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Effects of Consuming Foods Containing Lutein, Zeaxanthin, Dha, EPA, and Piperine from Long Pepper (Hihatsu) on Deep Vision:

A Placebo-Controlled, Randomized, Double-Blind, Parallel-Group Study

Koji FURUNISHI[1)]／**Nobuki OKADA**[2)]／**Yumiko IDE**[3)]／
Saeko YAGI[4)]／**Shigeru IMAI**[5)]

1) Kenso Factory Corporation
2) Marga Japan Corporation
3) Shibuya Kamiyama-cho Clinic
4) Jikoukai Yagi Hospital
5) Leverage Brain Inc.

● **Abstract**

Purpose: Lutein and zeaxanthin are pigments present in the eye macula that protect the retina from harmful light. This randomized, placebo-controlled, double-blind, parallel-group, study was conducted to investigate the effects of test foods containing DHA(Docosahexaenoic Acid), EPA(eicosapentaenoic acid), and piperine from long pepper (Hihatsu), in conjunction with lutein and zeaxanthin, on deep vision in healthy adults.
Methods: The study included healthy adult men and women. Sixty-four participants (32 in each group) were given test or placebo foods containing lutein, zeaxanthin, DHA, EPA, and piperine from long peppers (Hihatsu) for 8 weeks and VDT(Visual Display Terminals) work was performed before, 4 weeks after, and 8 weeks after intake. VAS(Visual analog scale) analog scale questionnaire was administered, and a weekly questionnaire was also conducted during the dietary intake period.
Results: Following 8 weeks of consumption, deep vision was significantly improved in the test food group compared to the placebo group immediately after VDT work. No adverse events or side effects attributable to consumption of the test food were observed during the study period.
Conclusion: Consumption of foods containing lutein, zeaxanthin, DHA, EPA, and piperine from long peppers (Hihatsu) helped maintain deep vision in healthy participants.
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Key words: lutein, zeaxanthin, DHA, EPA, piperine from honeysuckle, deep vision, VAS

INTRODUCTION

In our modern lifestyles, the prevalence of working with electronic displays, commonly referred to as video display terminal (VDT) work, has been linked to an increased incidence of visual function impairment due to the undue strain placed on the eyes[1)~3)]. Workers engaged in VDT work for long periods of time are aware of various nonspecific symptoms (eye fatigue, redness, and pain) called eyestrain, which has attracted attention as a major occupational health problem[4)~7)]. Furthermore, in this age of widespread smartphone usage, every person possesses a smartphone, and prolonged screen viewing has become a commonplace occurrence. Smartphone screens are also electronic displays, and the eye focus adjustment disorder caused by continuous screen viewing is called smartphone presbyopia[8)]. Suzuki et al[9)]. investigated the duration of smartphone use among university students and reported that users who spent more time using smartphones tended to have reduced eye regulation and a longer near point of adjustment. Thus, the daily implementation of the VDT workload is thought to decrease eye function and lead to a decrease in the quality of daily life. In addition, the VDT work is thought to affect deep vision, which measures stereoscopic perception by keeping the eyes fixed at the same focal position for long periods of time.

VDT work causes deterioration of eye function and worsening of subjective symptoms such as eyestrain. Studies on the reduction of eye strain caused by VDT work through the intake of food have been conducted, and their efficacy has been reported for various

food components, including anthocyanins[11)12)] and astaxanthin[13)14)].

Xanthophyll pigments accumulate in the central retina of the eye, 1.5 to 2.0 mm in diameter. This area, called the macula, is the site where lutein and zeaxanthin are formed via cell-tissue association[15)16)]. Lutein is transported to and developed in the macula via oral intake. The developed macula is believed to buffer the stimulation of short-wavelength visible light, such as blue light, and limit the risk of ocular tissue damage[17)18)]. It has also been reported that macular pigment-filled retinas improve visibility by enhancing contrast sensitivity for eyes, particularly for individuals frequently engaged in VDT work[19)]. In addition, deep vision is not only relevant to VDT work but may also be relevant to sports vision. Sports vision refers to the visual function important for sports activities. Previous studies found a correlation between athletes and high athletic performance in various sports and vision. The required vision abilities vary from sport to sport. For example, deep vision to estimate the distance between moving objects and dynamic visual acuity to track the movement of a moving object are considered necessary for various visual functions during competitions. We have previously investigated the inhibitory effects of foods containing lutein, zeaxanthin, DHA, EPA, and piperine derived from Japanese cyprinid diets on the reduction of deep vision after an extensive VDT workload. We used foods that contain lutein, zeaxanthin, Japanese cyprinid extract, and fish oil, and found significant improvement in deep vision. Therefore, this study aimed to confirm the effects of foods containing lutein, zeaxanthin, and piperine from lLong pepper (Hihatsu) on the reduction in deep vision that occurs after a deep vision VDT workload. Furthermore, a stratified analysis of the results was performed.

I SUBJECTS AND METHODS

1. Test design

This was a randomized, placebo-controlled, and parallel-group trial (1:1 allocation ratio of the test food intake group to the target food intake group). In accordance with the principles outlined in the Declaration of Helsinki (amended in October 2013), this study was conducted following the Ethical Guidelines for Life Sciences and Medical Research Involving Human Subjects (Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labour and Welfare, notice enacted March 23, 2021) and the Guidance on Ethical Guidelines for Life Sciences and Medical Research Involving Human Subjects (Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labour and Welfare, notice enacted April 16, 2021). The study plan was reviewed and approved by the Ethics Review Committee of the Japan Council for Clinical Research (JCCR) on July 21, 2022. The study was then pre-registered with UMIN-CTR (UMIN000048088) on July 21, 2022, under the study title "Verification study of the effects of test food intake on deep vision" before the implementation of the study. The study protocol has not yet been published. This study was conducted at the Shibuya Kamiyama-cho Clinic from July 2022 to November 2022. No major changes were made to the study protocol during the study period.

2. Test participants

The participants met the selection criteria listed below and did not meet the exclusion criteria. The selection criteria were:1) healthy adult male and female participants aged 20 years or older but less than 65 years; 2) participants with corrected distance vision of 0.6 or better; 3) participants who were able to use the electronic daily logging application (e-DCA); and 4) participants who were fully informed of the purpose and content of this study, understood the content, volunteered freely, and agreed in writing to participate. Exclusion criteria were as follows: (1) Patients with serious diseases of the brain, liver, kidney, heart, lungs, digestive organs, blood, endocrine system, or metabolic system; (2) Patients with diseases requiring constant medication or with a history of serious diseases requiring medication; (3) Patients currently undergoing treatment at a medical institution for diseases of the digestive system or with a history of digestive system surgery (excluding appendicectomy); (4) Patients with ophthalmological diseases, a history of ophthalmological surgery, or who regularly use eye drops for treatment or prevention; (5) Patients with a history of hypersensitivity to test food ingredients; (6) Patients who regularly use pharmaceuticals, health foods, or supplements; (7) Pregnant, lactating, or women intending to become pregnant during the study period; (8) Patients who have a history of lifestyle-related diseases, and (9) those who were judged to be unsuitable as participants based on their answers to the lifestyle questionnaire. In addition, this study was conducted at Shibuya Kamiyama-cho Clinic.

3. Intervention

The test foods were soft capsule foods containing lutein, zeaxanthin, DHA, EPA, and piperine from long peppers (Hihatsu) or a placebo food in which lutein, zeaxanthin, DHA, EPA, and piperine from long peppers (Hihatsu) were replaced by crystalline cellulose. There were no differences in the appearance or taste of the two foods. **Table 1** shows the compositions of the test foods. The participants consumed four capsules of the assigned test or placebo food once daily with water or lukewarm water for 8 weeks.

4. Outcomes

The primary outcome measure was deep vision. The secondary outcome was the VAS questionnaire on the

Table 1　Composition of test food

Test product name		Test food	Placebo food
Additive		Marigold pigment oil（lutein＋zeaxanthin）, DHA purified fish oil（DHA＋EPA）, Long pepper（Hihatsu）extract powder	Dextrin, safflower oil, olive oil, glycerin fatty acid ester, glycerin fatty acid ester-2, beeswax
Involved component	Lutein	10 mg/4 tablets	−
	Zeaxanthin	2 mg/4 tablets	−
	DHA	398.7 mg/4 tablets	−
	EPA	36.3 mg/4 tablets	−
	Piperine from Long pepper（Hihatsu）	120 μg/4 tablets	−
Form		softgel capsule	softgel capsule
Daily		4 tablets（2160 mg）/day	4 tablets（2160 mg）/day

state of the physical condition before and after VDT work. In addition, changes in physical condition during the intake period were set as safety endpoints. The outcome measures did not change at the start of the study.

1) Deep vision
Deep visual acuity was measured six times before, immediately after, and 20 min after the end of the VDT work using a visual testing device (Kowa AS-27a Kowa Co., Ltd., Tokyo, Japan) in a three-rod method, and the average value of the errors was adopted. Deep vision was measured before, 4 weeks after, and 8 weeks after ingestion, before VDT work, immediately after work, and 20 min after the end of work.

2) VAS questionnaire on the state of experience before and after VDT work
The VAS method was used to interview the participants about their experiential state with respect to “eye fatigue,” “eye focus adjustment,” and “headache” before VDT work, immediately after work, and 20 minutes after the end of work. Additionally,100 mm line segments were interviewed at both ends, with the best state being 0 mm and the worst state 100 mm, and the length of the line segments was measured and evaluated by the amount of change from the VDT work before VDT. The VAS questionnaire was administered before, 4 weeks after, and 8 weeks after intake, before VDT work, immediately after work, and 20 minutes after the end of work.

3) Safety endpoints
As a safety test, the participants were asked to record changes in their physical condition and medication use during the intake period using a daily logbook.

5. Test Method
Participants underwent a VDT workload test at the time of their visit to the clinic, in which they played an Othello game for 45 min without moving the screen, followed by 15 min of free viewing of a news site on a smartphone. This task was repeated twice (for 2 hours). VAS questionnaires were administered before, immediately after, and 20 minutes after the VDT task was completed to measure the participants’ deep vision and their current state of experience.

During the intake period, the participants were asked to respond to a daily diary on their health and intake status, and once a week, a VAS questionnaire on quality of life was also administered. The study period was August 1, 2022, with a follow-up end date of November 4, 2022

6. Sample size
The sample size was calculated from the results of the previous study by calculating the difference and standard deviation between the test food intake group and the placebo food intake group for the primary endpoint of deep visual acuity, and the required sample size was calculated with a power of 80% and a significance level of 5%. In addition, the required sample size per group was set to 30 or more to account for dropouts.

7. Randomization
Randomization was performed by generating random numbers using computer software. Initially, a pre-test involving 111 patients who visited the clinic was conducted. After the pre-test, 64 participants were preferentially selected from among those who met the selection criteria, did not meet the exclusion criteria, and had good results in the depth vision test. After selecting the participants, the allocation manager created an allocation table using the stratified blocked randomization method, with sex, age, BMI, deep vision, and corrected vision as stratification factors. The allocation manager confirmed the unidentifiability of each test product and affixed a sticker with the subject’s ID to the packaging surface of the test and placebo foods. Throughout the study’s duration, the allocation list was securely

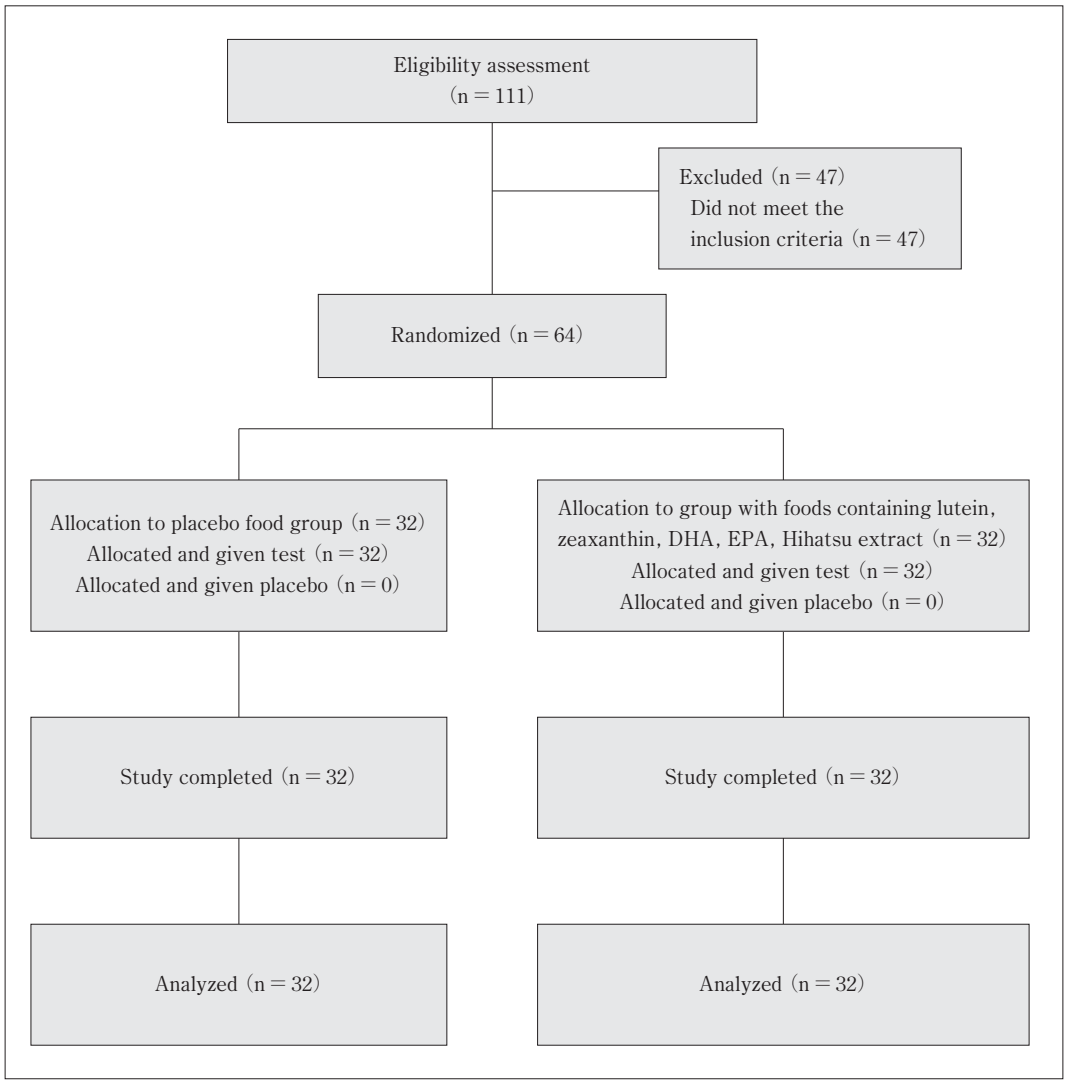

Fig. 1 Subject Selection Flow

maintained within a locked cabinet, sealed to prevent any unauthorized access. The allocation manager refrained from opening the list until the conclusion of the study, thus upholding the principle of double-blindness, which was maintained for both the participants and the study implementers.

8. Statistical analysis

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc.) The values used in the analysis were the actual measured values or the change from before intake, and the results are presented as mean ± standard deviation. The two-sided significance level for all tests was set at 5%. The primary endpoint of the deep visual acuity was measured. The VAS score of the questionnaire (VAS change from before VDT work is shown as the value immediately after VDT work and 20 minutes after VDT work). Statistical analysis was performed using the Student's t-test between the test food intake group and the placebo food intake group without considering multiplicity. No additional analyses, such as subgroup or adjusted analyses, were performed in this study.

II RESULTS

The subject selection process is illustrated in **Fig. 1**. Of the 118 participants who consented to participate in the study, 111 participants who came for a pre-test were pre-tested, and 64 participants were preferentially selected from those who met the selection criteria and did not meet the exclusion criteria and had good results in the depth vision test. Thirty-two participants in the test food intake group and 32 participants in the placebo food intake group were assigned at the start of the study. The study was completed with no dropouts, discontinuations, or loss to follow-up. All 64 participants were included in the efficacy analysis population, and the participants consumed the assigned foods.. The safety analysis population consisted of 64 participants who had consumed the study foods at least once. The participants'

Table 2　Subject background

	Test food（n＝32） Average ± Std. Dev.	Placebo food（n＝32） Average ± Std. Dev.	p value
Age	45.53 ± 12.05	43.81 ± 11.15	0.4805
Near vision（logMAR）	− 0.0386 ± 0.1570	− 0.0803 ± 0.1034	0.2148
Distant vision（logMAR）	0.00255 ± 0.07803	0.01107 ± 0.09018	0.6877
Deep visual acuity	5.64 ± 4.46	6.60 ± 6.08	0.4699
Male/Female	14/12	12/20	0.7994

Table 3　Results of deep vision

Timepoint	Load period	Group	# of cases	Average ± Std. Dev.	Intergroup comparison
					t-test
SCR	Before VDT task	Test food Placebo food	32 32	5.64 ± 4.46 6.60 ± 6.08	0.4699
	Immediately after VDT task	Test food Placebo food	32 32	17.10 ± 11.82 20.38 ± 13.24	0.3013
	20 minutes after VDT task	Test food Placebo food	32 32	5.98 ± 6.41 6.45 ± 6.57	0.7737
4 W	Before VDT task	Test food Placebo food	32 32	9.79 ± 11.48 8.58 ± 11.43	0.6746
	Immediately after VDT task	Test food Placebo food	32 32	20.50 ± 16.65 17.41 ± 13.08	0.4117
	20 minutes after VDT task	Test food Placebo food	32 32	10.84 ± 11.93 7.66 ± 10.76	0.2660
8 W	Before VDT task	Test food Placebo food	32 32	8.05 ± 11.04 9.59 ± 13.88	0.6245
	Immediately after VDT task	Test food Placebo food	32 32	10.79 ± 5.45 14.10 ± 7.59	0.0492
	20 minutes after VDT task	Test food Placebo food	32 32	7.32 ± 9.14 7.83 ± 9.57	0.828

SCR, screening; W, weeks; VDT, visual display terminal

backgrounds in the efficacy analyses are listed in **Table 2**.

1. Efficacy Evaluation

1) Deep vision

Table 3 lists the results of deep vision. Deep vision is a visual function that captures the perspective, solidity, depth, and dynamics of an object. In the Screening test, there were no significant differences between the two groups before VDT work, immediately after VDT work, and 20 minutes after VDT work. At week 8, the test food intake group showed a significant difference after VDT work. Specifically, at week 8, the test food group showed a significant improvement compared to the placebo group, with a shorter error margin immediately after VDT work.

2) VAS questionnaire on post-VDT experience

Table 4 presents the results of the VAS questionnaire assessing participants' post-VDT work experiences. Regarding the change from before to immediately after VDT work after 8 weeks of intake, the test food intake group showed significantly fewer symptoms and suppression of headache symptoms than the placebo food intake group.

2. Safety Evaluation

Subjective symptoms, adverse events, and adverse reactions were reported by both groups which were recorded in the daily logbook and during hospital visits, as part of the safety evaluation. Specifically, 19 incidents were documented among seven participants in the test food intake group, while 13 events were recorded in nine

Table 4 VAS questionnaire on patient response after VDT task (Difference in VAS scores before and after VDT task)

Immediately after VDT work

Item	Group	4 W	8 W	Intergroup p value	
				4 W	8 W
Eye fatigue	Test food Placebo food	19.94 ± 16.84 16.84 ± 16.39	17.78 ± 14.64 20.16 ± 18.65	0.4011	0.8298
Eye focusing	Test food Placebo food	12.69 ± 22.42 11.72 ± 17.51	11.16 ± 20.33 14.34 ± 18.02	0.7933	0.9038
Headache	Test food Placebo food	3.34 ± 12.66 6.19 ± 14.97	2.28 ± 11.22 4.66 ± 9.90	0.8079	0.0044

20 minutes after VDT work

Item	Group	4 W	8 W	Intergroup p value	
				4 W	8 W
Eye fatigue	Test food Placebo food	9.16 ± 17.96 4.00 ± 15.63	8.78 ± 21.67 10.72 ± 14.94	0.3402	0.1771
Eye focusing	Test food Placebo food	6.38 ± 17.11 2.78 ± 15.07	2.91 ± 20.25 5.81 ± 17.97	0.4933	0.1811
Headache	Test food Placebo food	2.25 ± 11.93 2.84 ± 12.02	0.91 ± 9.26 3.47 ± 11.06	0.5857	0.1834

VDT, visual display terminal; W, weeks

participants from the placebo food intake group. Several participants developed colds or other symptoms, mainly during the intake period, but all were mild, and a causal relationship with study participation was ruled out by the study investigators. Based on the above, the safety of the consumption of the test foods over an 8-week period was considered acceptable.

III CONSIDERATIONS

The purpose of this randomized, placebo-controlled, and parallel-group study was to investigate the effects of test foods containing DHA, EPA, and piperine from long peppers (Hihatsu), in addition to lutein and zeaxanthin, on eye function in healthy adults. The results showed that deep vision improved immediately after VDT in the 8th week of intake. The results showed that the test food was effective in improving deep vision.

In addition to lutein and zeaxanthin, DHA, EPA, and Hijatsu extract were added to the test food. According to a study conducted by Kizawa et al on healthy adults, foods containing lutein, zeaxanthin, and astaxanthin were associated with reduced occurrences of “difficulty in focusing the eyes” and “difficulty in seeing objects up close, nearby, and letters” after VDT work, compared to placebo foods. This study also showed that headaches immediately after VDT work were significantly reduced in the test food intake group compared to the placebo food intake group in the subjective evaluation. Improvements in deep vision may also be related to spatial perception such as depth perception. The inclusion of DHA and EPA, in addition to lutein and zeaxanthin, in the test food may have resulted in an improvement in depth perception and the perception index. Spatial perception is an essential aspect of daily life. For example, when driving a car, spatial awareness is essential for assessing the distance to the vehicle in front, the gap to the adjacent vehicle, and the proximity to the stop line. Moreover, deep vision is of considerable importance in various sports contexts. For example, in basketball free throws and baseball hitting, it is important to accurately perceive the distance to move toward a predetermined time or target. The results of this study show the possibility of improving the spatial perception abilities needed in daily life. The limitations of this study include the fact that the participants were VDT workers and the mechanism of action was not fully investigated. It is expected that the results for participants other than VDT workers and the mechanism of action will be elucidated in the future. In this study, 19 adverse events occurred in seven participants in the test food intake group, but none of them were causally related to the intake of the test food; therefore, the intake of the test food was considered to be more beneficial than harmful.

CONCLUSION

This randomized, placebo-controlled, and parallel-group study was conducted to investigate the effects of an 8-week intake of foods containing lutein, zeaxanthin, DHA, EPA, and piperine from long peppers (Hihatsu) on visual function after VDT work and safety during the intake period in healthy adult male and female participants. The outcomes of this study showed that food intake containing lutein, zeaxanthin, DHA, EPA, and piperine from Japanese spruce improved deep visual acuity after VDT work. In addition, as for the change in the state of feeling immediately after VDT work from before VDT work, the test foods significantly suppressed headache symptoms compared to the placebo foods after 8 weeks of intake, indicating that the test foods also help suppress unpleasant symptoms caused by VDT work.

Conflict of Interest: This study was funded by Marga Japan, Inc. The commission was paid to the testing organization. The funding agency provided the test foods. The authors included employees of the test provider, Marga Japan Inc. The authors declare no conflicts of interest.

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