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.

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

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**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].

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

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 Bioelasticity 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|                |           |            |                   | pre-VDT use post-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 \(n = 15\))

| Age   | Placebo group | Active group |
|-------|---------------|--------------|
|       | Men | Women | Men | Women |
| 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 \(n = 15\))

|                      | Placebo group \((n = 16)\) | Active group \((n = 16)\) | \(P\) value |
|----------------------|-----------------------------|---------------------------|-------------|
| 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 | Active group | EMM | 95% CI− | 95% CI+ | P value |
|-------------------------|---------------|--------------|-----|---------|---------|---------|
|                         | (n = 15)      | (n = 15)     |     |         |         |         |
|                         | 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    | 0.858   |
|                         | 6w – Scr      | −1.2         | 12.9| −3.2    | 8.2     | 0.612   |
| Dominant eye            | Scr           | 35.6         | 13.1| 39.6    | 11.1    | 0.368   |
|                         | 6w            | 32.4         | 13.1| 34.6    | 12.3    | 0.945   |
|                         | 6w – Scr      | −3.2         | 13.4| −5.0    | 8.9     | 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    | 0.902   |
|                         | 6w – Scr      | 0.8          | 14.7| −1.4    | 10.3    | 0.638   |
| Right eye               | Scr           | 34.0         | 14.1| 39.2    | 10.8    | 0.266   |
|                         | 6w            | 32.5         | 13.5| 34.7    | 12.3    | 0.654   |
|                         | 6w – Scr      | −1.5         | 10.4| −4.5    | 9.0     | 0.408   |
| Left eye                | Scr           | 35.4         | 14.3| 39.4    | 11.5    | 0.402   |
|                         | 6w            | 34.5         | 14.0| 37.5    | 15.8    | 0.886   |
|                         | 6w – Scr      | −0.8         | 17.1| −1.9    | 10.4    | 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    | 0.774   |
|                         | 6w – Scr      | 1.4          | 9.1 | 1.3     | 8.5     | 0.978   |
| Dominant eye            | Scr           | 32.0         | 13.2| 35.1    | 14.5    | 0.545   |
|                         | 6w            | 33.8         | 14.4| 35.4    | 12.3    | 0.821   |
|                         | 6w – Scr      | 1.8          | 10.4| 0.3     | 8.0     | 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
---|---|---|---|---|---
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 3–4. The results of BUT and Schirmer’s value

|                              | Average of both eyes |Dominant eye | Non-dominant eye | Right eye | Left eye |
|------------------------------|---------------------|-------------|------------------|-----------|----------|
| **Tear film break-up time (BUT) (s)** |                     |             |                  |           |          |
| **Active group (n = 15)**    | Scr 5.6 4.3         | Scr 5.6 4.2 | Scr 5.6 4.3      | Scr 5.7 4.3 | Scr 5.5 4.3 |
|                             | 6w 7.1 3.6          | 6w 7.5 3.7  | 6w 7.5 3.7       | 6w 7.6 3.6 | 6w 6.6 3.9 |
|                             | 6w – Scr 1.5 3.7    | 6w – Scr 1.9 4.2 | 6w – Scr 1.1 3.6 | 6w – Scr 1.9 4.2 | 6w – Scr 1.1 3.6 |
| **Schirmer’s value (mm)**    |                     |             |                  |           |          |
| **Active group (n = 15)**    | Scr 10.7 9.6        | Scr 10.3 9.6 | Scr 11.1 10.5    | Scr 9.5 9.0 | Scr 11.9 10.9 |
|                             | 6w 12.2 10.8        | 6w 10.3 12.4 | 6w 12.1 10.3     | 6w 11.8 10.7 | 6w 12.5 12.0  |
|                             | 6w – Scr 1.5 4.0    | 6w – Scr 0.9 4.0 | 6w – Scr 0.9 4.0 | 6w – Scr 2.3 5.4 | 6w – Scr 0.6 5.0 |
|                             | 6w – Scr 0.7 6.7    | 6w – Scr 0.7 6.7 | 6w – Scr 0.9 6.9 | 6w – Scr 0.7 6.9 | 6w – Scr 0.7 6.9 |
| **EMM**                     |                     |             |                  |           |          |
|                             | Mean 95% 95% | Mean 95% 95% | Mean 95% 95% | Mean 95% 95% | Mean 95% 95% |
|                            | SD Cl–  Cl+ | SD Cl–  Cl+ | SD Cl–  Cl+ | SD Cl–  Cl+ | SD Cl–  Cl+ |
|                            | –  –  –  | –  –  –  | –  –  –  | –  –  –  | –  –  –  |

The data are presented as the mean and standard deviation (SD).
The data of differences between active and placebo groups (Active 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      | 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                 | 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                  | 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      | 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)         | 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                  | 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      | 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                       | 0.4        | 8.2      | 3.6        | 6.7      | 2.6     | -3.0    | 8.2     | 0.344   |
| Right shoulder                 | 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                  | 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 (active 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 were 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.
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    | 4.0 | 2.0 | 3.0     | 0.974   |
|                      | 6w     | 4.0 | 2.5 | 4.0    | 4.0 | 3.0 | 4.0     | 0.911   |
|                      | 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 body | 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)*

|                          | Median | Q1  | Q3  | Median | Q1  | Q3  | P value |
|--------------------------|--------|-----|-----|--------|-----|-----|---------|
|                          |        |     |     |        |     |     |         |
| 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   |

### Active group *(n = 15)*

|                          | Median | Q1  | Q3  | Median | Q1  | Q3  | P value |
|--------------------------|--------|-----|-----|--------|-----|-----|---------|
|                          |        |     |     |        |     |     |         |
| 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   |

### Stiffness in the neck and shoulders

|                          | Median | Q1  | Q3  | Median | Q1  | Q3  | P value |
|--------------------------|--------|-----|-----|--------|-----|-----|---------|
| 6w                       | 4.0    | 4.0 | 5.0 | 4.0    | 4.0 | 5.0 | 0.890   |
| 6w − Scr                 | 0.0    | −1.0| 0.0 | 0.0    | −0.5| 0.0 | 0.838   |

### Feeling fatigued in the back of the eyes

|                          | Median | Q1  | Q3  | Median | Q1  | Q3  | P value |
|--------------------------|--------|-----|-----|--------|-----|-----|---------|
| 6w                       | 3.0    | 3.0 | 4.0 | 4.0    | 3.0 | 5.0 | 0.523   |
| 6w − Scr                 | 0.0    | −1.0| 1.0 | 0.0    | −0.5| 0.0 | 0.496   |

### Laziness

|                          | Median | Q1  | Q3  | Median | Q1  | Q3  | P value |
|--------------------------|--------|-----|-----|--------|-----|-----|---------|
| 6w                       | 2.0    | 1.0 | 3.0 | 2.0    | 1.5 | 3.5 | 0.617   |
| 6w − Scr                 | 0.0    | −0.5| 1.0 | 1.0    | 0.0 | 1.0 | 0.403   |

### Inability to concentrate

|                          | Median | Q1  | Q3  | Median | Q1  | Q3  | P value |
|--------------------------|--------|-----|-----|--------|-----|-----|---------|
| 6w                       | 3.0    | 1.0 | 3.0 | 3.0    | 2.0 | 4.0 | 0.412   |
| 6w − Scr                 | 0.0    | −1.0| 1.5 | 0.0    | 0.0 | 1.0 | 0.403   |

### Objects feeling cold to the hands and feet

|                          | Median | Q1  | Q3  | Median | Q1  | Q3  | P value |
|--------------------------|--------|-----|-----|--------|-----|-----|---------|
| 6w                       | 1.0    | 1.0 | 3.0 | 1.0    | 1.0 | 3.0 | 0.971   |
| 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)*

|                          | Median | Q1  | Q3  | Median | Q1  | Q3  | P value |
|--------------------------|--------|-----|-----|--------|-----|-----|---------|
|                          |        |     |     |        |     |     |         |
| A tiredness sensation in the eyes
| 6w                       | 0.0    | −0.5| 1.0 | 1.0    | 0.0 | 1.0 | 0.650   |
| 6w − Scr                 | 0.0    | −1.0| 1.0 | 1.0    | 0.0 | 1.0 | 0.452   |

| A sensation of dry eyes
| 6w                       | 1.0    | 0.0 | 1.0 | 1.0    | 1.0 | 2.0 | 0.212   |
| 6w − Scr                 | 0.0    | 0.0 | 0.5 | 1.0    | 0.0 | 1.0 | 0.034*  |

| Objects appear to be blurred, hazy, or doubled
| 6w                       | 0.0    | −1.0| 0.5 | 0.0    | 0.0 | 1.0 | 0.283   |
| 6w − Scr                 | 0.0    | −1.0| 2.0 | 1.0    | −1.0| 1.0 | 0.456   |

| Watery eyes
| 6w                       | 0.0    | 0.0 | 2.0 | 1.0    | 0.0 | 2.0 | 0.929   |
| 6w − Scr                 | 0.0    | −1.0| 1.0 | 0.0    | −1.0| 1.0 | 0.595   |

| A sensation of trouble in focusing
| 6w                       | 0.0    | −1.0| 0.5 | 0.0    | −1.0| 1.0 | 0.734   |
### Placebo group (n = 15) vs. Active group (n = 15)

| Common Symptom                                      | Median (Placebo) | Q1 | Q3 | Median (Active) | Q1 | Q3 | P value |
|-----------------------------------------------------|------------------|----|----|-----------------|----|----|---------|
| The eyes                                            | 6w               | 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 hand and nearby or fine print | Scr             | 0.0| 0.0| 1.0             | 0.0| 0.0| 0.0| 0.571   |
|                                                     | 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 eyes             | Scr              | 0.0| 0.0| 2.0             | 1.0| 0.0| 1.0| 0.756   |
|                                                     | 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 and feet          | Scr              | 0.0| −0.5| 0.0             | 0.0| −0.5| 1.0| 0.514   |
|                                                     | 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 group

Table 4–2. The results of the urinalysis

|                                | 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 \(^{\text{n = 15}}\) | Active group \(^{\text{n = 15}}\) | \(P\) 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 \((\times10^6)/\mu L\) | Men: 430-570   | Scr  | 457.4  | 46.0   | 476.0  | 70.6   | 0.400              |
|                         | Women: 380-500  | Scr  | 462.3  | 45.1   | 484.8  | 74.4   | 0.550              |
| Hemoglobin (g/dL)       | Men: 13.5-17.5 | Scr  | 13.7   | 1.5    | 14.0   | 1.7    | 0.649              |
|                         | Women: 11.5-15.0| Scr  | 13.7   | 1.5    | 14.2   | 1.8    | 0.260              |
| Hematocrit value (%)    | Men: 39.7-52.4 | Scr  | 42.1   | 3.8    | 43.1   | 4.1    | 0.485              |
|                         | Women: 34.8-45.0| Scr  | 42.1   | 3.5    | 43.6   | 4.8    | 0.482              |
| Platelet count \((\times10^9)/\mu L\) | 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              |
| Test                        | 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      | 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   |
| LAP (U/L)                   | Men: 45-81      | Scr 48.0 10.7          | 50.7 6.1              | 0.407   |
|                             | Women: 37-61    | 45.9 9.6               | 49.7 6.9              | 0.451   |
| 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  | 291.0 59.2             | 316.3 45.4            |         |
| 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| 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  | 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   | 126.9 94.0            | 115.2 68.3            |         |
| 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   |
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) Mean | Placebo group (n = 15) SD | Active group (n = 15) Mean | Active group (n = 15) SD | P value |
|--------------------------|-----------------------------|---------------------------|----------------------------|--------------------------|---------|
|                          |                             |                           |                            |                          |         |
| left eye                 |                             |                           |                            |                          |         |
| 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 S.

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% Cl− | 95% Cl+ | 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 (%) |       |       |       |        |         |         |         |
| 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 (%) |       |       |       |        |         |         |         |
| 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 |
|------------------------------|--------------------------|--------------------------|-----|---------|---------|---------|
| **Percentage of pupillary response** |                          |                          |     |         |         |         |
| Scr                          | 3.4                      | 3.5                      | —   | —       | —       | 0.364   |
| 6w                           | 3.4                      | 3.5                      | 0.0 | -0.2    | 0.3     | 0.810   |
| 6w – Scr                     | 0.0                      | 0.0                      | 0.0 | -0.3    | 0.3     | 0.991   |
| **Percentage of pupillary response/near point** |                          |                          |     |         |         |         |
| Scr                          | 1.5                      | 1.7                      | —   | —       | —       | 0.019*  |
| 6w                           | 1.6                      | 1.8                      | 0.1 | -0.1    | 0.3     | 0.220   |
| 6w – Scr                     | 0.1                      | 0.1                      | 0.0 | -0.2    | 0.2     | 0.976   |

**Changes between pre- and post-VDT use (post-VDT use – pre-VDT use)**

|                              | Placebo group (eye = 26) | Active group (eye = 30) | EMM | 95% CI- | 95% CI+ | P value |
|------------------------------|--------------------------|--------------------------|-----|---------|---------|---------|
| **Percentage of pupillary response (%)** |                          |                          |     |         |         |         |
| Scr                          | -1.3                     | -3.4                     | -2.6 | 0.1     | 6.3     | 0.413   |
| 6w                           | -0.9                     | 1.1                      | 1.8  | -0.4    | 1.5     | 0.233   |
| 6w – Scr                     | 0.4                      | 4.5                      | 4.1  | -1.4    | 9.6     | 0.140   |
| **Percentage of pupillary response/near point (%/D)** |                          |                          |     |         |         |         |
| Scr                          | -0.1                     | -0.3                     | -0.4 | 0.6     | 2.0     | 0.155   |
| 6w                           | -0.2                     | 0.4                      | 0.6  | -0.4    | 1.5     | 0.233   |
| 6w – Scr                     | -0.1                     | 0.7                      | 0.8  | -0.3    | 2.0     | 0.155   |
| **Percentage of pupillary response (logarithmic conversion)** |                          |                          |     |         |         |         |
| Scr                          | 0.0                      | -0.1                     | -0.3 | -0.1    | 0.3     | 0.043*  |
| 6w                           | -0.1                     | 0.0                      | 0.1  | -0.1    | 0.3     | 0.043*  |
| 6w – Scr                     | 0.0                      | -0.1                     | 0.1  | -0.1    | 0.3     | 0.043*  |

The data are presented as the mean and standard deviation (SD).
The data of differences between active and placebo groups (Active 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 ORTHOMIC, 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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