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The effect of consuming an anthocyanin-containing supplement derived from Bilberry (*Vaccinium myrtillus*) on eye function: A Randomized, Double-Blind, Placebo-Controlled Parallel Study

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ABSTRACT

Objective: The purpose of this study was to determine the effects of 6-week consumption of anthocyanin-containing supplement on eye function.

Methods: This was a randomized, placebo-controlled, double-blind, parallel-group comparison study involving 32 healthy Japanese adults with eye fatigue after using visual display terminals (VDTs). Subjects were randomly allocated into either the active group (bilberry-derived anthocyanin 43.2 mg per capsule) or placebo group using a random number generator. Subjects consumed either one active or placebo capsule once a day for 6 weeks. The primary outcome measured was the change in percentage of pupillary response pre- and post-VDT use, whereas the secondary outcomes were tear film break-up time, Schirmer's value, muscle hardness, and subjective symptoms. Experimental data was analyzed using Student's t-test, the two-way analysis of covariance, or Mann–Whitney U-test.

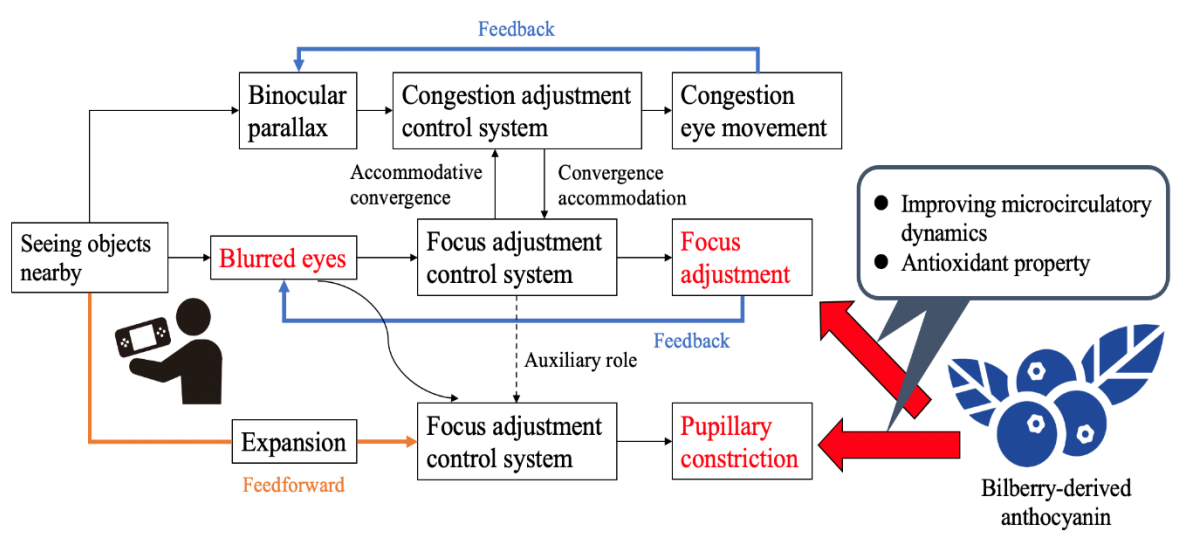
Results: Each group included 15 subjects in the efficacy analysis. The active group showed a significant

improvement in the logarithmic conversion values of the percentage of pupillary response (active group: 0.2 ± 0.4, placebo group: 0.0 ± 0.3; P = 0.043) and pupillary response/near point (active group: 0.1 ± 0.4, placebo group: −0.1 ± 0.3; P = 0.049) pre- and post-VDT use at 6 weeks compared with the placebo group in a subgroup analysis per eye. No adverse events were reported.

Conclusions: The consumption of the supplement containing anthocyanins extracted from bilberry for 6 weeks inhibited the decrease in the accommodative function caused by oxidative stress due to VDT use.
Trial registration: UMIN-CTR: UMIN000037039.

Foundation: BGG Japan Co., Ltd. and Arysta Health and Nutrition Sciences Corp.

Keywords: accommodative function; anthocyanin; bilberry; visual display terminals



INTRODUCTION

In the modern age, visual display terminal (VDT) devices such as personal computers and smartphones are widely used and have become necessary in our daily life. However, many people report fatigue and pain in their eyes while using VDTs, especially after longer periods of time [1]. In fact, VDT use is known to decrease blink frequency [2], resulting in eye dryness [3]; decreased accommodative function, headache, and shoulder stiffness have also been reported [4]. In addition, blue light generated by light-emitting diodes, one of the major light sources of VDTs, is thought to be a cause of eye strain and dryness because of the time-dependent production of reactive oxygen species (ROS) in photoreceptor cells [5,6]. Furthermore, it has been reported that rhodopsin, which is involved in transmitting visual information to the brain, is deactivated when under oxidative stress due to

abundant ROS [7]. Therefore, reducing the ocular cytotoxicity caused by ROS may be important in preventing and reducing eye strain and dryness.

Bilberry (*Vaccinium myrtillus*) is a perennial plant of the family Ericaceae, and is found throughout the northern and eastern parts of Europe [8]. Clinical studies have shown that bilberry-derived anthocyanins aids in relieving eye fatigue [9,10] and in improving object focus, contrast sensitivity [11], and tear fluid quality [12]. Bilberry contains a higher level of anthocyanins than other berries [8]. Anthocyanins contained in bilberries such as delphinidin 3-galactoside, delphinidin 3-glucoside, and cyanidin 3-glucoside (C3G) possess antioxidant properties [13]. In particular, C3G has been reported to show high scavenging effects against hydrogen peroxide, a member of ROS [14]. In addition, C3G can bind to deactivated rhodopsin [15], promoting its regeneration after light absorption [16,17]. We hypothesize that consumption of bilberry-derived anthocyanins may improve eye function.

The discomfort caused by VDT use may be due to mental fatigue, such as decreased perceptual functioning because of attenuated arousal levels [18]. In fact, the intake of bilberry-containing foods has been shown to improve eye fatigue by reducing fatigue of the central nervous system [19]. In addition, oculomotor fatigue may lead to a secondary change in innervation to the postural muscles in the neck, shoulder, and upper back, and the change resulting in discomfort in these areas [20]. Although multiple factors contribute to eye fatigue [20], the relationship between shoulder muscle hardness and eye fatigue has not yet been examined well. Therefore, this study aimed to verify the effect of bilberry extract on eye fatigue caused by the decline of accommodative functions and its correlation with shoulder stiffness.

METHODS

Study design: This was a randomized, double-blind, placebo-controlled parallel study. The allocation ratio was 1:1. The study protocol was approved by the independent Ethics Committee of the Medical Corporation Seishinkai, Takara Clinic, on May 27, 2019 (approval no. 1905-1904-BJ02-02-TC), and the protocol was approved by the University Hospital Medical Information Network Clinical Trials Registry (UMIN000037039). 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. The examinations were conducted at the Ario Nishiarai Eye Clinic (Tokyo, Japan).

Subjects: Inclusion criteria were as follows: (1) healthy Japanese adults experiencing eye fatigue during and/or after VDT use; (2) corrected visual acuity of both eyes with 1.0 acuity or more [21] and who do not use contact lenses, or who can switch to using eye glasses during the test period; (3) considered eligible to participate in the study by the principal physician judging from the results of a blood test; and (4) relatively larger drop in the percentage of pupil constriction (average of both eyes) pre- and post-VDT use (playing a video game for 60 min) at screening (Scr; examination before ingestion).

Exclusion criteria were as follows: (a) currently being treated for malignancy, heart failure, or myocardial infarction; (b) using a pacemaker or an implantable cardioverter defibrillator; (c) currently being treated for cardiac arrhythmia, hepatic, renal, or cerebrovascular disease, or for chronic diseases such as rheumatism, diabetes mellitus, hyperlipidemia, or hypertension; (d) diagnosed with or experiencing presbyopia; (e) the presence of ophthalmopathy, entropion, or trichiasis; (f) currently using eye drops for the treatment of an eye disease; (g) the presence of ametropia and without proper treatment of orthoptics; (h) underwent laser eye surgery (LASIK); (i) the presence of an irregular astigmatism; (j) eye strain without accommodation function, including a neurological deficit; (k) daily consumption of "foods for specified health uses," "foods with function claims," or other functional foods/beverages; (l) regular use of medications, including herbal medicines and/or supplements; (m) allergic reaction to medications and/or products that contain the study components; (n) being pregnant, lactating, or planning to become pregnant; (o) enrollment in other clinical trials within the last 3 months before agreeing to participate in this study; and (p) 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). The study protocol was comprehensively explained to all the subjects. Written informed consent was obtained from all subjects before being enrolled at the ORTHOMEDICO Inc. office. No subject was part of the sponsoring or funding companies.

Intervention: An active hard capsule included 43.2 mg of bilberry-derived anthocyanins (equivalent to 120 mg of bilberry extract powder [BGG Japan Co., Ltd., Tokyo, Japan]) and the placebo capsule was composed of starch. The determination of the anthocyanins and free anthocyanidins content in the supplements by high performance liquid chromatography in accordance with European Pharmacopoeia. Subjects were asked to consume either one active (the active group) or placebo capsule (the placebo group) daily with water once a day after breakfast for 6 weeks. The Ethics Committee declared both capsules have identical color, odor, and flavor. In addition, subjects were prohibited to consume anthocyanin-containing foods such as fresh berries outside the intervention.

Outcomes: Table 1 describes the schedule for this study. Subjects visited the clinic and underwent examinations at Scr and 6w. VDT use at these timeframes consisted of playing a video game with a handheld game console for 60 min. Assessments were done pre- and post-VDT use at Scr and 6w, and the changes between pre- and post-VDT use (post-VDT use minus pre-VDT use) were calculated. The tear film break-up time (BUT) test, visual acuity test, Schirmer's test, and safety assessments were performed only pre-VDT use. The primary outcome of this study is the percentage of pupillary response set for investigating the effects of VDT on eye fatigue, so other items not related to pupillary response were included only before VDT loading. The

assessments associated with the eyes were evaluated as the average of both dominant and non-dominant eyes. The dominant eye of each subject was determined by the hole-in-card test [22].

Primary outcome:

Change of percentage of pupillary response (average of both dominant and non-dominant eyes) between pre- and post-VDT use at Scr and 6w.

The percentage of pupillary responses was evaluated using the TriIRIS C9000 (Hamamatsu Photonics K.K., Shizuoka, Japan), which is a near-point measuring device. The percentage of pupillary response obtained from the TriIRIS C9000 corresponds to the moving distance of the accommodative target; therefore, the accommodative function of the pupil of healthy subjects could be quantified by the percentage of pupillary response measured at near point [23,24]. The percentage of pupillary response could be calculated from equation (1) [23,24].

$$\frac{\text{maximum lateral diameter of the pupil} - \text{minimum lateral diameter of the pupil}}{\text{maximum lateral diameter of the pupil}} \times 100 \quad (1)$$

The symptoms of eye fatigue caused by VDT use include reduced pupillary constriction and mydriasis, whereas enhancement of the accommodative function is indicated by an increased percentage of pupillary response.

Secondary outcomes

All data obtained from the measurement of the percentage of pupillary response, except for the primary outcome, were considered the secondary outcome. The percentage of pupillary response was evaluated by using the TriIRIS C9000 and calculated using equation (1).

BUT was measured after administering fluorescein into the eyes of subjects and instructing them not to blink. The time between the last blink and the appearance of the first dry spot was observed and measured using a slit lamp SL-1800 (NIDEK CO., LTD., Aichi, Japan).

Schirmer's value (the amount of lacrimal fluid) was measured using Schirmer Tear Test Color Bar (Katena Products Inc., New Jersey, U.S.A.).

Muscle hardness was measured using the Bio elasticity meter PEK-1 (Imoto Machinery Co., Ltd., Kyoto, Japan). Measurements were performed pre- and post-VDT use, once on each of both shoulders.

The subjective symptoms of eye fatigue were evaluated using a questionnaire (the Likert scale method). Subjects expressed subjective sensations of the following symptoms: a tiredness sensation in the eyes; a sensation of dry eyes; objects appear to be blurred, hazy, or doubled; watery eyes; a sensation of trouble in focusing the eyes; difficulty in seeing objects in one's hand and nearby or fine print; easily feeling dazzled by light; stiffness in the neck and shoulders; feeling fatigued in the back of the eyes; laziness; inability to concentrate; and objects feeling cold to the hands and feet. All these questions were assessed on a 6-point scale of 1 (strongly disagree) to 6 (strongly agree). Although Likert scales of more than 6 points are low accuracy because of limitations in human working memory capacity[25], 6-point scales should be used as they permit the possibility of increased measurement precision [26]. Therefore, we chose a 6-point scale.

Table 1. Schedule of enrollment, intervention, and assessments.

	Enrollment	Screening (Scr)		Selection	Allocation	Start intake (0w)	Six weeks after intake 6w)	
		pre-VDT use	post-VDT use				pre-VDT use	post-VDT use
ENROLLMENT:								
Eligibility screen	●			●				
Informed consent	●							
Dominant eye test (the hole-in-card test)	●							
Allocation					●			
INTERVENTIONS:								
Active group						⟷	⟷	
Placebo group						⟷	⟷	
ASSESSMENTS:								
Accommodative function test		●	●				●	●
Tear film break-up time test		●					●	
Visual acuity test		●					●	
Schirmer's test		●					●	
Muscle hardness test		●	●				●	●
Questionnaire		●	●				●	●
Tonometry		●					●	
Physical examination	●	●					●	
Urinalysis		●					●	
Blood analysis		●					●	
Medical questionnaire		●					●	
Diary records						⟷	⟷	

Filled circles (●) represent the timing of execution of each item; VDT, visual display terminal

Safety evaluation

Safety evaluations were assessed during a physical examination, urinalysis, blood analysis, visual acuity test, and tonometry (Tables 4-1–4-4). All subjects were asked to fill out a medical questionnaire regarding their health conditions at each examination. Additionally, subjects were asked to keep a daily record of consumption of the supplement, health conditions, use of medications, and their lifestyle.

Sample size: The study was designed to detect a significant difference in the pupillary response (average of both eyes) pre- and post-VDT use at Scr and 6 weeks after intake (6w) when the difference in mean between the active and placebo groups was 0.4 and the standard deviation was 0.37. These values are based on the preceding study of Nakata A *et al*. (2016) [27], which evaluated the pupillary response by the same equipment as this study. The sample size was calculated with an assumed significance level (α) of 0.05 and statistical power (1−β) of 0.80, leading to 30 subjects (15 subjects per group). The dropout and violation of compliance rules during the test period were considered, and two extra subjects were added to each group to have 16 subjects per group, 32 subjects in total.

Enrollment, randomization, and blinding: Of the 69 subjects who signed informed consent forms, 32 subjects were considered eligible by the physician. Subjects with relatively larger drop in the percentage of pupil constriction (average of both eyes) between pre- and post-VDT use at Scr were selected as priority subjects for enrollment in this study. Subjects were equally and randomly allocated to either the active group or placebo group (n = 16 per group), following a computer-generated randomized list managed by an allocation controller who was not directly involved in this study. The allocation adjustment factors were sex, age at the Scr, and the percentage of pupil constriction (average of both eyes) between pre- and post-VDT use at Scr. Furthermore, the subjects, physician, assessor of outcomes, and others who were associated with this study were not aware of the group assignments and were not involved in the allocation. Additionally, the allocation controller locked the assignment sheet until the key-opening day.

Statistical analysis: Subjects visited the clinic twice, and the outcomes were assessed at Scr and 6w. The data at Scr were set as baseline, and the data at the baseline were subtracted from post-intervention (6w) values and reported as the change in value (6w − Scr). In addition, subjects' background data were aggregated on the basis of sex, age, and physical characteristics (height, non-specific IgE, and dominant eye), and the active group was demographically compared with the placebo group using Student's *t*-test.

The primary and secondary outcomes except for subjective symptoms (pupillary response, BUT, Schirmer's value, and muscle hardness), physical examination, urinalysis, blood analysis, visual acuity test, and tonometry were presented as their mean ± standard deviation and analyzed using Student's *t*-test at baseline and 6w − Scr. Furthermore,

we analyzed the data at 6w using the two-way analysis of covariance (ANCOVA). When ANCOVA was used for data analyses, we used the baseline values as a covariate and the group as factors. Moreover, data on subjective symptoms were presented as their median and interquartile range (first and third quartiles [Q1 and Q3, respectively]) and analyzed using the Mann–Whitney U-test. Furthermore, urinalysis and blood analysis data were assigned codes wherein "1" was identified as within the normal range and "0" was identified as outside the normal range. The data were expressed as the number of subjects and analyzed using the chi-square test. A subgroup analysis per eye was conducted in the same way.

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

RESULTS

Subjects: The study flowchart and subjects' dropout are illustrated in Figure 1. We recruited subjects for this study from June 13, 2019, to November 12, 2019, and conducted it from August 19, 2019, to January 31, 2020. At the case review meeting after the intervention, two subjects who did not come to the examination at 6w and did not receive a supplement intervention after allocation were judged as ineligible for analysis and were excluded. Upon key opening, we found that one of those two subjects was from the active group and the other was from the placebo group. The number of subjects who underwent the full analysis set (FAS) and safety analysis population was 15 (8 men and 7 women) in the active group and 15 (7 men and 8 women) in the placebo group. The background and age distribution of the study subjects are shown in Tables 2-1 and 2-2. There were no significant differences between the background factors of both groups.

In addition, a subgroup analysis per eye was performed in this study. The dominant eye differs from individual to individual, with 58%–80% of those in Japan having a dominant right eye [28–31]. Regarding the FAS of this study, 80% of the active group and 60% of the placebo group had a dominant right eye (Table 2-2). A previous study on Japanese VDT workers showed decreased visual acuity, accommodation, and accommodative function in the right eye; thus, the effects of VDT use may be more likely to appear in the dominant eye [32]. It has been reported that the dominant eye has a significantly higher rate of accommodation tension speed than the non-dominant eye and that the dominant eye is more likely to see nearby. It is thought that the dominant eye tenses up more than the non-dominant eye and precedes the non-dominant eye by quickly adjusting to the near side [32]. However, there was no statistically significant difference between the dominant and non-dominant eyes in terms of intervention effect on the FAS. Therefore, we analyzed one case as a single eye. The results of this analysis can be seen in Figure 1.

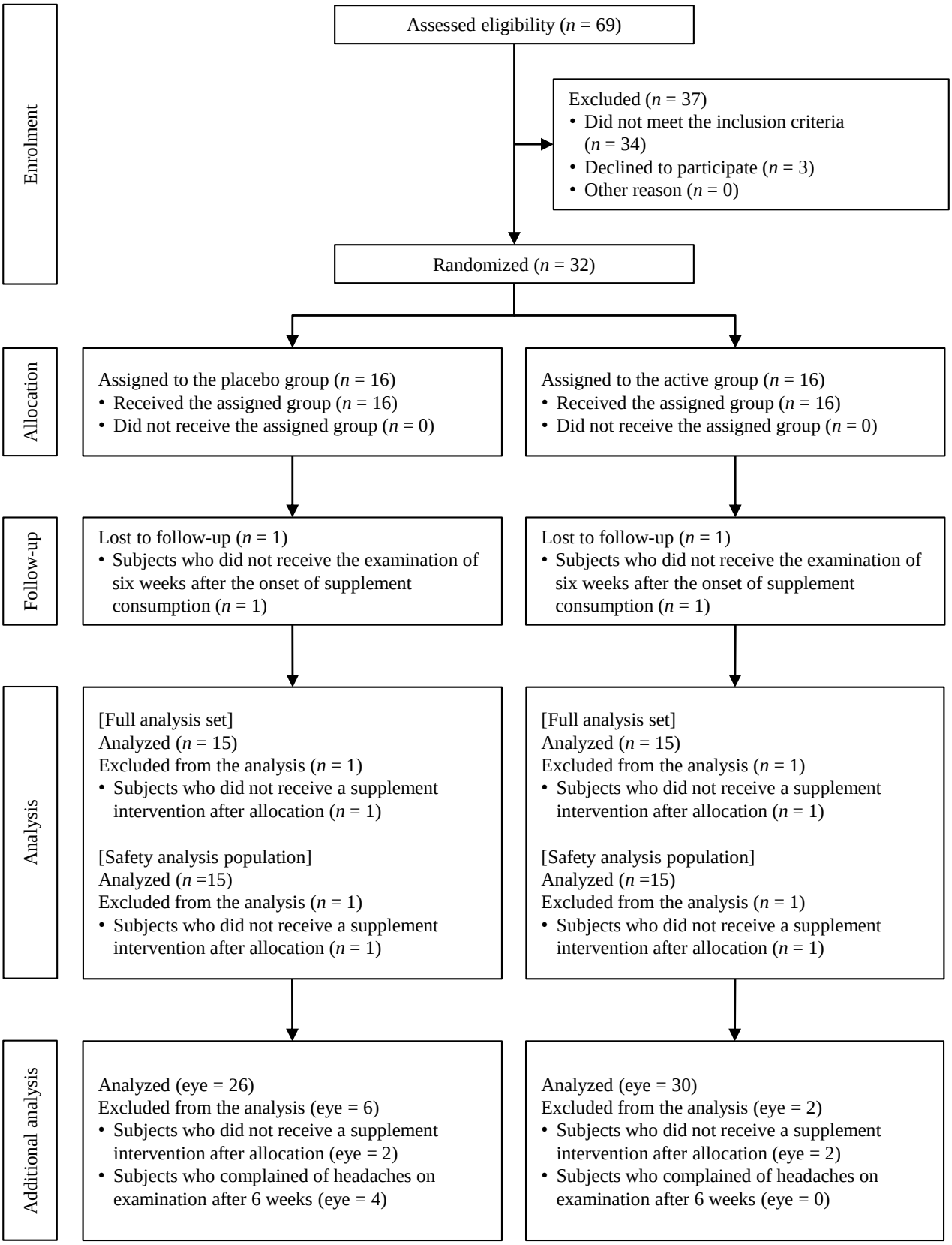


Figure 1. Flowchart of subjects in this study.

Table 2–1. Subject background (sex and age)

Age	Placebo group		Active group	
	Men	Women	Men	Women
Allocated subjects (Active group *n* = 16, Placebo group *n* = 16)				
20-29	1	1	2	2
30-39	5	3	2	3
40-49	2	4	4	3
FAS, full analysis set; SAF, safety analysis population (Active group *n* = 15; Placebo group *n* = 15)				
20-29	1	1	2	2
30-39	4	3	2	2
40-49	2	4	4	3

The data are presented as the number of subjects.

The data are assessed by the chi-square test.

Table 2–2. Subject background information (age, height, non-specific IgE, and dominant eye)

	Placebo group (*n* = 16)	Active group (*n* = 16)	*P* value
Allocated subjects (Active group *n* = 16; Placebo group *n* = 16)			
Age[a] (years)	37.7 ± 7.1	36.8 ± 9.3	0.766
Height[a] (cm)	163.9 ± 7.9	168.4 ± 6.1	0.080
Non-specific IgE[a] (IU/mL)	430.7 ± 1302.5	115.7 ± 146.8	0.344
Dominant eye[b]			
Right eye/Left eye	9/7	13/3	0.252
FAS, full analysis set; SAF, safety analysis population (Active group *n* = 15; Placebo group *n* = 15)			
Age[a] (years)	37.8 ± 7.3	36.9 ± 9.6	0.767
Height[a] (cm)	163.4 ± 8.0	168.6 ± 6.3	0.058
Non-specific IgE[a] (IU/mL)	449.4 ± 1345.9	120.0 ± 150.9	0.354
Dominant eye[b]			
Right eye/Left eye	9/6 (60.0%)	12/3 (80.0%)	0.427

a. The data are presented as the mean ± standard deviation; b. The data are presented as the number of subjects.

IgE, immunoglobulin E; The data are assessed by Student's *t*-test.

FAS

(1) **Accommodative function, BUT, Schirmer's value**

There were no significant differences between the groups (Tables 3–1–3–3).

Table 3–1. The results of accommodative function

		Placebo group (n = 15)			Active group (n = 15)			EMM	95% CI−	95% CI+	P value
		Mean	**SD**		**Mean**	**SD**					
Percentage of the pupillary response pre-VDT use (%)											
Average of both eyes	Scr	34.7	13.3		39.3	10.8		—	—	—	0.303
	6w	33.5	13.3		36.1	13.5		−0.7	−8.7	7.3	0.858
	6w − Scr	−1.2	12.9		−3.2	8.2		−2.0	−10.1	6.1	0.612
Dominant eye	Scr	35.6	13.1		39.6	11.1		—	—	—	0.368
	6w	32.4	13.1		34.6	12.3		−0.3	−8.3	7.7	0.945
	6w − Scr	−3.2	13.4		−5.0	8.9		−1.9	−10.4	6.7	0.659
Non-dominant eye	Scr	33.8	15.2		38.9	11.2		—	—	—	0.297
	6w	34.6	14.3		37.6	15.7		−0.6	−9.9	8.7	0.902
	6w − Scr	0.8	14.7		−1.4	10.3		−2.2	−11.7	7.3	0.638
Right eye	Scr	34.0	14.1		39.2	10.8		—	—	—	0.266
	6w	32.5	13.5		34.7	12.3		−1.6	−8.7	5.5	0.654
	6w − Scr	−1.5	10.4		−4.5	9.0		−3.0	−10.3	4.3	0.408
Left eye	Scr	35.4	14.3		39.4	11.5		—	—	—	0.402
	6w	34.5	14.0		37.5	15.8		0.7	−9.3	10.7	0.886
	6w − Scr	−0.8	17.1		−1.9	10.4		−1.1	−11.7	9.5	0.839
Percentage of the pupillary response post-VDT use (%)											
Average of both eyes	Scr	31.6	12.9		35.9	14.0		—	—	—	0.392
	6w	33.0	13.7		37.2	12.7		0.9	−5.5	7.3	0.774
	6w − Scr	1.4	9.1		1.3	8.5		−0.1	−6.7	6.5	0.978
Dominant eye	Scr	32.0	13.2		35.1	14.5		—	—	—	0.545
	6w	33.8	14.4		35.4	12.3		−0.7	−7.3	5.8	0.821
	6w − Scr	1.8	10.4		0.3	8.0		−1.5	−8.5	5.4	0.655
Non-dominant eye	Scr	31.2	13.1		36.7	13.9		—	—	—	0.278

		Placebo group (n = 15)			Active group (n = 15)			EMM	95% CI−	95% CI+	P value
		Mean	**SD**		**Mean**	**SD**					
	6w	32.2	13.4		39.0	14.3		2.6	−4.5	9.8	0.457
	6w − Scr	0.9	9.0		2.3	10.5		1.4	−6.0	8.7	0.707
Right eye	Scr	31.5	14.2		34.7	14.1		—	—	—	0.543
	6w	34.0	14.0		36.0	12.9		−0.5	−6.5	5.6	0.880
	6w − Scr	2.5	8.9		1.3	8.2		−1.2	−7.6	5.2	0.706
Left eye	Scr	31.8	12.0		37.1	14.2		—	—	—	0.273
	6w	32.0	13.7		38.3	13.9		2.4	−5.2	10.1	0.523
	6w − Scr	0.2	10.4		1.2	10.4		1.0	−6.8	8.8	0.792
Change in percentage of pupillary response pre- and post-VDT use (post-VDT use − pre-VDT use, %)											
Average of both eyes	Scr	−3.1	6.7		−3.4	5.4		—	—	—	0.879
	6w	−0.5	4.9		1.1	7.5		1.5	−3.2	6.3	0.514
	6w − Scr	2.5	7.9		4.5	11.0		1.9	−5.2	9.1	0.583
Dominant eye	Scr	−3.6	7.6		−4.5	6.9		—	—	—	0.718
	6w	1.4	4.4		0.8	4.9		−0.9	−4.2	2.4	0.595
	6w − Scr	5.0	9.5		5.3	10.4		0.3	−7.1	7.8	0.931
Non-dominant eye	Scr	−2.5	8.4		−2.2	5.4		—	—	—	0.914
	6w	−2.4	7.4		1.4	12.7		3.8	−4.1	11.7	0.334
	6w − Scr	0.1	8.6		3.7	14.3		3.6	−5.3	12.4	0.416
Right eye	Scr	−2.5	6.9		−4.5	6.8		—	—	—	0.428
	6w	1.5	4.3		1.3	5.6		−0.6	−4.3	3.2	0.755
	6w − Scr	4.1	7.9		5.9	10.7		1.8	−5.2	8.8	0.605
Left eye	Scr	−3.6	9.0		−2.3	5.5		—	—	—	0.628
	6w	−2.6	7.4		0.8	12.5		3.3	−4.5	11.1	0.395
	6w − Scr	1.0	10.5		3.1	13.9		2.1	−7.1	11.3	0.648

The data are presented as the mean and standard deviation (SD).

The data of differences between active and placebo groups (Δactive group − placebo group) are presented as the estimated marginal means (EMM), whereas the 95% confidence interval (95% CI) was based on the EMM.

The data at Scr and 6w – Scr were analyzed using Student's *t*-test. As for the data at 6w, Scr values are utilized as covariates, and the group is utilized as a factor in the ANCOVA.

Table 3–2. The results of percentage of pupillary response / near point

		Placebo group (*n* = 15)			Active group (*n* = 15)			EMM	95% CI−	95% CI+	*P* value
		Mean	SD		Mean	SD					
Percentage of pupillary response/near point pre-VDT use (%/D)											
Average of both eyes	Scr	5.2	2.0		5.9	1.2		—	—	—	0.236
	6w	5.2	1.5		5.6	1.5		0.2	−0.9	1.3	0.704
	6w − Scr	0.0	2.2		−0.3	1.5		−0.3	−1.7	1.1	0.642
Dominant eye	Scr	5.4	2.4		6.0	1.6		—	—	—	0.430
	6w	5.1	1.5		5.4	1.1		0.2	−0.8	1.2	0.634
	6w − Scr	−0.3	2.7		−0.6	1.4		−0.3	−1.9	1.3	0.730
Non-dominant eye	Scr	4.9	2.2		5.8	1.1		—	—	—	0.170
	6w	5.4	1.7		5.9	2.0		0.2	−1.2	1.5	0.826
	6w − Scr	0.4	2.1		0.0	2.0		−0.4	−1.9	1.1	0.621
Right eye	Scr	5.0	2.1		6.0	1.6		—	—	—	0.193
	6w	5.1	1.6		5.4	1.1		0.0	−1.0	0.9	0.990
	6w − Scr	0.0	1.9		−0.6	1.4		−0.6	−1.8	0.7	0.352
Left eye	Scr	5.3	2.4		5.9	1.0		—	—	—	0.428
	6w	5.4	1.6		5.9	2.0		0.4	−1.0	1.8	0.536
	6w − Scr	0.0	2.9		0.0	2.0		−0.1	−1.9	1.8	0.945
Percentage of pupillary response/near point post-VDT use (%/D)											
Average of both eyes	Scr	4.9	1.7		5.6	1.3		—	—	—	0.219
	6w	5.2	2.0		6.1	1.8		0.5	−0.8	1.8	0.427
	6w − Scr	0.3	1.9		0.4	1.8		0.2	−1.2	1.6	0.794
Dominant eye	Scr	5.0	1.9		5.5	1.5		—	—	—	0.485
	6w	5.3	2.2		5.7	1.4		0.1	−1.1	1.3	0.812
	6w − Scr	0.3	2.2		0.2	1.2		−0.1	−1.4	1.3	0.924
Non-dominant eye	Scr	4.8	1.7		5.8	1.2		—	—	—	0.078
	6w	5.0	1.8		6.4	2.5		1.0	−0.7	2.7	0.232

		Placebo group (*n* = 15)		Active group (*n* = 15)		EMM	95% CI−	95% CI+	*P* value
		Mean	SD	Mean	SD				
	6w − Scr	0.2	2.0	0.6	2.5	0.4	−1.3	2.1	0.622
Right eye	Scr	4.8	1.9	5.4	1.6	—	—	—	0.364
	6w	5.4	2.0	5.8	1.4	0.1	−1.0	1.1	0.911
	6w − Scr	0.5	1.8	0.4	1.2	−0.2	−1.3	1.0	0.771
Left eye	Scr	5.0	1.7	5.8	1.1	—	—	—	0.127
	6w	5.0	2.0	6.3	2.5	1.1	−0.7	2.9	0.212
	6w − Scr	0.0	2.3	0.5	2.6	0.5	−1.3	2.3	0.564
Change in percentage of pupillary response/near point pre- and post-VDT use (post-VDT use − pre-VDT use) (%/D)									
Average of both eyes	Scr	−0.3	0.8	−0.3	1.2	—	—	—	0.956
	6w	0.0	0.8	0.4	1.8	0.5	−0.5	1.5	0.347
	6w − Scr	0.2	1.0	0.7	2.6	0.5	−1.0	2.0	0.495
Dominant eye	Scr	−0.4	1.2	−0.5	1.5	—	—	—	0.791
	6w	0.3	1.0	0.3	1.1	0.1	−0.7	0.9	0.869
	6w − Scr	0.6	1.3	0.9	2.3	0.2	−1.2	1.6	0.753
Non-dominant eye	Scr	−0.1	1.0	−0.1	1.0	—	—	—	0.793
	6w	−0.3	1.1	0.5	2.9	0.9	−0.8	2.6	0.276
	6w − Scr	−0.2	1.1	0.6	3.4	0.8	−1.1	2.7	0.404
Right eye	Scr	−0.2	1.0	−0.5	1.5	—	—	—	0.483
	6w	0.3	1.0	0.4	1.1	0.0	−0.8	0.9	0.916
	6w − Scr	0.5	1.2	0.9	2.3	0.4	−0.9	1.8	0.529
Left eye	Scr	−0.4	1.2	−0.1	1.0	—	—	—	0.468
	6w	−0.4	1.0	0.5	2.9	1.0	−0.7	2.6	0.248
	6w − Scr	0.0	1.3	0.5	3.4	0.6	−1.4	2.5	0.544

The data are presented as the mean and standard deviation (SD).
The data of differences between active and placebo groups (Δactive group − placebo group) are presented as the estimated marginal means (EMM), whereas the 95% confidence interval (95% CI) was based on the EMM.
The data at Scr and 6w – Scr were analyzed using Student's *t*-test. As for the data at 6w, Scr values are utilized as covariates, and the group is utilized as a factor in the ANCOVA.

Table 3–3. The results of BUT and Schirmer's value

		ebo group (*n* =15)		Active group (*n* = 15)		EMM	95% CI−	95% CI+	*P* value
		Mean	SD	Mean	SD				
Tear film break-up time (BUT) (s)									
Average of both eyes	Scr	5.6	4.3	6.5	3.7	—	—	—	0.544
	6w	7.1	3.6	7.2	3.5	−0.2	−2.6	2.2	0.858
	6w − Scr	1.5	3.7	0.7	4.3	−0.8	−3.8	2.2	0.604
Dominant eye	Scr	5.6	4.2	7.3	4.1	—	—	—	0.282
	6w	7.5	3.7	7.5	3.4	−0.5	−3.1	2.1	0.684
	6w − Scr	1.9	4.2	0.3	4.5	−1.7	−5.0	1.6	0.307
Non-dominant eye	Scr	5.6	4.3	5.7	3.9	—	—	—	0.930
	6w	6.7	3.8	6.9	3.7	0.2	−2.3	2.7	0.867
	6w − Scr	1.1	3.6	1.2	4.2	0.1	−2.8	3.1	0.926
Right eye	Scr	5.7	4.3	7.1	4.0	—	—	—	0.364
	6w	7.6	3.6	7.5	3.4	−0.5	−3.0	2.1	0.705
	6w − Scr	1.9	4.2	0.5	4.6	−1.5	−4.8	1.8	0.371
Left eye	Scr	5.5	4.3	5.9	4.1	—	—	—	0.795
	6w	6.6	3.9	6.9	3.7	0.1	−2.3	2.6	0.909
	6w − Scr	1.1	3.6	1.0	4.2	−0.1	−3.0	2.9	0.963
Schirmer's value (mm)									
Average of both eyes	Scr	10.7	9.6	11.4	9.1	—	—	—	0.847
	6w	12.2	10.8	11.3	9.7	−1.5	−5.2	2.1	0.391
	6w − Scr	1.5	4.0	−0.1	5.4	−1.6	−5.1	2.0	0.376
Dominant eye	Scr	10.3	9.6	11.6	10.4	—	—	—	0.718
	6w	12.3	12.4	12.1	10.4	−1.4	−6.2	3.3	0.546
	6w − Scr	2.0	6.3	0.5	6.2	−1.5	−6.1	3.2	0.523
Non-dominant eye	Scr	11.1	10.5	11.1	9.2	—	—	—	1.000
	6w	12.1	10.3	10.4	9.4	−1.7	−5.7	2.4	0.404
	6w − Scr	0.9	4.0	−0.7	6.7	−1.7	−5.8	2.4	0.414
Right eye	Scr	9.5	9.0	11.8	9.9	—	—	—	0.505
	6w	11.8	10.7	11.8	10.4	−2.2	−6.5	2.1	0.307
	6w − Scr	2.3	5.4	0.0	5.9	−2.3	−6.6	1.9	0.269
Left eye	Scr	11.9	10.9	10.9	9.7	—	—	—	0.792
	6w	12.5	12.0	10.7	9.4	−0.9	−5.4	3.6	0.678
	6w − Scr	0.6	5.0	−0.2	6.9	−0.8	−5.3	3.7	0.720

The data are presented as the mean and standard deviation (SD).

The data of differences between active and placebo groups (Δactive group − placebo group) are presented as the estimated marginal means (EMM), whereas the 95% confidence interval (95% CI) was based on the EMM.

The data at Scr and 6w – Scr were analyzed using Student's *t*-test. As for the data at 6w, Scr values are utilized as covariates, and the group is utilized as a factor in the ANCOVA

(2) **Muscle hardness:**

The results of the muscle hardness are shown in Table 3-4.

After the 6-week intervention, the muscle hardness of both shoulders post-VDT use in the active group was significantly higher than in the placebo group (active group, 66.8 ± 4.9 mm/10; placebo group, 61.9 ± 4.0 mm/10; P = 0.007). Also, the muscle hardness of the right shoulder post-VDT use in the active group was

significantly higher than that in the placebo group (active group, 66.6 ± 5.4 mm/10; placebo group, 62.2 ± 4.6 mm/10; *P* = 0.027). Moreover, the muscle hardness of the left shoulder post-VDT use in the active group was significantly higher than the placebo group (active group, 66.9 ± 5.7 mm/10; placebo group, 61.6 ± 4.7 mm/10; *P* = 0.011).

Table 3–4. The results of muscle hardness test

		Placebo group (n = 15)		Active group (n = 15)		EMM	95% CI−	95% CI+	P value
		Mean	SD	Mean	SD				
Muscle hardness pre-VDT use (mm/10)									
Average of both shoulders	Scr	57.7	3.7	58.1	4.8	—	—	—	0.786
	6w	59.2	5.5	61.2	4.3	1.8	−1.6	5.2	0.279
	6w − Scr	1.5	5.1	3.1	4.7	1.6	−2.1	5.3	0.378
Right shoulder	Scr	57.9	3.7	57.9	5.1	—	—	—	0.968
	6w	60.4	5.8	61.1	5.2	0.7	−3.3	4.7	0.719
	6w − Scr	2.5	5.9	3.2	5.9	0.7	−3.7	5.1	0.758
Left shoulder	Scr	57.5	4.8	58.3	5.0	—	—	—	0.657
	6w	57.9	6.4	61.3	5.8	2.9	−1.3	7.1	0.167
	6w − Scr	0.4	5.6	2.9	6.2	2.5	−1.9	7.0	0.252
Muscle hardness post-VDT use (mm/10)									
Average of both shoulders	Scr	59.5	4.3	60.1	5.7	—	—	—	0.734
	6w	61.9	4.0	66.8	4.9	4.8	1.4	8.2	0.007**
	6w − Scr	2.4	5.6	6.7	6.8	4.2	−0.5	8.9	0.075
Right shoulder (mm/10)	Scr	59.3	5.6	59.7	6.9	—	—	—	0.841
	6w	62.2	4.6	66.6	5.4	4.3	0.5	8.1	0.027*
	6w − Scr	2.9	7.3	6.9	7.4	3.9	−1.6	9.4	0.154
Left shoulder	Scr	59.7	3.9	60.5	5.2	—	—	—	0.638
	6w	61.6	4.7	66.9	5.7	5.3	1.3	9.2	0.011*
	6w − Scr	1.9	5.9	6.5	7.5	4.5	−0.5	9.6	0.075
Changes in muscle hardness pre- and post-VDT use (post-VDT use − pre-VDT use) (mm/10)									
Average of both shoulders	Scr	1.8	3.0	2.0	4.7	—	—	—	0.889
	6w	2.7	6.5	5.6	3.6	2.9	−0.9	6.7	0.125
	6w − Scr	1.0	8.2	3.6	6.7	2.6	−3.0	8.2	0.344
Right shoulder	Scr	1.4	4.6	1.8	6.2	—	—	—	0.842
	6w	1.8	6.6	5.5	5.4	3.9	−0.3	8.0	0.070
	6w − Scr	0.4	10.2	3.7	9.1	3.3	−4.0	10.5	0.364
Left shoulder	Scr	2.1	3.9	2.1	4.3	—	—	—	1.000
	6w	3.7	7.7	5.7	6.5	2.0	−3.4	7.4	0.453
	6w − Scr	1.5	8.5	3.5	6.8	2.0	−3.8	7.8	0.485

The data are presented as the mean and standard deviation (SD).

The data of differences between active and placebo groups (Δactive group − placebo group) are presented as the estimated marginal means (EMM), whereas the 95% confidence interval (95% CI) was based on the EMM.

The data at Scr and 6w – Scr were analyzed using Student's *t*-test. As for the data at 6w, Scr values are utilized as covariates, and the group is utilized as a factor in the ANCOVA.

***P* < 0.01 and **P* < 0.05 vs. the placebo group.

(3) **Subjective symptoms (questionnaire)**

The results of the questionnaire are shown in Table 3–5.

After the 6-week intervention, the scale numbers of the question "A sensation of dry eyes" post-VDT use had a median of 1.0 (Q1–Q3, 0.0–1.0) in the active group and 0.0 (Q1–Q3, 0.0–0.5) in the placebo group. This was significantly higher in the active group than in the placebo group (P = 0.034)

Table 3–5. The results of the questionnaire

		Placebo group (n = 15)			Active group (n = 15)			P value
		Median	**Q1**	**Q3**	**Median**	**Q1**	**Q3**	
Pre-VDT use								
A tiredness sensation in the eyes	Scr	4.0	3.0	5.0	4.0	3.0	4.5	0.959
	6w	3.0	2.5	4.0	4.0	3.0	4.0	0.708
	6w − Scr	−1.0	−1.0	0.5	0.0	−1.5	1.0	0.846
A sensation of dry eyes	Scr	3.0	2.0	3.0	2.0	2.0	3.0	0.911
	6w	4.0	2.5	4.0	4.0	3.0	4.0	0.974
	6w − Scr	1.0	0.0	1.0	1.0	0.0	1.5	0.863
Objects appear to be blurred, hazy, or doubled	Scr	2.0	1.5	3.5	2.0	1.5	3.5	0.992
	6w	3.0	1.5	4.0	3.0	2.0	4.0	0.891
	6w − Scr	1.0	0.0	1.0	0.0	−0.5	2.0	0.910
Watery eyes	Scr	2.0	2.0	3.5	3.0	2.0	3.5	0.493
	6w	3.0	2.0	3.5	3.0	2.0	3.5	0.947
	6w − Scr	0.0	−1.0	1.0	0.0	−1.5	1.5	0.861
A sensation of trouble in focusing the eyes	Scr	2.0	1.0	2.5	2.0	2.0	3.0	0.227
	6w	3.0	2.0	3.5	3.0	2.0	4.0	0.903
	6w − Scr	1.0	0.0	1.0	0.0	−1.0	1.0	0.384
Difficulty in seeing objects in one's hand and nearby or fine print	Scr	1.0	1.0	2.0	2.0	1.5	3.0	0.128
	6w	2.0	1.0	3.0	2.0	1.0	3.5	0.960
	6w − Scr	0.0	0.0	1.0	0.0	0.0	0.0	0.300
Easily feeling dazzled by the light	Scr	2.0	1.0	4.0	2.0	1.5	3.5	0.873
	6w	2.0	1.0	3.0	2.0	1.0	3.0	0.943
	6w − Scr	0.0	−1.5	1.5	0.0	−1.0	0.0	0.951
Stiffness in the neck and shoulders	Scr	4.0	2.0	5.0	4.0	3.0	5.0	0.418
	6w	4.0	2.5	4.5	4.0	3.0	5.0	0.471
	6w − Scr	0.0	−1.0	1.5	0.0	−1.0	1.0	0.779
Feeling fatigued in the back of the	Scr	3.0	1.0	3.5	3.0	2.0	4.0	0.433

		Placebo group (n = 15)			Active group (n = 15)			P value
		Median	**Q1**	**Q3**	**Median**	**Q1**	**Q3**	
eyes	6w	3.0	2.0	4.0	3.0	2.0	3.5	0.830
	6w – Scr	0.0	−1.0	1.0	0.0	−1.0	1.0	0.500
Laziness	Scr	2.0	1.0	3.5	3.0	1.0	3.0	0.883
	6w	2.0	1.5	2.5	2.0	1.0	3.0	0.963
	6w – Scr	0.0	−1.0	0.5	0.0	−1.0	0.5	0.956
Inability to concentrate	Scr	2.0	1.0	3.5	2.0	1.0	4.0	0.829
	6w	2.0	2.0	3.0	2.0	1.5	3.0	0.999
	6w – Scr	0.0	−1.0	1.0	0.0	−0.5	1.0	0.910
Objects feeling cold to the hands and feet	Scr	2.0	1.0	2.5	2.0	1.0	3.0	0.908
	6w	3.0	1.0	4.0	3.0	1.0	4.0	0.827
	6w – Scr	0.0	0.0	2.0	0.0	0.0	1.0	0.870
Post-VDT use								
A tiredness sensation in the eyes	Scr	4.0	4.0	4.5	4.0	4.0	5.0	0.291
	6w	4.0	3.0	4.0	5.0	4.0	5.5	0.137
	6w – Scr	0.0	−0.5	0.5	0.0	−1.0	1.0	0.738
A sensation of dry eyes	Scr	4.0	3.0	4.5	4.0	3.5	4.0	0.542
	6w	3.0	2.0	4.5	4.0	4.0	5.0	0.115
	6w – Scr	0.0	−1.0	1.0	0.0	−0.5	1.0	0.563
Objects appear to be blurred, hazy, or doubled	Scr	2.0	1.0	3.0	3.0	2.0	4.0	0.390
	6w	3.0	2.0	4.0	3.0	3.0	4.0	0.778
	6w – Scr	0.0	0.0	2.5	1.0	0.0	2.0	0.794
Watery eyes	Scr	4.0	2.5	4.5	4.0	3.5	4.5	0.777
	6w	4.0	2.5	5.0	3.0	3.0	4.5	0.864
	6w – Scr	0.0	−1.5	1.0	0.0	−1.0	0.5	0.624
A sensation of trouble in focusing the eyes	Scr	2.0	1.0	3.0	2.0	2.0	3.5	0.227
	6w	3.0	1.0	4.5	3.0	2.0	4.0	0.852
	6w – Scr	0.0	0.0	2.0	0.0	0.0	1.5	0.709
Difficulty in seeing objects in one's hand and nearby or fine print	Scr	2.0	1.0	3.0	2.0	2.0	3.0	0.294
	6w	2.0	1.0	3.0	3.0	2.0	4.0	0.111
	6w – Scr	0.0	0.0	0.5	0.0	0.0	1.0	0.461
Easily feeling dazzled by the light	Scr	3.0	2.0	4.0	2.0	1.5	3.0	0.160

		Placebo group (n = 15)			Active group (n = 15)			P value
		Median	**Q1**	**Q3**	**Median**	**Q1**	**Q3**	
	6w	2.0	1.0	4.0	2.0	1.5	3.5	0.793
	6w – Scr	−1.0	−2.0	1.5	0.0	0.0	0.0	0.445
Stiffness in the neck and shoulders	Scr	4.0	4.0	5.0	4.0	4.0	5.0	0.890
	6w	4.0	3.5	5.0	4.0	3.0	5.0	0.745
	6w – Scr	0.0	−1.0	0.0	0.0	−0.5	0.0	0.838
Feeling fatigued in the back of the eyes	Scr	3.0	3.0	4.0	4.0	3.0	5.0	0.523
	6w	3.0	2.5	4.0	4.0	4.0	5.0	0.057
	6w – Scr	0.0	−1.0	1.0	0.0	0.0	1.0	0.496
Laziness	Scr	2.0	1.0	3.0	2.0	1.5	3.5	0.617
	6w	2.0	1.0	3.0	3.0	2.0	4.0	0.068
	6w – Scr	0.0	−0.5	1.0	1.0	0.0	1.0	0.403
Inability to concentrate	Scr	3.0	1.0	3.0	3.0	2.0	4.0	0.412
	6w	3.0	1.0	3.5	3.0	2.0	4.0	0.225
	6w – Scr	0.0	−1.0	1.5	0.0	0.0	1.0	0.758
Objects feeling cold to the hands and feet	Scr	2.0	1.0	3.0	1.0	1.0	3.0	0.971
	6w	1.0	1.0	4.0	2.0	1.0	4.0	0.955
	6w – Scr	0.0	0.0	1.5	0.0	0.0	1.0	0.854
Changes between pre- and post-VDT use (post-VDT use – pre-VDT use)								
A tiredness sensation in the eyes	Scr	0.0	−0.5	1.0	1.0	0.0	1.0	0.650
	6w	0.0	−0.5	1.0	1.0	0.0	2.0	0.183
	6w – Scr	0.0	−1.0	1.0	1.0	0.0	1.0	0.452
A sensation of dry eyes	Scr	1.0	0.0	1.0	1.0	1.0	2.0	0.212
	6w	0.0	0.0	0.5	1.0	0.0	1.0	0.034*
	6w – Scr	−1.0	−1.5	0.0	−1.0	−1.0	0.0	0.444
Objects appear to be blurred, hazy, or doubled	Scr	0.0	−1.0	0.5	0.0	0.0	1.0	0.283
	6w	0.0	0.0	1.0	0.0	−0.5	1.0	0.964
	6w – Scr	0.0	−1.0	2.0	−1.0	−1.0	1.0	0.456
Watery eyes	Scr	0.0	0.0	2.0	1.0	0.0	2.0	0.929
	6w	0.0	0.0	2.5	1.0	0.0	2.0	0.628
	6w – Scr	0.0	−1.0	1.0	0.0	−1.0	1.0	0.595
A sensation of trouble in focusing	Scr	0.0	−1.0	0.5	0.0	−1.0	1.0	0.734

		Placebo group (n = 15)			Active group (n = 15)			P value
		Median	**Q1**	**Q3**	**Median**	**Q1**	**Q3**	
the eyes	6w	0.0	0.0	1.0	0.0	−0.5	1.0	0.993
	6w − Scr	0.0	−1.0	1.5	1.0	−1.0	1.0	0.993
Difficulty in seeing objects in one's	Scr	0.0	0.0	1.0	0.0	0.0	0.0	0.571
hand and nearby or fine print	6w	0.0	−0.5	0.0	0.0	0.0	1.0	0.221
	6w − Scr	0.0	−1.0	0.0	0.0	0.0	1.0	0.110
Easily feeling dazzled by the light	Scr	0.0	0.0	1.0	0.0	−0.5	0.5	0.507
	6w	0.0	0.0	1.0	0.0	0.0	1.0	0.957
	6w − Scr	0.0	−1.0	1.5	0.0	0.0	1.0	0.730
Stiffness in the neck and shoulders	Scr	0.0	−0.5	2.0	0.0	0.0	0.5	0.439
	6w	0.0	0.0	1.0	0.0	0.0	1.0	0.805
	6w − Scr	0.0	−1.0	1.0	0.0	−1.0	1.0	0.609
Feeling fatigued in the back of the	Scr	0.0	0.0	2.0	1.0	0.0	1.0	0.756
eyes	6w	0.0	−1.0	1.5	1.0	0.5	1.5	0.089
	6w − Scr	0.0	−1.5	0.0	0.0	0.0	1.0	0.061
Laziness	Scr	0.0	−1.0	0.0	0.0	−1.0	0.0	0.955
	6w	0.0	-0.5	1.0	0.0	0.0	1.5	0.158
	6w − Scr	0.0	0.0	1.0	1.0	0.0	1.5	0.243
Inability to concentrate	Scr	0.0	−0.5	0.0	0.0	0.0	1.5	0.318
	6w	0.0	−1.0	1.0	0.0	0.0	1.5	0.234
	6w − Scr	0.0	−1.0	1.0	0.0	−0.5	1.0	0.735
Objects feeling cold to the hands	Scr	0.0	−0.5	0.0	0.0	−0.5	1.0	0.514
and feet	6w	0.0	−1.0	0.0	0.0	−1.0	0.0	0.911
	6w − Scr	0.0	−1.0	1.0	0.0	−1.0	0.0	0.505

The data are presented as median (Median), first quartile (Q1), and third quartile (Q3).

1, strongly disagree; 2, disagree; 3, slightly disagree; 4, slightly agree; 5, agree; 6, strongly agree

*P < 0.05 vs. the placebo group.

(4) **Safety assessment:**

Even though some items in the safety assessment indicated significant differences between groups, the mean values were still within the adequate or reference ranges, and these were not medically problematic. In addition, no adverse effects were observed with continued ingestion of the supplement (Tables 4–1–4–4).

Table 4–1. The results of the physical examination

		Placebo group (*n* = 15)		Active group (*n* = 15)		*P* value
		Mean	SD	Mean	SD	
Body weight (kg)	Scr	58.9	9.5	65.3	9.9	0.080
	6w	59.2	9.2	65.1	9.2	0.796
BMI (kg/m²)	Scr	22.0	2.5	23.0	3.5	0.357
	6w	22.1	2.4	22.9	3.3	0.543
Body fat percentage (%)	Scr	22.6	5.4	23.6	7.5	0.693
	6w	22.7	5.5	23.3	7.4	0.729
Systolic blood pressure (mmHg)	Scr	107.6	9.0	114.3	14.6	0.138
	6w	110.4	10.8	111.5	10.9	0.174
Diastolic blood pressure (mmHg)	Scr	68.3	7.4	73.9	11.2	0.122
	6w	72.4	10.7	72.1	11.8	0.017*
Pulse rate (bpm)	Scr	70.7	7.6	66.6	7.6	0.148
	6w	70.6	8.5	68.2	6.4	0.918
Body temperature (°C)	Scr	36.4	0.3	36.5	0.3	0.508
	6w	36.3	0.3	36.5	0.2	0.231

The data are presented as the mean and standard deviation (SD); The data at Scr were analyzed using Student's *t*-test. As for the data at 6w, Scr values are utilized as covariates, and the group is utilized as a factor in the ANCOVA.

*$P < 0.05$ vs. the placebo groupT

T**able 4–2.** The results of the urinalysis

	Reference range		Placebo group (*n* = 15)		Active group (*n* = 15)		*P* value
			Within the reference range	Outside the reference range	Within the reference range	Outside the reference range	
Protein	(−)	Scr	14	1	13	2	1.000
		6w	14	1	15	0	1.000
Glucose	(−)	Scr	15	0	15	0	N.A.
		6w	15	0	15	0	N.A.
Urobilinogen	(±)	Scr	15	0	15	0	N.A.
		6w	15	0	15	0	N.A.
Bilirubin	(−)	Scr	15	0	15	0	N.A.
		6w	15	0	15	0	N.A.
pH	5.0-7.5	Scr	15	0	14	1	1.000
		6w	14	1	15	0	1.000
pH	(−)	Scr	12	3	13	2	1.000
		6w	12	3	14	1	0.598
Ketone bodies	(−)	Scr	15	0	15	0	N.A.
		6w	15	0	15	0	N.A.

The data are presented as the number of subjects.

N.A., not available

Table 4–3. The results of the blood analysis

	Reference range		Placebo group (*n* = 15)		Active group (*n* = 15)		*P* value
			Mean	SD	Mean	SD	
Leukocyte count (/μL)	3300-9000	Scr	5100.0	1489.5	5440.0	1746.3	0.571
		6w	5526.7	1204.4	5193.3	1455.3	0.149
Erythrocyte count (×10⁴/μL)	Men: 430-570 Women: 380-500	Scr	457.4	46.0	476.0	70.6	0.400
		6w	462.3	45.1	484.8	74.4	0.550
Hemoglobin (g/dL)	Men: 13.5-17.5 Women: 11.5-15.0	Scr	13.7	1.5	14.0	1.7	0.649
		6w	13.7	1.5	14.2	1.8	0.260
Hematocrit value (%)	Men: 39.7-52.4 Women: 34.8-45.0	Scr	42.1	3.8	43.1	4.1	0.485
		6w	42.1	3.5	43.6	4.8	0.482
Platelet count (×10⁴/μL)	14.0-34.0	Scr	26.6	3.0	26.1	6.3	0.783
		6w	27.0	3.4	27.7	6.2	0.246
MCV (fl)	85-102	Scr	92.5	5.1	91.5	7.6	0.696
		6w	91.5	6.2	90.7	8.1	0.795
MCH (pg)	28.0-34.0	Scr	30.0	2.0	29.6	2.9	0.650
		6w	29.7	2.6	29.5	3.1	0.277
MCHC (%)	30.2-35.1	Scr	32.5	1.3	32.4	1.3	0.744
		6w	32.4	1.4	32.5	0.9	0.484
Percentage of neutrophils (%)	40.0-75.0	Scr	60.1	7.5	58.6	6.2	0.570
		6w	63.0	10.8	58.6	7.0	0.245
Percentage of lymphocytes (%)	18.0-49.0	Scr	30.6	7.2	33.5	6.4	0.251
		6w	28.0	8.7	32.4	7.6	0.354
Percentage of monocytes (%)	2.0-10.0	Scr	6.0	1.3	5.0	1.7	0.094
		6w	5.5	1.3	5.6	1.2	0.310
Percentages of eosinophils (%)	0.0-8.0	Scr	2.7	1.7	2.3	1.2	0.507
		6w	2.9	2.8	2.7	2.0	0.936
Percentages of basophils (%)	0.0-2.0	Scr	0.6	0.4	0.5	0.4	0.303
		6w	0.6	0.3	0.7	0.4	0.482
Neutrophil count (/μL)	-	Scr	3090.0	1042.8	3276.2	1446.2	0.689
		6w	3527.5	1192.1	3102.2	1190.1	0.153
Lymphocyte count (/μL)	-	Scr	1532.9	501.2	1735.2	269.5	0.179
		6w	1512.4	517.8	1619.7	333.1	0.436
Monocyte count (/μL)	-	Scr	302.2	108.6	266.7	103.8	0.368
		6w	304.6	96.0	289.8	90.8	0.847
Eosinophil count (/μL)	-	Scr	146.4	126.5	137.9	105.6	0.843
		6w	149.8	130.1	150.4	135.5	0.849
Basophil count (/μL)	-	Scr	28.5	13.4	24.0	18.6	0.449
		6w	32.3	18.2	31.4	12.7	0.980
AST (GOT) (U/L)	10-40	Scr	19.0	4.2	20.2	9.6	0.660
		6w	20.1	6.5	20.3	7.7	0.637

	Reference range		Placebo group (*n* = 15)		Active group (*n* = 15)		*P* value
			Mean	SD	Mean	SD	
ALT (GPT) (U/L)	5-45	Scr	16.9	10.1	19.7	19.3	0.615
		6w	19.6	16.8	19.4	13.3	0.615
γ-GT (γ-GTP) (U/L)	Men: ≤80	Scr	20.1	18.3	20.1	12.3	0.991
	Women: ≤30	6w	19.7	14.3	19.5	10.5	0.950
ALP (U/L)	100-325	Scr	168.9	41.4	168.9	50.8	1.000
		6w	168.7	37.0	168.0	49.1	0.887
LD (LDH) (U/L)	120-240	Scr	172.3	27.8	180.8	26.8	0.403
		6w	164.5	29.6	174.7	19.3	0.451
LAP (U/L)	Men: 45-81	Scr	48.0	10.7	50.7	6.1	0.407
	Women: 37-61	6w	45.9	9.6	49.7	6.9	0.195
Total bilirubin (mg/dL)	0.2-1.2	Scr	0.85	0.29	0.85	0.22	1.000
		6w	0.87	0.35	0.89	0.29	0.878
Direct bilirubin (mg/dL)	0.0-0.2	Scr	0.08	0.04	0.10	0.04	0.178
		6w	0.09	0.05	0.09	0.05	0.394
Indirect bilirubin (mg/dL)	0.2-1.0	Scr	0.77	0.27	0.75	0.20	0.818
		6w	0.78	0.33	0.79	0.26	0.720
Cholinesterase (ChE) (U/L)	Men: 234-493	Scr	290.6	57.9	305.9	53.5	0.457
	Women: 200-452	6w	291.0	59.2	316.3	45.4	0.177
Total protein (g/dL)	6.7-8.3	Scr	7.2	0.2	7.0	0.4	0.245
		6w	7.2	0.3	7.1	0.4	0.460
Urea nitrogen (mg/dL)	8.0-20.0	Scr	11.7	3.2	12.1	2.1	0.662
		6w	12.2	3.4	12.7	2.7	0.849
Creatinine (mg/dL)	Men: 0.61-1.04	Scr	0.71	0.12	0.77	0.10	0.140
	Women: 0.47-0.79	6w	0.71	0.12	0.79	0.09	0.196
Uric acid (mg/dL)	Men: 3.8-7.0	Scr	4.5	1.2	5.5	1.2	0.027*
	Women: 2.5-7.0	6w	4.7	1.4	5.5	1.2	0.399
CK (U/L)	Men: 60-270	Scr	119.4	57.4	119.7	63.6	0.990
	Women: 40-150	6w	126.9	94.0	115.2	68.3	0.570
Sodium (mEq/L)	137-147	Scr	139.5	1.1	140.5	2.2	0.155
		6w	138.5	1.4	139.8	2.1	0.234
Potassium (mEq/L)	3.5-5.0	Scr	4.4	0.4	4.5	0.5	0.659
		6w	4.7	0.3	4.8	0.3	0.825
Chloride (mEq/L)	98-108	Scr	101.1	2.6	102.1	1.4	0.174
		6w	100.9	2.0	102.3	2.0	0.170
Calcium (mg/dL)	8.4-10.4	Scr	9.2	0.3	9.2	0.2	0.717
		6w	9.3	0.3	9.4	0.3	0.497
Inorganic phosphorus (mg/dL)	2.5-4.5	Scr	3.3	0.5	3.2	0.5	0.485
		6w	3.3	0.3	3.2	0.4	0.398
Serum iron (μg/dL)	Men: 50-200	Scr	97.5	44.3	105.0	40.4	0.633

	Reference range		Placebo group (*n* = 15)			Active group (*n* = 15)			*P* value
			Mean	SD		Mean	SD		
	Women: 40-180	6w	88.7	36.9		110.7	33.2		0.099
Serum amylase (U/L)	40-122	Scr	80.6	24.4		82.7	22.9		0.807
		6w	78.1	19.1		89.2	24.8		0.051
Total cholesterol (mg/dL)	120-219	Scr	185.9	19.6		204.3	26.0		0.037*
		6w	182.3	21.9		206.6	31.2		0.305
HDL cholesterol (mg/dL)	Men: 40-85 Women: 40-95	Scr	64.3	13.7		67.1	13.6		0.579
		6w	64.0	14.6		68.7	13.6		0.381
LDL cholesterol (mg/dL)	65-139	Scr	103.2	17.5		119.2	24.3		0.048*
		6w	104.9	22.8		122.9	27.5		0.795
Triglyceride (mg/dL)	30-149	Scr	69.1	38.5		78.2	51.6		0.587
		6w	63.3	36.8		73.1	45.5		0.728
Glucose (mg/dL)	70-109	Scr	80.7	4.6		80.5	5.4		0.943
		6w	79.9	5.4		84.5	8.1		0.020*
HbA1c (NGSP) (%)	4.6-6.2	Scr	5.2	0.3		5.3	0.2		0.127
		6w	5.2	0.3		5.3	0.2		0.485
Glycoalbumin (%)	12.3-16.5	Scr	13.7	1.1		13.4	1.2		0.499
		6w	13.5	0.9		13.2	1.0		0.847

The data are presented as the mean and standard deviation (SD).

The data at Scr were analyzed using Student's *t*-test. As for the data at 6w, Scr values are utilized as covariates, and the group is utilized as a factor in the ANCOVA.

*$P < 0.05$ vs. the placebo group.

Table 4–4. The results of visual acuity and intraocular pressure

		Placebo group (*n* = 15)			Active group (*n* = 15)			*P* value
		Mean	SD		Mean	SD		
Visual acuity								
Visual acuity of average of both eyes	Scr	0.54	0.38		0.62	0.57		0.652
	6w	0.53	0.39		0.57	0.50		0.566
Visual acuity of dominant eye	Scr	0.54	0.43		0.62	0.56		0.665
	6w	0.53	0.48		0.57	0.51		0.603
Visual acuity of non-dominant eye	Scr	0.54	0.38		0.62	0.57		0.653
	6w	0.53	0.39		0.57	0.51		0.750
Visual acuity of right eye	Scr	0.49	0.39		0.63	0.56		0.455
	6w	0.47	0.41		0.57	0.51		0.802
Visual acuity of	Scr	0.59	0.41		0.61	0.57		0.885

		Placebo group (*n* = 15)			Active group (*n* = 15)			*P* value
		Mean	SD		Mean	SD		
left eye	6w	0.59	0.45		0.57	0.51		0.558
Intraocular pressure								
Intraocular pressure of average of both eyes (mmHg)	Scr	14.5	4.3		14.2	5.0		0.854
	6w	14.5	3.6		14.0	4.0		0.638
Intraocular pressure of dominant eye (mmHg)	Scr	14.9	4.5		14.3	5.0		0.732
	6w	14.3	3.2		14.2	4.2		0.615
Intraocular pressure of non-dominant eye (mmHg)	Scr	14.2	4.4		14.1	5.1		0.985
	6w	14.8	4.2		13.8	3.9		0.270
Intraocular pressure of right eye (mmHg)	Scr	15.0	4.2		14.4	5.1		0.720
	6w	15.0	4.1		14.0	4.1		0.542
Intraocular pressure of left eye (mmHg)	Scr	14.0	4.6		14.0	5.0		0.994
	6w	14.0	3.3		13.9	4.0		0.884

The data are presented as the mean and standard deviation (SD).
The data at Scr were analyzed using Student's *t*-test. As for the data at 6w, Scr values are utilized as covariates, and the group is utilized as a factor in the ANCOVA.

Subgroup analysis (the accommodative function tabulated for each eye): The accommodative function tabulated for each eye can be seen in Table 5.

At Scr, the percentage of pupillary response/near point (%/D) and the percentage of pupillary response/near point (logarithmic conversion) pre-VDT use of the active group were significantly higher than those of the placebo group (active group, 5.9 ± 1.3; placebo group, 4.8 ± 1.8; $P = 0.012$, active group, 1.8 ± 0.2; placebo group, 1.5 ± 0.4; $P = 0.004$). Furthermore, at Scr, the percentage of pupillary response/near point (%/D) was significantly lower than that of the placebo group (active group, 5.6 ± 1.4; placebo group, 4.7 ± 1.7; $P = 0.039$). Also, the logarithmic conversion of the percentage of pupillary response/near point (%/D) was significantly lower than that of the placebo group (active group, 1.7 ± 0.3; placebo group, 1.5 ± 0.4; $P = 0.019$).

In the amount of change (6w − Scr), the logarithmic conversion of the percentage of pupillary response and pupillary response/near point (%/D) pre-VDT use of the active group was significantly lower than that of the placebo group. The logarithmic conversion of the percentage of pupillary response of active group was −0.1 ± 0.3, and that of placebo group was 0.1 ± 0.4 ($P = 0.045$). On the other hand, the logarithmic conversion of the percentage of pupillary response/near point of active group was −0.1 ± 0.3, and that of placebo group was 0.1 ± 0.4 ($P = 0.035$). Moreover, the logarithmic conversions of the percentage of pupillary response and of the percentage of pupillary response/near point between pre- and post-VDT use of the active group were significantly lower than those of the placebo group. The logarithmic

conversion of the percentage of pupillary response of active group was 0.2 ± 0.4 and that of placebo group was 0.0 ± 0.3 (*P* = 0.043). Whereas, the logarithmic conversion of the percentage of pupillary response/near point of active group was 0.1 ± 0.4, and that of placebo group was −0.1 ± 0.3 (*P* = 0.049).

Table 5. The results of accommodative function tabulated for each eye

		Placebo group (eye = 26)		Active group (eye = 30)		EMM	95% CI−	95% CI+	*P* value
		Mean	SD	Mean	SD				
Pre-VDT use									
Percentage of pupillary response (%)	Scr	33.8	12.9	39.3	11.0	—	—	—	0.090
	6w	35.8	12.1	36.1	14.0	−3.7	−9.3	1.8	0.182
	6w − Scr	2.0	11.3	−3.2	9.6	−5.2	−10.8	0.4	0.067
Percentage of pupillary response/near point (%/D)	Scr	4.8	1.8	5.9	1.3	—	—	—	0.012*
	6w	5.3	1.3	5.6	1.6	0.0	−0.8	0.8	0.987
	6w − Scr	0.5	2.0	−0.3	1.7	−0.8	−1.8	0.2	0.108
Percentage of pupillary response (logarithmic conversion)	Scr	3.4	0.5	3.6	0.3	—	—	—	0.067
	6w	3.5	0.4	3.5	0.4	−0.1	−0.3	0.1	0.156
	6w − Scr	0.1	0.4	−0.1	0.3	−0.2	−0.4	0.0	0.045*
Percentage of pupillary response/near point (logarithmic conversion)	Scr	1.5	0.4	1.8	0.2	—	—	—	0.004**
	6w	1.6	0.3	1.7	0.2	0.0	−0.1	0.1	0.907
	6w − Scr	0.1	0.4	−0.1	0.3	−0.2	−0.4	0.0	0.035*
Post-VDT use									
Percentage of pupillary response (%)	Scr	32.5	13.3	35.9	14.0	—	—	—	0.362
	6w	34.9	12.8	37.2	13.2	−0.2	−4.8	4.4	0.926
	6w − Scr	2.4	9.2	1.3	9.2	−1.1	−6.1	3.8	0.648
Percentage of pupillary response/near point (%/D)	Scr	4.7	1.7	5.6	1.4	—	—	—	0.039*
	6w	5.2	1.8	6.1	2.0	0.5	−0.5	1.5	0.326
	6w − Scr	0.4	2.0	0.4	2.0	0.0	−1.0	1.1	0.942

		Placebo group (eye = 26)		Active group (eye = 30)		EMM	95% CI−	95% CI+	*P* value
		Mean	SD	Mean	SD				
Percentage of pupillary response (logarithmic conversion)	Scr	3.4	0.5	3.5	0.5	—	—	—	0.364
	6w	3.4	0.7	3.5	0.5	0.0	−0.2	0.3	0.810
	6w − Scr	0.0	0.6	0.0	0.3	0.0	−0.3	0.3	0.991
Percentage of pupillary response/near point (logarithmic conversion)	Scr	1.5	0.4	1.7	0.3	—	—	—	0.019*
	6w	1.6	0.5	1.8	0.3	0.1	−0.1	0.3	0.220
	6w − Scr	0.1	0.6	0.1	0.3	0.0	−0.2	0.2	0.976
Changes between pre- and post-VDT use (post-VDT use − pre-VDT use)									
Percentage of pupillary response (%)	Scr	−1.3	5.4	−3.4	6.2	—	—	—	0.180
	6w	−0.9	6.2	1.1	9.5	1.8	−2.6	6.3	0.413
	6w − Scr	0.4	7.1	4.5	12.3	4.1	−1.4	9.6	0.140
Percentage of pupillary response/near point (%/D)	Scr	−0.1	0.8	−0.3	1.3	—	—	—	0.439
	6w	−0.2	0.9	0.4	2.2	0.6	−0.4	1.5	0.233
	6w − Scr	−0.1	0.8	0.7	2.9	0.8	−0.3	2.0	0.155
Percentage of pupillary response (logarithmic conversion)	Scr	0.0	0.2	−0.1	0.2	—	—	—	0.117
	6w	−0.1	0.3	0.0	0.3	0.1	−0.1	0.3	0.167
	6w − Scr	0.0	0.3	0.2	0.4	0.2	0.0	0.4	0.043*
Percentage of pupillary response/near point (logarithmic conversion)	Scr	0.0	0.2	−0.1	0.2	—	—	—	0.437
	6w	−0.1	0.3	0.1	0.3	0.1	0.0	0.3	0.066
	6w − Scr	−0.1	0.3	0.1	0.4	0.2	0.0	0.4	0.049*

The data are presented as the mean and standard deviation (SD).
The data of differences between active and placebo groups (Δactive group − placebo group) are presented as the estimated marginal means (EMM) and the 95% confidence interval (95% CI) based on EMM.
The data at Scr and 6w – Scr were analyzed using Student's *t*-test. As for the data at 6w, Scr values are utilized as covariates, and the group is utilized as a factor in the ANCOVA.
***P* < 0.01 and **P* < 0.05 vs. the placebo group.

DISCUSSION

It is shown that visual acuity, accommodation, and accommodative function of the right eye are decreased in Japanese VDT workers such as the staff of a newspaper, so VDT use is more likely to affect the dominant eye more [32]. However, in this study, we found no clinically meaningful difference between the dominant and non-dominant eyes; thus, we analyzed one case as a single eye. In the analysis per eye, there was a significant difference in the change of the logarithmic conversion of the percentage of pupillary response pre- and post-VDT use between the two groups (0.0 in the placebo group and 0.2 in the active group, 0.96% and 1.18% as the measured value, respectively); the percentage of pupillary response in the active group was larger than that in the placebo group by 0.2 (1.23%). Therefore, consumption of the 43.2 mg of bilberry-derived anthocyanin was confirmed to inhibit the decline in accommodative function caused by VDT use and improve eye function.

One of the symptoms of eye strain caused by VDT use is a decrease in both pupillary constriction and mydriasis [33]. Pupillary constriction increases the depth of focus and contributes to the expansion of the clear vision region [34,35]. In other words, smooth pupillary constriction increases the range of distance that the subject can clearly see. In addition, the accommodative function is reported to be determined from the near-point percentage of pupillary response [24,36], and an improvement in the percentage of pupillary response can be interpreted as an improvement in the accommodative function. Clinical trials studying the consumption of anthocyanin-containing foods and eye function found that anthocyanin levels between 43.2 and 240.0 mg improved the percentage of pupillary response and the high-frequency component; both being indices of accommodative function [9,10,27,37–39]. In this study, the active capsule contained 43.2 mg/day of anthocyanin. Our results support the previous studies, but we found that lower doses of anthocyanin than in preceding studies improved the accommodative function.

Another study involving crocetin, which could also be effective for improving eye fatigue, showed that consumption of the supplement improved the microcirculatory dynamics of nutrient delivery to the ciliary muscles and to the pupillary sphincter and dilator by increasing microcirculatory blood flow [40]. Authors of that study thought the increase of microcirculatory blood flow induced the release of the tension of the muscles related to pupillary response and eye movement and consequently improved eye fatigue [40]. In addition, bilberry extract and bilberry-derived anthocyanin were reported to have a vasodilating effect by increasing the release of nitric oxide [5]; this effect was assumed to relax the ciliary muscle. Therefore, the focus adjustment function of the eyes improved because of the relaxation of muscles involved in the pupillary response. This was made possible because the intake of supplement in this study improved microcirculatory dynamics.

Under ROS-abundant conditions due to VDT use, the function of rhodopsin is reduced in photoreceptor cells [41]. Rhodopsin is involved in transmitting visual information to the brain with conformational changes on light absorption [42]. The degradation and synthesis of rhodopsin are reversible, but the delay in resynthesis

weakens visual function [43]. In other words, the decrease of eye function during and/or after VDT use happens not only because of the decrease of accommodative function but also because of the decrease of degradation and resynthesis of rhodopsin. Thus, the improvement in accommodative function after consumption of anthocyanin-containing foods may have also contributed to improved rhodopsin function. However, our results differed from previous studies using anthocyanin-containing foods likely due to the low composition of anthocyanidin glycosides (39.0 mg/capsule) contained in our supplement, causing a difference in redox balance after the intervention. Future studies could take into account the changes in the redox balance of the body after consumption of the supplement.

Although muscle hardness and subjective symptoms were used to evaluate shoulder stiffness in this study, there were various issues regarding the correlation between muscle hardness and shoulder stiffness. Shoulder stiffness is evaluated by a combination of psychological and physiological evaluations such as muscle hardness and subjective symptoms [44]. Furthermore, no consistency was found between the results of muscle hardness and subjective symptoms of "Stiffness in the neck and shoulders" in this study, but there was a relationship between hemodynamics and shoulder stiffness [45]. As for the hemodynamics, because some devices can assess blood flow rate, it is possible to verify if the supplement truly can alleviate shoulder stiffness by assessing the blood flow rate together with the bioelasticity meter in future studies.

CONCLUSIONS

The 6-week consumption of the supplement containing bilberry-derived anthocyanin on eye function in the healthy Japanese adult subjects with eye fatigue during and after VDT use was investigated in this study. Upon consumption of the supplement, improvements in the percentage of pupillary response and pupillary response/near point pre- and post-VDT use were observed. Furthermore, consumption of this supplement was found to be safe under the conditions of this study.

List of abbreviations: ANCOVA: analysis of covariance, BUT: tear film break-up time, FAS: full analysis set, ROS: reactive oxygen species, VDT: visual display terminal

Competing interests: The sponsors of this study, BGG Japan Co., Ltd. and Arysta Health and Nutrition Sciences Corp., entrusted ORTHOMEDICO, Inc., with conducting the study. Takahiro Sekikawa and Yuki Kizawa are employees of BGG Japan Co., Ltd.; Atsushi Takeoka and Takuji Sakiyama are employees of Arysta Health and Nutrition Sciences Corp.; and Yanmei Li is a member of Beijing Gingko-Group Biological Technology Co., Ltd. Takahiro Yamada (MD) is a staff of the Ario Nishiarai Eye Clinic. Takahiro Yamada was the principal investigator and monitored all the conditions of the subjects.

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