished data).

In this study, to verify the effect of this multiple dietary supplement to improve accommodative ability, a randomized placebo-controlled double-blind study was conducted for 45 to 64 year-old men and women who felt eye strain on a daily basis.

2. MATERIALS AND METHODS

2.1. Ethics

This study was approved by the clinical research ethical committee at FANCL Corporation and also by the ethical review board at Huma R&D Ltd. in terms of ethical, scientific and medical appropriateness, and then conducted. It was performed in compliance with ethical principles

based on the Declaration of Helsinki, "Ethical Guidelines for Epidemiological Studies (Notification of Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare)". Under the instruction of the principal investigator (physician), the investigator explained the contents of the study, using the informed consent form to the subjects before conducting the study. The written consent was obtained from each subject who fully understood the contents of the study and agreed to participate in the study on a voluntary basis. This study was carried out at Kono Medical Clinic.

2.2. Study Subjects

Subjects who were enrolled in this study were paid volunteers recruited by HUMA CORP, and selected based on the following inclusion criteria; (i) men and women aged 45 years or more and less than 65 years, (ii) who were aware of eye strain on a daily basis, (iii) had no eye disease other than refractive error, and (iv) were able to keep the eyes open for 30 seconds or more without blinking. Moreover, people who fall under any of the following exclusion criteria, were eliminated; (i) presence or history of severe liver disease, digestive disorder or kidney disease, (ii) presence of soybean allergy or cuttlefish allergy, (iii) regular intake of health food or drug effective for asthenopia, (iv) otherwise judged by the principal investigator as unsuitable to participate in this study.

Before the participation, the contents of the study were fully explained to all the subjects, and their written consent was obtained from each subject.

2.3. Test Supplement

The composition of functional ingredients contained in the test supplement is shown in Table **1**. The test supplement was a soft gel capsule that contained astaxanthin, lutein, and bilberry extract and black soybean hull extract (as cyanidin-3-glucoside in both extracts), and DHA as functional ingredients. Placebo was a soft gel capsule that did not contain these ingredients. Capsules for both supplements looked identical. They were prepared according to the dose of two capsules per day. The daily dose of the test supplement was 10 mg of lutein, 4 mg of astaxanthin, 20 mg of bilberry extract, 26.5 mg of black soybean hull extract, and 50 mg of DHA. And that of cyanidin-3-glucoside derived from bilberry extract and black soybean hull extract was 2.3 mg.

Table 1. Functional ingredients of the test supplement.

Ingredients	mg/capsule	mg/day
Lutein	5.00	10.00
Astaxanthin	2.00	4.00
Bilberry extract	10.00	20.00
Black soybean hull extract	13.25	26.50
DHA	25.00	50.00

Each subject received four weeks' worth of test supplement or placebo packaged in an aluminum pouch, and took 2 capsules with water after supper every day during the test period.

2.4. Study Design

Study design was a randomized placebo-controlled double-blind comparison study.

Fifty subjects were randomly assigned to one of the two groups; 25 subjects to the test supplement group and 25 subjects to the placebo group after confirming there was no significant difference between the two groups based on gender, age, accommodative ability, and visual acuity checked at the pre-intake test (baseline).

Measurements and examination were conducted at the pre-intake test combined with a screening test, and after 4 weeks of intake, by questioning subjects, examining visual acuity, measuring near point accommodation (NPA) using an accommodometer, and checking subjective symptoms (a questionnaire comprising 15 items related to eye fatigue). At the baseline, the NPA of a dominant eye, a non-dominant eye and both eyes were respectively measured after the dominant eye and non-dominant eye of each subject were confirmed.

During the study period, the subjects followed our instructions: (i) do not change their eating habits and lifestyle, (ii) do not take any drug or quasi-drug for asthenopia, (iii) do not take any supplement other than the test supplement, (iv) do not change the type and frequency of eye drop use, and (v) do not change frequency of contact lens use. The intake status of the test supplement and the change in physical condition of the subjects during the intake period were observed every day,

using HUMA R&D's WEB diary system "Chronos.wiz".

2.5. Endpoints

2.5.1. Primary Endpoint

The primary endpoint was near point accommodation (NPA). Regarding the near point accommodation measurement, a near-point distance of both eyes, a dominant eye, and a non-dominant eye were respectively measured by KOWA NP accommodometer (Kowa Co., Ltd., Japan) ten times. And NPA (diopter; D) was calculated by dividing 1,000 by the mean near-point distance N (mm) of each. Measured values and differences (amount of change) were evaluated.

2.5.2. Secondary Endpoint

As the secondary endpoint, symptom scores and the amount of changes were evaluated, using the subjective symptoms questionnaire (questionnaire comprising 15 items of subjective symptoms related to eye fatigue), based on the items used in "Study on the Evaluation Method of Asthenopia" by Nakamura *et al.* [28]. Each subject graded on a 5-point scale (scores from 1 = no symptoms, to 5 = most severe symptoms) and entered by him/herself. The scores of each item were calculated.

2.6. Safety Evaluation

Based on the results of questioning at study visits, and the WEB diary recorded by the subjects during the study period, all adverse events that occurred during the 4-week intake period of the test supplement were analyzed and assessed for association with the test supplement. Regardless of the association with the test supplement or placebo, all the adverse events were also evaluated by type, severity, onset time and duration of each event, presence and details of medical treatment.

2.7. Analysis Set and Statistical Analysis

The analysis set was per protocol set (PPS). Before unblinding the treatment assignment, the cases were reviewed to determine the analysis set by the elimination of the following cases: (i) the test food intake rate was below 80%, (ii) a drug or quasi-drug for asthenopia was used, (iii) a case was found to have fallen under any of the exclusion criteria after the start of intake, and (iv) significant deviation from the protocol was observed.

The statistical analysis of near point accommodation was performed using a paired t-test to compare the values measured before and after 4 weeks' intake of the test supplement, and using an unpaired t-test to compare the measured values and differences (amount of change) after 4 weeks' intake, between the test supplement group and the placebo group. Regarding the subjective symptoms questionnaire, Wilcoxon rank sum test was used to compare scores before and after intake, and Wilcoxon signed rank test to compare the differences measured after 4 weeks' intake of the test supplement between the test supplement group and the placebo group. For each comparison, the following null hypothesis was tested: There was no change between before and after 4 weeks' intake, or between the test supplement group and the placebo group. When a significant difference was found, the null hypothesis was considered to be rejected.

IBM SPSS® Statistics ver.19 was used for the statistical analysis. With a level of significance of 5%, a significance probability of less than 5% in a two-tailed test ($p<0.05$) was considered to be statistically significant.

3. RESULTS

3.1. Subject Background

The flow diagram of participants in this study is shown in Figure **1**. A total of 102 volunteers underwent screening (pre-intake) tests, and 50 of them were chosen to participate in the study. Then, each of 25 subjects was assigned to either of the test supplement or the placebo groups. All the subjects completed this study to final visits. After reviewing the subjects, two of them were eliminated from the analysis: One subject of the test supplement group fall under the exclusion criteria (iv) because of a remarkably short amount of sleep the day before the visit after 4 weeks' intake, which was considered difficult to assess appropriately. One subject of the placebo group fall under the exclusion criteria (iii) because of being unable to keep the eyes open for 30 seconds without blinking at the visit after 4 weeks' intake. As a result, the PPS consisted of 48 subjects (24 in the test

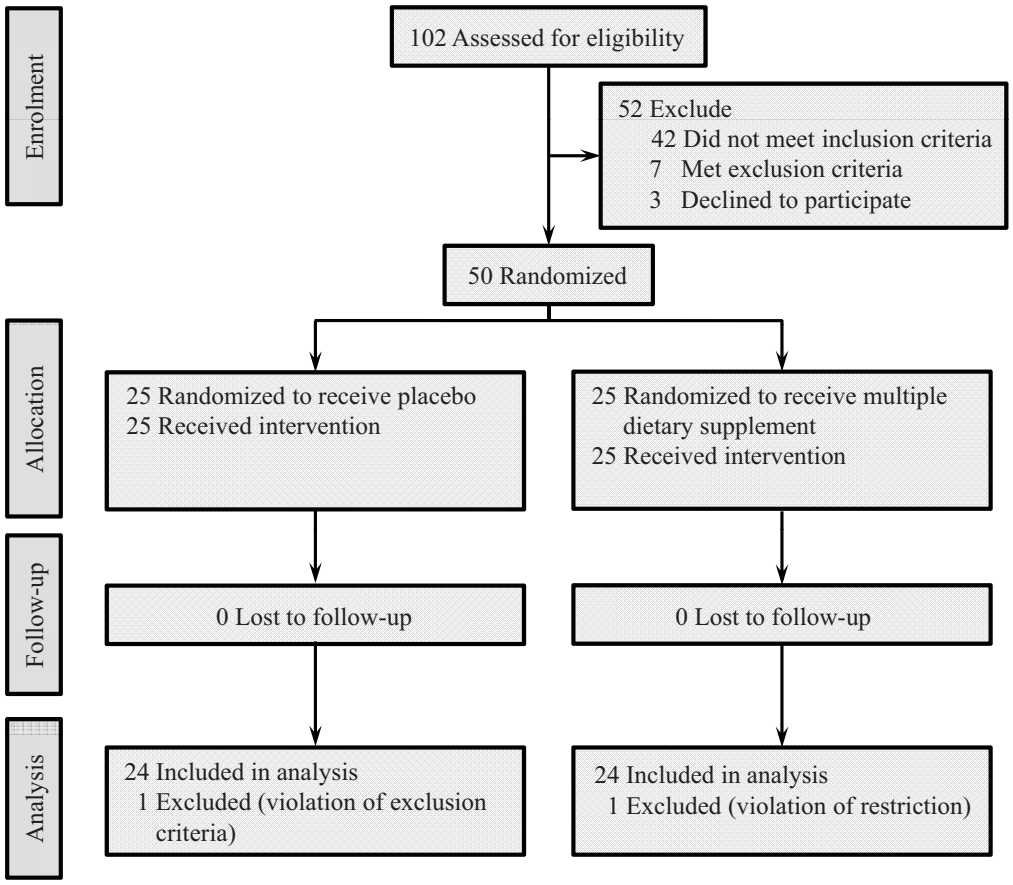

Fig. (1). Flow diagram of participants in this study. Multiple dietary supplement was contained lutein, astaxanthin, bilberry extract and black soybean extract (as source of cyanidin-3-glucoside), and docosahexaenoic acid (DHA).

Table 2. Baseline characteristics of the per protocol set.

		Placebo	Supplement	*P* value a)
Sex (male/female)		12/12	13/11	1.000
Age (years)		52.6±0.92	53.0±0.99	0.782
Dominant eye (right eye/left eye)		20/4	17/7	0.494
Perfect corrected VA (logMAR)	Dominant eye	0.090±0.036	0.129±0.042	0.478
	Non-dominant eye	0.130±0.036	0.164±0.046	0.571
	Both eyes	0.005±0.032	0.009±0.030	0.925
Near point Accommodation (diopter; D)	Dominant eye	5.425±0.490	5.483±0.407	0.927
	Non-dominant eye	5.042±0.379	5.575±0.375	0.322
	Both eyes	5.121±0.245	5.033±0.255	0.806

Values were expressed as mean±SE.
[a)] Between groups comparisons were assessed by unpaired t test or Fisher's extract test.

supplement group, and 24 in the placebo group). The intake rate of the test supplement in the PPS was 99.6% in the test supplement group, and 100% in the placebo group. There was no subject whose intake rate was below 80%.

Table **2** shows the background of subjects in the PPS. There was no significant difference between the two groups in any index. In the PPS, 5 subjects (4 in the test supplement group and 1 in the placebo group) wore contact lenses.

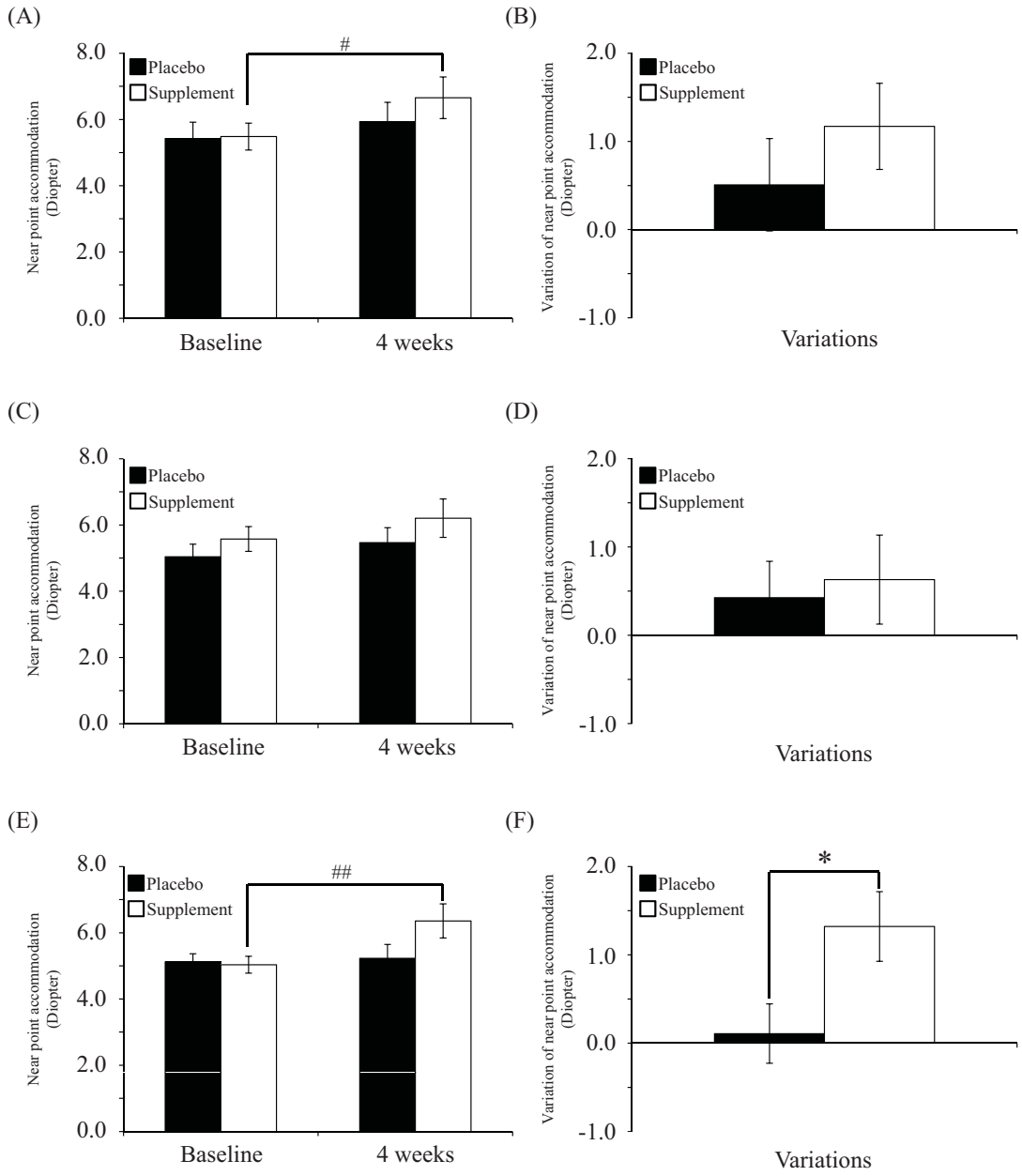

Fig. (2). Changes of near point accommodation before and after ingestion of test foods.
Measurement values of near point accommodation (NPA) of (**A**) dominant eye, (**C**) non-dominant eye, or (**E**) both eyes from before and after ingestion. Variations of the NPA of (**B**) dominant eye, (**D**) non-dominant eye, or (**F**) both eyes from before to after intervention. Values were expressed as means±SE. Inter-group comparison was analyzed using paired t-test between before and after ingestion (#P<0.05, ##P<0.01). Comparison of differences between placebo and supplement groups was assessed by unpaired t-test (*P<0.05, **P<0.01).

Regarding the secondary endpoint, in the PPS, only the subjects who scored four or more in the subjective symptoms questionnaire at the baseline were analyzed.

3.2. Primary Endpoint

The transition of near-point accommodation from pre-intake and the amount of changes are shown in Fig. (**2**).

The values in the test supplement group were 5.483±0.407 diopter (D) before intake, and 6.654±0.626 D after 4 weeks' (4 wk) intake for the dominant eye, which was a significant increase (p=0.025), 5.033±0.255 D before intake, and 6.354±0.515 D after 4 wk intake for both eyes, which was a significant increase (p=0.003), and 5.575±0.375 D before intake, and 6.204±0.58 D after 4 wk intake for the non-dominant eye, which

was not significant increase (p=0.225). On the other hand, no significant change was found in the placebo group; 5.425±0.490 D before intake, 5.933±0.585 D after 4 wk intake for the dominant eye (p=0.343), 5.042±0.379 D before intake, 5.467±0.451 D after 4 wk intake for the non-dominant eye (p=0.313), and 5.121±0.245 D before intake, 5.229±0.418 D after 4 wk intake for both eyes (p=0.750). Furthermore, values measured after 4 wk intake were not significantly different between the two groups; 5.933±0.585 D in the placebo group and 6.654±0.626 D in the test supplement group for the dominant eye (p=0.405), 5.467±0.451 D in the placebo group and 6.204±0.580 D in the test supplement group for the non-dominant eye (p=0.321), 5.229±0.418 D in the placebo group and 6.354±0.515 D in the test supplement group for both eyes (p=0.097). However, regarding variation from baseline, 1.321±0.394 D in the test supplement group was a significantly higher value as compared to 0.108±0.336 D in the placebo group (p=0.023) in case of both eyes, which showed improvement effect of the test supplement on accommodative ability.

3.3. Secondary Endpoint

The pre to post transition and amount of change in scores on the subjective symptoms questionnaire are shown in Table **3**.

In the test supplement group, significant decreases were found in 5 items; “eye strain” (4.045±0.045 before intake, 3.500±0.158 after 4wk intake p=0.002), “blurred vision” (4.083±0.083 before intake, 3.083±0.313 after 4 wk intake, p=0.016), “stiff shoulders or neck” (4.381±0.109 before intake, 3.667±0.199 after 4 wk intake, p=0.004), “heaviness of head” (4.111±0.111 before intake, 2.889±0.423 after 4 wk intake, p=0.031), and “difficult to focus on objects” (4.400±0.163 before intake, 3.500±0.269 after 4 wk intake, p=0.031).

In contrast, in the placebo group, significant decreases were found only in 2 items; “eye strain” (4.292±0.095 before intake, 3.708±0.185 after 4 wk intake, p=0.012), and “difficult to see in low light condition” (4.111±0.111 before intake, 2.889±0.389 after 4 wk intake, p=0.016).

In comparison between the groups test supplement and placebo after 4 wk intake, the test supplement group showed significant decreases in 2 items; “blurred vision” (4.000±0.226 in the placebo group, 3.083±0.313 in the test supplement group, p=0.033), and “stiff shoulders or neck” (4.300±0.179 in the placebo group, 3.667±0.199 in the test supplement group, p=0.026). The test supplement group also showed a significant decrease in the variations from baseline compared to placebo group (-0.050±0.135 in the placebo group, -0.714±0.197 in the test supplement group, p=0.009). From these results, the effect of the test supplement to improve subjective symptoms was demonstrated.

3.4. Safety

Safety was evaluated for 50 subjects (25 in the supplement group and 25 in the placebo group) who started taking the test supplement or placebo.

A total of 20 adverse events occurred during the study period. They were minor events such as common cold or headache. None of them was judged to be related to the test supplement by the principal investigator.

4. DISCUSSION

In this study, a randomized placebo-controlled double-blind comparison study was conducted for 48 subjects (25 men and 23 women) aged 45 to 64 years (mean age: 52.8 years) who were aware of eye strain, in order to verify the effect of multiple dietary supplement (functional ingredients: lutein, astaxanthin, bilberry extract and black soybean hull extract (as cyanidin-3-glucoside in both extracts), and DHA) on accommodative ability of the eye. After 4 weeks’ intake of the multiple dietary supplement, improvement was observed in accommodative ability of the eye, and subjective symptoms related to asthenopia such as “blurred vision” and “stiff shoulders or neck”, compared with placebo. After 4 weeks’ intake of the multiple dietary supplement, alleviation was observed in three subjective symptoms; “eye strain”, “heaviness of head”, and “blurred vision when looking from near to far”, although, there was no significant difference compared with placebo.

Decrease in accommodative ability is supposedly caused by the loss of lens elasticity, and the loss of ciliary muscles that controls lens thickness [29-32]. The multiple dietary supplements used for

Table 3. Changes of subjective symptoms of eye fatigue from baseline to 4 weeks after ingestion.

Symptom	Group	n	Before	4 weeks after	*P* value[b)]	Variations
			mean±SE.	mean±SE.		mean±SE.
Eye strain	Placebo	24	4.292±0.095	3.708±0.185	0.012#	-0.583±0.199
	Supplement	22	4.045±0.045	3.500±0.158	0.002##	-0.545±0.143
	P value[a)]		0.049*	0.235		0.815
Ocular pain	Placebo	5	4.200±0.200	3.200±0.374	0.125	-1.000±0.316
	Supplement	4	4.000±0.000	2.500±0.645	0.250	-1.500±0.645
	P value		1.000	0.532		0.556
Blurred vision	Placebo	13	4.385±0.140	4.000±0.226	0.188	-0.385±0.213
	Supplement	12	4.083±0.083	3.083±0.313	0.016#	-1.000±0.302
	P value		0.160	0.033*		0.090
Teary eyes	Placebo	10	4.100±0.100	3.400±0.267	0.063	-0.700±0.260
	Supplement	6	4.000±0.000	3.000±0.258	0.063	-1.000±0.258
	P value		1.000	0.221		0.386
Eye redness	Placebo	6	4.000±0.000	3.500±0.224	0.250	-0.500±0.224
	Supplement	6	4.333±0.211	3.833±0.167	0.250	-0.500±0.224
	P value		0.455	0.545		1.000
Flickering vision	Placebo	7	4.286±0.184	3.714±0.286	0.125	-0.571±0.202
	Supplement	6	4.000±0.000	3.667±0.333	0.625	-0.333±0.333
	P value		0.462	1.000		0.796
Double vision	Placebo	7	4.429±0.202	4.429±0.202	1.000	0.000±0.218
	Supplement	4	4.250±0.250	3.500±0.289	0.500	-0.750±0.479
	P value		1.000	0.064		0.258
Stiff shoulders or neck	Placebo	20	4.350±0.109	4.300±0.179	1.000	-0.050±0.135
	Supplement	21	4.381±0.109	3.667±0.199	0.004##	-0.714±0.197
	P value		1.000	0.026*		0.009**
Frustrate	Placebo	6	4.167±0.167	3.667±0.494	0.500	-0.500±0.428
	Supplement	2	4.000±0.000	4.500±0.500	1.000	0.500±0.500
	P value		1.000	0.500		0.357
Heaviness of head	Placebo	5	4.200±0.200	3.600±0.510	0.500	-0.600±0.400
	Supplement	9	4.111±0.111	2.889±0.423	0.031#	-1.222±0.364
	P value		1.000	0.320		0.226
Head ache	Placebo	4	4.000±0.000	3.500±0.500	1.000	-0.500±0.500
	Supplement	5	4.200±0.200	3.600±0.600	0.500	-0.600±0.510
	P value		1.000	0.992		0.825

Table 3. contd….

Symptom	Group	n	Before	4 weeks after	*P* value[b)]	Variations
			mean±SE.	mean±SE.		mean±SE.
Difficult to see for small objects	Placebo	17	4.471±0.125	4.235±0.202	0.219	-0.235±0.136
	Supplement	14	4.357±0.133	4.143±0.206	0.375	-0.214±0.155
	P value		0.717	0.741		0.963
Difficult to focus on objects	Placebo	9	4.222±0.147	3.667±0.408	0.313	-0.556±0.377
	Supplement	10	4.400±0.163	3.500±0.269	0.031#	-0.900±0.277
	P value		0.628	0.539		0.397
Dazzle vision	Placebo	10	4.100±0.100	3.700±0.367	0.500	-0.400±0.340
	Supplement	11	4.273±0.141	3.727±0.195	0.125	-0.545±0.247
	P value		0.586	0.744		0.641
Difficult to see in low light condition	Placebo	9	4.111±0.111	2.889±0.389	0.016#	-1.222±0.364
	Supplement	8	4.500±0.189	3.500±0.378	0.063	-1.000±0.378
	P value		0.131	0.508		0.616

Subjects that scored four or more in each symptom at the baseline were analyzed. Values were expressed as mean±SE.
[a)] Comparison of differences between placebo and multiple dietary supplement groups was assessed by Wilcoxon rank sum test (**: $p<0.01$, *: $p<0.05$).
[b)] Inter-group comparison was assessed by the Wilcoxon singed rank test (##: $p<0.01$, #: $p<0.05$).

this study includes astaxanthin as well as bilberry extract and black soybean hull extract containing anthocyanin whose effect to improve accommodative ability or accommodation. Astaxanthin may have improved accommodation due to its relaxing effect on the ciliary muscles [8]. Astaxanthin is reported that it increases blood flow in the retinal capillaries [4] and inhibits the NF-κB signaling pathway in the ciliary body [12]. Nutrients are delivered to the ciliary body via the ciliary artery and the short posterior ciliary artery that are considered to have branched from the central retinal artery which branches from the ophthalmic artery. For this reason, increased retinal capillary blood flow may lead to increased blood flow through the ciliary muscle. Then, astaxanthin that has reached to the ciliary body, may suppress the increase of TNF-α by NF-κB signaling pathway in the ciliary body to prevent the loss of ciliary muscle function, which suggests astaxanthin should improve accommodative ability. The amount of astaxanthin contained in the multiple dietary supplement used in this study was 4 mg, less than that of intake (5 to 6 mg) used in other studies [9-11] on the effect of astaxanthin to improve an accommodative ability. However, in this study, the intake of the multiple dietary supplements improved accommodative ability or accommodation, which suggests the action of astaxanthin was augmented by other functional ingredients.

Anthocyanin found in bilberry extract and black soybean hull extract used in this multiple dietary supplement is known for its vasorelaxant effects [5] and ciliary muscle relaxant effects [7]. Moreover, some studies show improved accommodative ability with a higher amount of bilberry extract or black soybean hull extract [6, 13, 16]. Ciliary muscle relaxant effects of anthocyanin, which may be a functional ingredient of bilberry extract and black soybean hull extract, have been studied, using delphinidin-3-rutinoside or an anthocyanin found in black currant. And endothelin B receptor-mediated and NO/cGMP pathway-mediated mechanisms are suggested [33]. Therefore, cyanidin-3-glucoside, an anthocyanin found in bilberry extract and black soybean hull extract, may also have the potential to relax ciliary muscles in the pathway similar to delphinidin-3-rutinoside. As antioxidant effects of lutein on the macula and lens [25] are shown, it may augment the antioxidative properties of astaxanthin on the eyes. DHA is a major lipid in the retina [34], and simultaneously, contributes to the structural elements of the

ciliary body [21], which suggests that it should play a role in keeping the ciliary body healthy. This may mean that a lower dose of astaxanthin acts synergistically and additively with other functional ingredients in this multiple dietary supplement, but further studies on detailed mechanism of action are considered necessary. "Stiff shoulders or neck" among improved subjective symptoms in this study is a domain of that QOL, and is likely to be prompted by the worsening of visual symptoms such as presbyopia and asthenopia, which suggests QOL is enhanced by an improved accommodative ability.

There are many studies on functional ingredients including astaxanthin to improve accommodative ability. But most of them were conducted for subjects aged from their mid-twenties to around 40 years old before developing presbyopia. Kajita *et al*. reported the influence of astaxanthin on accommodative ability of aged and older subjects [15]. This study also shows that the use of multiple dietary supplements has potential to improve an accommodative ability and subjective symptoms related to asthenopia of aged and older subjects whose accommodative ability tends to decrease.

The multiple dietary supplement (test supplement) is contains low dose astaxanthin in addition of lutein with the reduction in the risk of advanced AMD [23], cyaniding-3-glucoside with the stimulatory effect on the regeneration of rhodopsin [18], and DHA, which improve dry eye disease [3]. The multiple dietary supplement may improve not only accommodative ability but also some eye diseases, which mean that the multiple dietary supplement is more effective on eye health than astaxanthin only.

We consider that this multiple dietary supplement will provide new therapeutic option for the treatment of presbyopia or eye fatigue.

5. CONCLUSION

In this study, 48 subjects aged 45 to 64 years (mean age: 52.8 years, 25 men and 23 women), who felt eye strain on a daily basis, took the multiple dietary supplement containing astaxanthin, lutein, bilberry extract and black soybean hull extract (as cyanidin-3-glucoside in both extracts), and DHA for four consecutive weeks, which suggested that the multiple dietary supplement should improve not only accommodative ability but also subjective symptoms related to asthenopia of aged and older subjects who felt eye strain. No clinically significant adverse events or side effects occurred during this study. There was no safety concern in the study conditions.

CONFLICT OF INTEREST

Costs of this study were covered by FANCL Corporation. There is no conflict of interest.

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