Effects of Black Currant Anthocyanoside Intake on Dark Adaptation and VDT Work-induced Transient Refractive Alteration in Healthy Humans

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Abstract

The effects of oral intake of a black currant anthocyanoside (BCA) concentrate on dark adaptation, video display terminal (VDT) work-induced transient refractive alteration, and subjective asthenopia symptoms (visual fatigue) were examined in a double-blind, placebo-controlled, crossover study with healthy human subjects. In a dark adaptation study, intake of BCA at three dose levels (12.5-, 20-, and 50 mg/subject, n = 12) appeared to bring about dose-dependent lowering of the dark adaptation threshold. Statistical analysis comparing the values before and after intake indicated there was a significant difference at the 50 mg dose (p= 0.011). Comparing the refraction values for the dominant eye, BCA intake (50 mg/subject, n = 21) resulted in no decrease in the average value after the visual task; whereas, a placebo trial resulted in a large decrease in the average value, resulting in borderline significance (p = 0.064). In the assessment of subjective asthenopia symptoms by questionnaire, significant improvement was recognized on the basis of the statements regarding the eye and lower back after BCA intake. (*Altern Med Rev* 2000;5(6):553-562)

Introduction

In the previous decade, the rapid spread of computers and video display terminals (VDTs) in the home and workplace has led to an increase in ocular and visual problems, including eye discomfort, blurring of distant objects, eye strain, and asthenopia (visual fatigue).^{1,2} Regarding nutritional mitigation of visual function problems, several dietary constituents, such as carotenoids,³ long-chain polyunsaturated fatty acids,⁴ and anthocyanosides⁵ have been shown to

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Figure 1: HPLC Profile of a Powdered Concentrate of BCA. Assignment: (a) D3G, (b) D3R, (c) C3G, and (d) C3R. Analysis was carried out under the conditions reported previously.¹⁰



enhance visual acuity. An anthocyanoside complex from bilberry (*Vaccinium myrtillus* L.) fruits has been reported to enhance visual acuity at night.⁶ This effect is reported to be exerted through acceleration of rhodopsin regeneration⁷ and/or activation of retinal enzymes.⁸ Although the number of scientific reports is few, this information has attracted much interest in screening anthocyanoside homologues to identify the active compound(s). This information also suggests that intake of anthocyanoside-rich foods may have previously unknown effects in terms of preventing visual problems attributable to working with computers and VDTs.

There are many kinds of anthocyanoside-rich foods and fruits, and the amounts and composition of anthocyanins differ depending on their origin and the preparation process. As the anthocyanoside composition is assumed to play an important role in terms of the physiological activity displayed (such as the French paradox⁹ of red wine and antioxidant activity¹⁰), it is important to evaluate the functions of anthocyanoside preparations with different compositions in order to clarify whether the composition affects physiological activity. Black currant (Ribes nigrum L.) fruits and juice, known to be rich in anthocyanosides, are commonly consumed in many parts of the world.¹¹ In the present study, a powdered concentrate of BCA was developed from a commercial source,¹² which was found to consist of four anthocyanoside components, having a simple composition compared with bilberry, which has fifteen anthocyanoside components. Its simple composition is assumed to facilitate evaluation of the structure-activity relationship in studies on the physiological activity and bioavailability of the components.

Accommodative alteration or transient myopic shift of refractive status following an extended and/or continuous near visual task is thought to be the most reliable indicator of visual fatigue.¹³ However, there have been no reports on the measurement of the alteration or shift after oral intake of anthocyanosides. This paper reports that oral intake of BCA brought about lowering of the dark adaptation threshold in a double-blind, placebo-controlled, crossover study with human subjects, and that the intake of BCA counteracted the transient myopic shift of refractive status after visual tasks such as VDT operation. In addition, assessment utilizing a questionnaire exhibited that BCA intake prevented or improved subjective symptoms of visual fatigue developing after the task.

Methods

Test sample of BCA

A powdered BCA concentrate was prepared from a commercially available black currant juice by the method developed in a previous study.¹² The concentrate contains 9.2percent BCA, consisting of delphinidin 3rutinoside (D3R, 4.61%), delphinidin 3-glucoside (D3G, 1.36%), cyanidin 3-rutinoside

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Table 1: The Composition of the Test Samples (capsules andjuice) used for the Dark Adaptation Study and the TransientRefractive Alteration Study.

	Type and c	omposition of	test sample,	g/subject
Component	Caps	ule	Juid	ce
	BCA	Placebo	BCA	Placebo
BCA concentrate (as BCA) Sucrose Citric acid Sodium citrate Blueberry flavor Cochineal Extract Gardenia Blue	0.540 ¹ (0.050) ¹ - - - - - - -	- (-) 0.540 ¹ - - - -	0.540 (0.050) 32.0 2.9 0.8 0.4 -	- (-) 32.0 2.9 0.8 0.4 0.5 0.2
Water	-	-	163.36	163.20

1. The amount in the case of a dose of 50 mg BCA/subject. In case of a dose of 25 or 12.5 mg of BCA, the value is reduced to a half and a quarter of that shown, respectively.

(C3R, 2.83%), and cyanidin 3-glucoside (C3G, 0.40%), as shown by the HPLC profile in Figure 1. As test samples, capsules were used for the dark adaptation study and juice was used for the transient refractive alteration study. For each study, a placebo was prepared so as to have a taste and color equivalent to those of the test sample. The compositions of the test samples and placebo preparations are shown in Table 1. All other nutrients, reagents, and chemicals used were purchased from commercial sources.

Dark Adaptation Study

Subjects

Twelve healthy volunteers (four males, eight females; body weight, 52-70 kg; age, 24-51; mean 33.3 years old) showing no pathological ocular signs participated in this study. The study was conducted according to the principles established in the Declaration of Helsinki and Tokyo; i.e., informed consent was obtained after full explanation of the nature of the procedure to each subject. On the day before the experiment subjects were asked to avoid caffeine, nicotine, foods rich in anthocyanosides (vegetables, fruits, and juice). All tests were started between 9 a.m. and 11 a.m.

Study design

This was a double-blind, placebo-controlled, crossover study in which the subjects were assigned to four groups: one group given placebo and three groups given doses of BCA concentrate (540, 270, and 135 mg/subject, corresponding to 50, 25, and 12.5 mg BCA/ subject). Each dose of BCA or placebo was orally ingested as six capsules.

The designated procedure is outlined as follows:

0:00 Measurement of dark adaptation (M_{da} 1) (before intake) 1:00 Intake of test sample (BCA or placebo) 3:00 Measurement of dark adaptation (M_{da} 2) (after intake)

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Subject	Age	Re	efraction	n (Dioptr	y)	Dominent	Correction
No.	and sex	R-Sph	R-Cyl	L-Sph	L-Cyl	eye	
1	21M	-0.89	-0.32	-0.75	-0.35	Right	
2	25M	0.33	-0.14	0.05	-0.17	Right	CL
3	21M	-0.66	-0.60	-0.08	-0.65	Right	
4	20F	-0.90	-0.46	-0.16	-0.31	Right	CL
5	20F	0.63	-0.40	1.40	-0.77	Right	
6	20F	-0.37	-1.39	-0.70	-0.92	Left	
7	20F	-0.20	-0.46	-0.50	-0.24	Left	
8	20M	0.15	-0.62	-0.36	-0.50	Right	
9	20F	-0.15	-0.36	-0.21	-0.26	Left	
10	22M	-1.08	-0.12	-0.85	-0.28	Right	
11	21M	-3.65	-0.40	-3.83	-0.24	Left	
12	20M	0.65	-0.40	0.57	-0.28	Left	
13	20F	-0.64	-0.27	-0.23	-0.41	Left	CL
14	24F	0.44	-0.99	0.36	-0.66	Left	CL
15	23F	-0.37	-0.45	0.35	-0.93	Right	
16	20M	0.11	-0.29	0.15	-0.22	Left	
17	20M	-0.09	-0.61	0.41	-0.71	Left	
18	20M	0.07	-0.96	-0.53	-0.17	Left	CL
19	20M	-1.92	-0.25	-0.71	-0.84	Left	CL
20	21M	-1.39	-0.30	-0.88	-0.44	Left	
21	22M	-0.44	-0.18	0.55	-1.22	Right	

Table 2: Age, Sex, and Refractive Status of IndividualSubjects in a Transient Refractive Alteration Study.

answer concerning the direction. The direction of the bars was changed randomly (vertical, horizontal, right diagonal, or left diagonal). In order to determine whether the subjects could resolve the bars they were asked to indicate their direction. Before each examination the light bar intensity was calibrated to 3.18 asb at 7 log units the on Goldmann-Weekers Adaptometer scale.

Transient Refractive Alteration Study *Subjects*

A total of 21 healthy subjects (age,

Variables Measured

Dark adaptation threshold was measured binocularly with a Goldmann-Weekers Adaptometer (Haag-Streit, Switzerland). After brief pre-adaptation in complete darkness for two minutes, the subjects looked for ten minutes into the center of a white sphere with luminance of 2000 asb (light adaptation). Next, during dark adaptation, a circular test field with 11 degrees of arc with dark and light bars (contrast 100%, width 1.5 cm) was presented centrally at a distance of 30 cm, and the dark adaptation threshold was measured during each ten seconds of the first ten minutes until the cone-rod break time, then each minute until 30 minutes after the start. The dark adaptation threshold was determined by increasing the light intensity of the light bars until the subject perceived the bars and gave the correct

20-25; mean 20.9 years old) were enrolled in the study. They were confirmed to be free of any ocular diseases, refractive errors (high myopia more than 4 diopter, hyperopia more than 1 diopter, or astigmatism of which the strongest curvature was more than 1.5 diopter), or presbyopia at the time of enrollment, as shown in Table 2. All of the subjects gave written informed consent according to the Declarations of Helsinki and Tokyo prior to the start of the study.

Study design

This was a double-blind, randomized, crossover study with subjects assigned to either a BCA or a placebo group. The subjects were asked to avoid caffeine, nicotine, and foods rich in anthocyanosides (vegetables, fruits, and juice) for 24 hours before the study.

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The subjects were given a cup of juice (200 mL) containing BCA concentrate (540 mg/ subject, corresponding to 50 mg of BCA/subject) or placebo, the compositions of which are shown in Table 1. Regarding the questionnaire for the assessment of subjective asthenopia symptoms, the subjects were instructed to respond by placing a mark showing the magnitude of the symptoms on a Visual Analog Scale (VAS).¹⁴

The designated procedure is outlined as follows: M_{tra} , M_{cff} , and M_{vas} denote the refraction values for the dominant eye, flicker values, and VAS magnitude of asthenopia symptoms, respectively:

0:00 Measurement of M_{tra} , M_{cff} , and $M_{vas}1$ (before the task)

0:10 Intake of juice (BCA or placebo)

2:10 Start of visual task

4:10 Completion of visual task

 $Measurement \ of \ M_{\rm tra}2, \ M_{\rm cff}2, \ and \ M_{\rm vas}2 \ (after \ the \ task)$

Variables Measured

The experimental near visual task consisted of a simple calculation test on a VDT, modifying the Kraepelin test method.¹⁵ The task was loaded for two hours without a pause, and measurements were carried out for three items. First, the spherical (R) and cylindrical (C) refraction of the dominant eye was measured by means of an autorefractometer (Nidek AR-600A, Japan) according to the method of Nakamura and Uosato.¹⁶ The refraction value for the dominant eye was evaluated in terms of the spherical equivalent of (S + C/2). Second, the $M_{\rm cff}$ value (flicker value) was measured using critical flicker fusion (CFF) determined via a Flicker 501 (Takei Kiki Kogyo, Japan) according to the method reported by Ogasawara et al.¹⁷ This measurement was carried out five times with the signal frequency

in UP mode and the mean of the five fusing values was determined. Third, subjective fatigue symptoms were assessed by a questionnaire consisting of five statements concerning the fatigue symptoms of the head and/or neck, arm, eye, shoulder, and lower back. The magnitude of each symptom was expressed in terms of VAS by having the subject place a mark on a 100 mm horizontal line indicating a continuum from no fatigue (left end) to strong fatigue (right end). The subject's response to each statement was defined as the distance (in millimeters) from the left end of the line to the mark.

Statistical Analyses

All measurements were performed before and after intake of the test samples (BCA concentrate or placebo). Comparison was made by means of a paired-t test.

Results BCA Test Sample

The anthocyanoside content of the BCA concentrate used in this study was 9.2 percent by HPLC analysis.¹² As shown in Figure 1, the concentrate consisted of four components: D3G, D3R, C3G, and C3R. Each component was quantified by HPLC. The results of analysis of the BCA test samples (capsules and juice) are shown in Table 1. In preparation of the juice-type test samples, sucrose, citric acid, and coloring pigments were added to make the placebo's taste and color identical to the BCA.

Dark Adaptation Study

Dark adaptation values for twelve healthy volunteers were measured as the visual threshold after 30 minutes of dark adaptation, before and two hours after intake of BCA (at three dose levels: 50, 25, and 12.5 mg/subject) or placebo. The results are summarized in Table 3. Figure 2 shows the typical profile of dark adaptation threshold values

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Dose of BCA	Dark adap	otation value, mean±	SD of log asb; (p value)	1
mg/subject	Before intake	After intake	Change, –M _{dae}	p value ²
0 (placebo)	2.056 ± 0.209 (1.000)	2.018 ± 0.218 (1.000)	-0.038 ± 0.106 (1.000)	0.244
12.5	$\begin{array}{c} 2.026 \pm 0.147 \\ (0.457) \end{array}$	2.004 ± 0.195 (0.761)	-0.023 ± 0.138 (0.733)	0.583
25	2.016 ± 0.170 (0.234)	1.980 ± 0.197 (0.264)	-0.037 ± 0.112 (0.983)	0.28
50	2.038 ± 0.186 (0.686)	1.923 ± 0.167 (0.014)	-0.115 ± 0.131 (0.171)	0.011

Table 3: Dark Adaptation Values Measured Before and After Intake of BCA or Placebo in a Dark Adaptation Study.

1. The results of statistical analysis, independently carried out in each vertical row, are shown in parentheses.

2. Statistical p value for "after intake" vs "before intake" in each horizontal row.

during the 30-minute period before and after BCA intake in the case of one subject. The mean standard deviation before intake ranged from 2.056 ± 0.209 (placebo) to 2.016 ± 0.170 (BCA, 25 mg/subject). No significant difference was found among the four groups as a matter of course. Comparison of the mean standard deviation after intake among the four groups showed a decrease from 2.018 ± 0.218 (placebo) to 1.923 ± 0.167 (BCA, 50 mg/subject) with increasing doses of BCA. This dosedependent decrease of the threshold was associated with expansion of the change in the refraction values for the dominant eye (M_{do}) from -0.038 ± 0.106 (placebo) to $-0.115 \pm$ 0.131 (BCA, 50 mg/subject), comparing the values before and after intake. At the BCA dose level of 50 mg/subject, compared to the placebo, there was a significant difference (p =0.014) in the threshold values after intake, but no significant difference in $M_{DA}(p = 0.171)$. In comparing the statistical p values obtained in analysis of the data after intake versus before intake among four dose levels of BCA, a significant difference (p = 0.011) was evident

at only one dose level of BCA (50 mg/subject).

Transient Refractive Alteration Study

The age, sex, and refractive status of individual subjects are shown in Table 2. Refraction values for the dominant eye (designated as diopter (D)), measured before $(M_{_{tra}}1)$ and after (M_{tra}^2) the visual task, and the changes ($M_{tra} = M_{tra}1 - M_{tra}2$) are summarized in Table 4. Comparing $M_{tra}2$ with $M_{tra}1$, BCA intake resulted in no decrease in the average value after the visual task (from M_{tra} 1 of -0.432 ± 0.602 D to M_{tra}2 of -0.402 ± 0.643 D, p = (0.598), whereas in the placebo trial after the visual task there was a large decrease in the refraction values, exhibiting borderline significance (from M_{tra} 1 of -0.384 ± 0.536 D to M_{tra} 2 of -0.503 ± 0.579 D, p = 0.064). Average changes (M_{tra}) following intake of BCA and placebo were -0.030 ± 0.252 D and $0.119 \pm$ 0.278 D, respectively, and a statistically significant difference was evident (p = 0.006).

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Figure 2: Typical Dark Adaptation Threshold Before (continuous line) and After (dotted line) BCA Intake in the Case of One Subject During 30 Minutes of Dark Adaptation.



The results concerning the flicker values and the assessment of subjective visual fatigue symptoms before and after the task in the subjects given BCA or placebo are summarized in Table 5. The average flicker values after the task were 34.39 ± 3.51 and $34.13 \pm$ 2.90 Hz, respectively, and the changes comparing the values before and after were small, within one Hz. Statistical analysis exhibited no significant difference in these parameters between BCA and placebo. Comparing the values obtained in assessment of subjective asthenopia symptoms for each of the five statements, all average values after the task were higher than those before the task in both the BCA and placebo groups. Furthermore, the average value in the case of every statement after BCA intake was smaller than that after placebo intake. Statistical analysis of the data on each statement showed a significant difference between BCA intake and placebo intake in two statements, one regarding the eye (after, 47.31 ± 24.72 , against 56.72 ± 25.24 mm, p = 0.078; change, 32.59 ± 18.94 against 42.14 ± 19.52 , p = 0.037) and the one regarding the lower back (after, 29.79 ± 27.15 , against 42.83 ± 33.55 mm, p =0.052; change, 19.16 ± 22.74 against 35.48 ± 30.87 mm, p =0.025).

Discussion

The present dark adaptation study, measuring the dark adaptation threshold after 30 minutes, shows the visual threshold after oral intake of BCA is lower than that before intake at every dose level (50, 25, and 12.5 mg/subject), and that intake of 50 mg results in a statistically significant lowering of the visual threshold (p = 0.011); the result is a statistically significant improvement in dark adaptation

with BCA (Table 3). The lowering of the threshold began in the first 10 minutes of cone break and continued until the end of the examination, 30 minutes after the start (Figure 2). As this feature is consistent with that observed in a study on bilberry anthocyanosides,⁴ oral intake of anthocyanosides is thought to affect preferably rod components involved in dark adaptation. However, not all studies have found positive improvements with bilberry extracts; for example, one in which researchers reported no improvement in night visual acuity between a concentrated bilberry anthocyanoside extract and placebo.¹⁸

In a transient refractive alteration study, it was shown that oral intake of BCA (50 mg/subject) has the effect of preventing myopic refractory shift after visual tasks on VDTs. This is the first scientific report of dietary anthocyanosides having such a preventive effect, except for a recent report¹⁹ on bilberry anthocyanosides which had the effect of promoting recovery of visual acuity in cases

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Table 4: Refraction Values for the Dominant Eye, Measured Before and After the Task in Subjects Given BCA or Placebo, and the Change in Refractive Alteration. ¹⁾

	Subject		BCA			Placebo	
Eye	no. and average ²⁾	Before (M _{tra} 1)	After ³⁾ (M _{tra} 2)	$\begin{array}{c} Change^{3)} \\ (\Delta M_{tra}) \end{array}$	Before (M _{tra} 1)	After ³⁾ (M _{tra} 2)	Change ³⁾ (∆M _{tra})
	1	-0.91	-1.14	0.23	-0.99	-1.10	0.11
	2	-0.08	-0.07	-0.01	0.04	-0.60	0.64
	3	-1.13	-1.01	-0.12	-1.29	-0.96	-0.33
	4	-0.77	-0.83	0.06	-0.85	-0.86	0.01
	5	0.57	0.59	-0.02	0.33	0.27	0.06
	6	-0.96	-0.95	-0.01	-0.74	-1.18	0.44
	7	-0.22	-0.81	0.59	-0.14	-0.68	0.54
	8	-0.01	0.53	-0.54	-0.28	0.07	-0.35
	9	-0.47	-0.30	-0.17	-0.50	-0.45	-0.05
	10	-0.20	-0.44	0.24	-0.28	-0.58	0.30
	11	-0.80	-0.73	-0.07	-0.66	-0.85	0.19
	12	0.47	0.43	0.04	0.66	0.46	0.20
	13	-0.81	-0.67	-0.14	-0.61	-0.77	0.16
	14	-0.08	0.11	-0.19	-0.08	-0.25	0.17
	15	-0.15	-0.28	0.13	-0.21	-0.25	0.04
	16	0.40	0.20	0.20	0.34	0.11	0.23
	17	0.05	0.21	-0.16	0.19	0.49	-0.30
	18	-0.58	-0.51	-0.07	-0.32	-0.58	0.26
	19	-1.75	-1.63	-0.12	-1.13	-1.44	0.31
	20	-1.29	-1.37	0.08	-1.20	-1.42	0.22
	21	-0.35	0.22	-0.57	-0.35	0.00	-0.35
	Mean	-0.432	-0.402 ^a	-0.030 ^{a)}	-0.384	-0.503 ^b	0.119 ^{b)}
	±SD	±0.602	±0.643	±0.252	±0.536	±0.579	±0.278

1) M_{tra} 1 and M_{tra} 2 are the measured values and DM_{tra} is the change (M_{tra} 1- M_{tra} 2).

2) Expressed as mean ±SD

3) Statistical analysis of M_{tra} 2 and DM_{tra} was independently carried out for each dominant eye comparing the BCA and placebo groups.

 $^{a,b,a),b)}$ Values with different superscript letters are significantly different (p<0.05). Superscript letters without and with parentheses show the results of statistical analysis of M_{tra}^2 and DM_{tra} , respectively.

of pseudomyopia in primary school students upon oral intake every day for eight weeks.

These results suggest that intake of dietary anthocyanosides may serve as a new means of preventing myopic refractory shift during visual tasks or promoting visual recovery. If so, effects such as improvement of dark adaptation and rhodopsin regeneration can be added to the known visual functions of The former showed no difference between BCA and placebo, and the latter provided interesting information through analysis of the five statements. As shown in Table 5, all average values after the task were found to be smaller than those before the task, thus the task applied was considered to give the subject an adequate load to induce visual fatigue. BCA intake resulted in a significant difference in

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Although the raw data could be explained as a hyperopic shift rather than prevention of myopic shift, that may be due to the circumstantial conditions of the subjects. The subjects in this study were not confined for the entire day, and the alteration measurement had to be carried out in afternoon, the even though some of the subjects had engaged in deskwork in the morning. Together

anthocyanosides.

with the alteration measurement, flicker values were measured and subjective symptoms of visual fatigue were assessed because a temporary shift in refraction is reported to comprise the major part of visual fatigue.²⁰ **Table 5:** Flicker Values and Assessment of Subjective Asthenopia Symptoms, Measured Before and After the
 Task in Subjects Given BCA or Placebo, and the Change in Transient Refractive Alteration.¹⁾

Item &		BCA			Placebo	
statement	Before	After ²⁾	Change ²⁾	Before	After ²⁾	Change ²⁾
Flicker value, Hz	34.95 ± 3.16	34.39 ± 3.51	0.56 ± 1.15	34.72 ± 2.99	34.13 ± 2.90	0.59 ± 1.22
Asthenopia symptoms, mm						
head & neck	12.70 ± 13.45	40.08 ± 24.86	27.38 ± 18.39	8.34 ± 11.87	44.09 ± 26.09	35.75 ± 24.96
arm	10.21 ± 17.82	36.15 ± 25.86	25.94 ± 29.61	4.32 ± 6.27	41.76 ± 29.33	37.44 ± 28.43
eye	14.72 ± 15.55	47.31 ± 24.72	32.59 ± 18.94^{a}	14.59 ± 17.98	56.72 ± 25.24	42.14 ± 19.52^{b}
shoulder	15.12 ± 15.65	49.66 ± 27.97	34.54 ± 25.75	10.95 ± 17.42	54.31 ± 29.31	43.36 ± 30.91
low back	10.63 ± 15.92	29.79 ± 27.15^{a}	$19.16 \pm 22.74^{a)}$	7.35 ± 9.25	42.83 ± 33.55^{b}	$35.48 \pm 30.87^{b)}$
) Expressed as mean ± SD.	-		-			

a. b. a). b) Values with different superscript letters are significantly different (p<0.05). Superscript letters without and with

parentheses indicate the results of statistical analysis of the values after the test and the change in values, respectively.

terms of prevention of fatigue of the eye and low back, as shown by means of the questionnaire; a result which supports the report indicating that a temporary shift in refraction comprises the major part of visual fatigue.¹¹ The lack of change in flicker values indicates that BCA intake does not affect the prechiasmal visual pathway, the optic nerve, or the activity of the central nervous system,¹⁹ as measured by CFF.

In conclusion, oral intake of BCA brought about a reduction of the dark adaptation threshold and promoted recovery from or served to prevent VDT work-induced transient refractive alteration and subjective symptoms of visual fatigue in healthy subjects. These findings suggest the possibility of prevention of VDT work-induced asthenopia through intake of dietary anthocyanosides. Additional research on the structure-activity relationship responsible for the physiological activity and bioavailability of anthocyanosides is warranted.

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