Original Research

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Dietary Supplementation with a Combination of Lactoferrin, Fish Oil, and *Enterococcus faecium* WB2000 for Treating Dry Eye: A Rat Model and Human Clinical Study

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ABSTRACT **Purpose: To examine the effect of a combined dietary supplement containing fish oil, lactoferrin, zinc, vitamin C, lutein, vitamin E, γ-aminobutanoic acid, and *Enterococcus faecium* WB2000 on dry eye. Methods: A preliminary study in a rat model and a prospective, randomized, double-blind, placebo-controlled study in humans were conducted. Forty Japanese volunteers aged 22 to 59 years were randomized into combined dietary supplement (2 capsules/day; 20 participants) and placebo (vehicle; 19 participants) groups and treated once daily for 8 weeks. Rats received the combined dietary supplement components (10 or 50 mg/kg orally) or vehicle (2% DMSO), and dry eye was mechanically induced for 2 days. Tear production was measured in rats after dry eye was induced. Humans were assessed at baseline and weeks 4 and 8 post-supplementation based on keratoconjunctival epithelial damage; fluorescein tear film breakup time; tear production; biochemical data; information regarding subjective dry eye symptoms by answering a questionnaire; and information regarding adverse events via medical interviews. Results: Supplementation dose-dependently mitigated the decrease in tear production in rats. Among subjects with confirmed dry eye, clinical symptoms improved at weeks 4 and 8 more significantly in the supplementation group than in the placebo group ($P<.05$). The rate of increase in the Schirmer value was greater in the supplementation group. No adverse events occurred. Conclusion: Supplementation improved objective and subjective dry eye symptoms.**

KEY WORDS dietary supplements, dry eye, Schirmer test, tears

I. INTRODUCTION

Dry eye disease is very prevalent and is recognized as a growing public health problem that degrades the daily quality of life.[1,2] Although dry eye is a multifactorial disease, aging is one of the most important factors in its pathogenesis, and the incidence of dry eye has increased recently in the aged modern society.[1]

Currently, anti-aging medicine has advanced, but intervention in the aging process remains clinically challenging. The free radical theory is thought to partially explain aging,[3] and the aging process could potentially be controlled through the management of reactive oxygen species (**ROS**). To this end, dietary supplements have been developed as a major preventive approach for diseases of aging. Several dietary constituents, such as n-3 long-chain fatty acids, carotenoids (e.g., lutein), polyphenols (e.g., anthocyanins), lactoferrin, and antioxidants, are reportedly effective in improving visual acuity and other ocular functions.[4-8]

Local treatment such as eye drops and punctal plug insertion have been the primary options to date for management of dry eye. Nutraceutical approaches utilizing micronutrient supplements for therapy or prevention of dry eye are currently under investigation, and numerous positive

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OUTLINE

data have been reported. Recently, we developed a dietary combined supplement for the prevention and treatment of dry eye using research based on these anti-aging theories.

In the present study, we first investigated the efficacy of the mixed components of the supplement on tear production in a rat model. We then investigated the efficacy of the combined supplement on dry eye signs and symptoms in human subjects in a randomized, double-blind, placebo-controlled trial.

II. MATERIALS AND METHODS

A. Animal Study

1. Ethics

All procedures in the study conformed to the principles outlined in the Guide for the Care and Use of Laboratory Animals published by the USA National Institutes of Health (NIH Publication No. 85-23, revised 1996). All procedures were approved by the Institutional Animal National Care and Use Committee of Keio University School of Medicine (permission No. 11008-[2]) and were performed according to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

Dry eye was induced in rats by placing the animals on a swing, as described previously.[9] Briefly, a series of treatments were performed under dry conditions at a room temperature of 23 ± 2°C, relative humidity of 25 ± 5%, and constant air flow of 2−4 m/s. Each rat was placed on a swing for 7.5 h daily between 9 AM and 5 PM for 2 days. A mixture of 40.0% fish oil, 25.0% lactoferrin, 9.2% zinc gluconate, 6.7% vitamin C, 2.5% marigold extract, 2.0% vitamin E, 1.7% rice germ extract, and 1.7% *Enterococcus faecium* WB2000 was orally administered at a dose of 10 or 50 mg/kg/day prior to placement on the swing. Tear production was measured with a phenol red thread (Zone-Quick; Showa Yakuhin kako, Japan) under topical anesthesia comprising 0.4% oxybuprocaine hydrochloride (Santen Pharmaceutical, Japan). The thread was placed at the temporal upper eyelid margin for 1 min, and the length of the moistened area from its edge was measured to within 1 mm.

2. Measurement of Reactive Oxygen Species Production from the Lacrimal Gland

ROS production was assessed with ROS-sensitive fluorescence indicator 2′, 7′-dichlorofluorescein diacetate (DCFH-DA, Molecular Probes, USA). The excited lacrimal gland (**LG**) was immersed in cold phosphate-buffered saline (**PBS**) (25 mg tissue/mL) and homogenized. The homogenized tissue was incubated in DCFH-DA for 1 h at 37°C. The preparations were washed three times with PBS by centrifugation at 1000*g* for 3 min. The fluorescence of 2′,7′-dichlorofluorescein (**DCF**), the oxidation product of DCFH-DA, was measured at a 485-nm excitation and 530-nm emission.

B. Human Study

1. Ethics

This study adhered to the guidelines of the Declaration of Helsinki (amended in 2008) and the Ethical Guidelines for Epidemiological Research (enacted by the Japanese Government in 2004). The study protocol was approved by the Shirasawa Clinical Research Center Ethical Review Board. All subjects received a full explanation of the study procedures, and written informed consent was obtained from each subject prior to enrolment. To ensure privacy, all records were identified by an anonymous subject identification number.

2. Study Design

This study was a prospective, randomized, double-blind, placebo-controlled, parallel design dietary-supplement trial. It was designed to assess the efficacy and safety of a combined dietary supplement containing fish oil (source of eicosapentaenoic acid [**EPA**] and docosahexaenoic acid [**DHA**]), lactoferrin, zinc, vitamin C, lutein, vitamin E, γ-aminobutanoic acid (**GABA**), and *E. faecium* WB2000 in alleviating acute dry eye, as evaluated by ophthalmic examination, and subjective symptoms, as evaluated by a questionnaire.

The study was performed from July 2014 to September 2014 at the Haneginomori Eye Clinic in Tokyo, Japan. The study was registered with the University Hospital Medical Information Network in Japan (UMIN000014447).

3. Subjects

Male and female Japanese volunteers between 20 and 60 years of age who reported subjective symptoms of dry eye were recruited. Individuals were excluded according to the following criteria: current or previous severe ocular disease(s) such as strabismus, cataract, or glaucoma; a risk of developing seasonal allergy between July and September; LASIK operation within the previous 3 months; or allergy to the test supplement. Participants were also excluded if they routinely used other supplements that contained the same ingredients as those of the combined dietary supplement, were currently taking medicine to improve vision, or were receiving medical treatment. Volunteers receiving chronic medical treatment for their ocular symptoms were also excluded. The participants were allowed to withdraw from the study at any time for any reason. Additional exclusion criteria were as follows: a history of any other serious disease requiring medical treatment, participation in another clinical trial within 1 month prior to the start of the present study, pregnancy or lactation during the study period, and the presence of any health disorders based on the questionnaire results.

4. Ophthalmic Examination

The tear film breakup time (**TFB**UT) was measured, and keratoconjunctival epithelial damage based on fluorescein staining scores of the cornea and conjunctiva was assessed by two experienced investigators (M.K. and S.I.), who specialized in ocular surface diseases, particularly dry eye diseases, as described in a previous report.[10] For these examinations, 2 μL of preservative-free 1% fluorescein dye was instilled into each eye using a micropipette in order to avoid altering the tear dynamics. The Schirmer test was performed without topical anesthesia, following all other examinations. Tear Production Measuring Strips (Scaled; Showa Yakuhin Kako Co., Ltd., Tokyo, Japan) were placed for 5 minutes at the outer one-third of the temporal lower conjunctival fornix. The strips were then removed, and the length (mm) of wet filter paper was recorded. To avoid the interference of conjunctivocorneal staining on the Schirmer test results, the Schirmer test was performed after a 10-minute interval. The data were measured at baseline and at weeks 4 and 8 after beginning the intervention.

5. Assessment of Subjective Symptoms Self-Reported via a Questionnaire

The Dry Eye-Related Quality-of-Life Score (**DEQS**) questionnaire was administered at baseline, week 4, and week 8.[11] The DEQS questionnaire comprises 15 questions: six questions assess the ocular symptoms, and nine questions assess the effect of dry eye disease on the quality of life. The six questions related to ocular symptoms query the respondents regarding the presence and severity of foreign body sensation, dry sensation, pain or soreness, ocular fatigue, eyelid heaviness, and eye redness. The frequency of symptoms are scored from 0 (none) to 4 (highest frequency); the severity of symptoms, from 1 (low) to 4 (high).

6. Diagnosis of Dry Eye Disease

Participants were classified as having "definite dry eye disease (**DED**)," "probable DED," or "non-DED" based on the results of clinical examinations (Schirmer test, fluorescein and lissamine green staining, and TFBUT) and a completed symptom questionnaire. A diagnosis of DED was made according to the latest Japanese Dry Eye Diagnostic Criteria (2006), which include the following: 1) the presence of dry eye symptoms, 2) the presence of qualitative or quantitative disturbance of the tear film (Schirmer test of 5 mm or less or BUT of 5 s or less), and 3) the presence of keratoconjunctival epithelial damage (total score of fluorescein staining of at least 3 points). If all three criteria were met, a diagnosis of "definite DED" was made. Participants who fulfilled two of the three criteria were categorized as having "probable DED," and those who met none or only one of the three criteria were categorized into the "non-DED" group.[10]

7. Serum Biochemical Analysis

Biochemical assessment was performed at baseline and week 8.

8. Intervention

Subjects were administered combined dietary supplement capsules (Table 1; Wakamoto Pharmaceutical Co. Ltd., Tokyo, Japan) or placebo capsules containing only

Table 1. Ingredients of the experimental combined dietary supplement

Ingredient	Amount (mg per two capsules)
Lactoferrin	135
EPA	81
DHA	54
Lutein	3
Enterococcus faecium WB2000	10
Vitamin C	40
Vitamin E	8
Zinc	7
γ-aminobutanoic acid (GABA)	0.5

EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

vehicle (Table 1). Both capsule types were packaged in an opaque bag for blinding. All eligible subjects were randomly assigned (1:1) to receive the combined dietary supplement capsules (supplementation group) or the placebo capsules (placebo group). The subjects were required to take two capsules once daily after dinner with sufficient water for 8 weeks. Subjects were also instructed to self-record their allocated capsule intake status and any adverse events in a study diary during the entire intervention period.

9. Safety Assessment

Subjects were instructed to report any adverse events during a medical interview conducted at every visit during the study period. Biochemical assessment as described above was also used to assess safety.

10. Statistical Analysis

In this pilot study, the mean difference in Shirmer value change in pre- and post-medication between placebo and supplementation group was set at 5 mm, and the

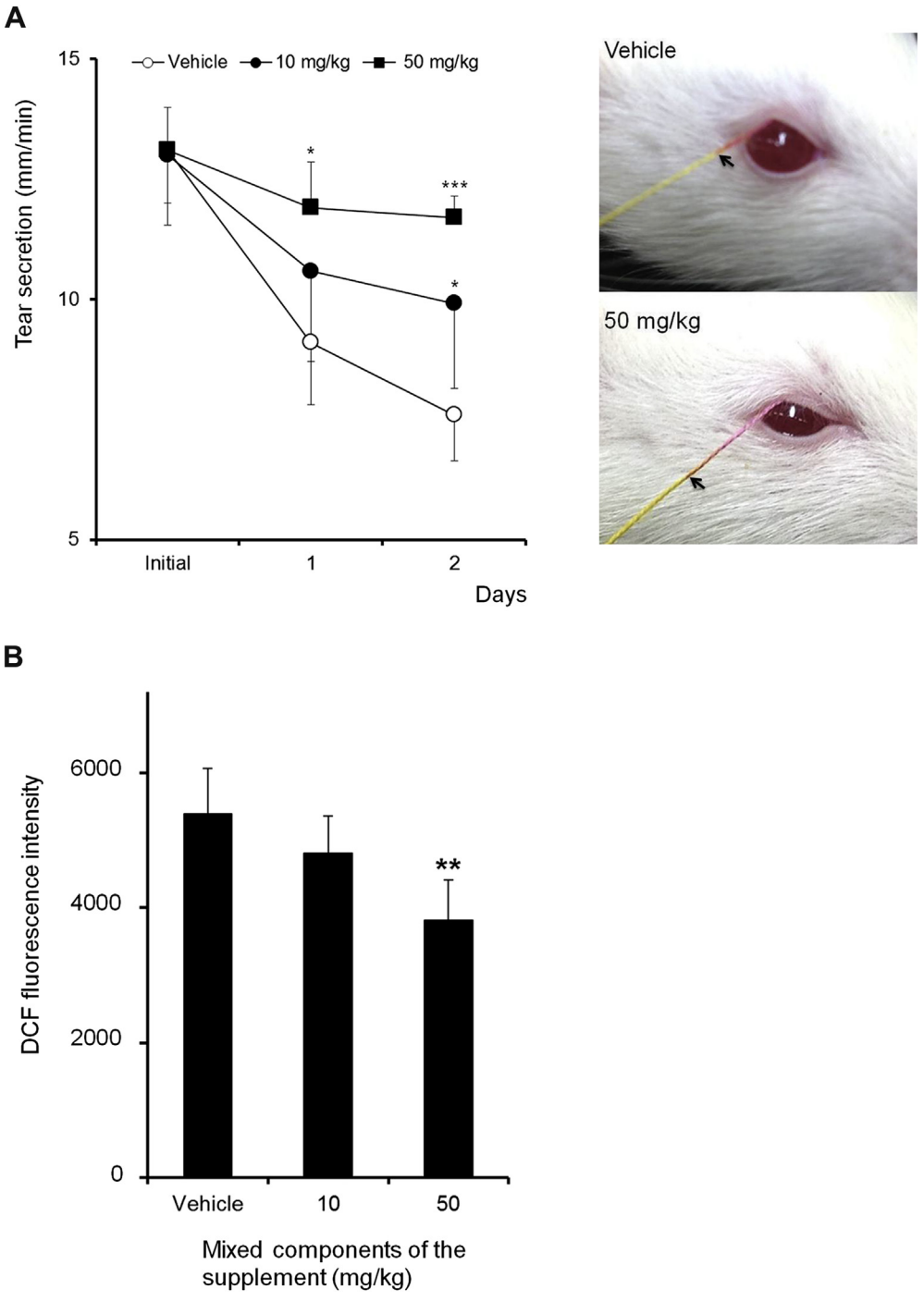

Figure 1. Effect of mixed supplement components on tear production and ROS formation from the lacrimal gland in a rat dry eye model. (A) Tear secretion and (B) ROS formation from the lacrimal gland. The mixed supplement components were dissolved in 2% DMSO at 0.4 mg/ml (10 mg/kg) or 2 mg/ml (50 mg/kg) and orally administered at 1 ml daily for 2 days. The 2% DMSO served as the vehicle control. Right panel: Tear production was measured using a cotton thread. Left panels: The length of wetting (arrows) in the cotton thread by tear secretion in the vehicle (top) and 50 mg/kg groups (bottom). Data are presented as the mean ± standard deviation (n=5 per group). *$P<.05$, **$P<.01$, ***$P<.001$ versus the vehicle.

Table 2. Baseline subject characteristics in the placebo and supplementation groups

Characteristic	Total	Supplement group	Placebo group	*P* value
Number of subjects	40	20	20	
Age (mean, range)	42.35 ± 8.09 (22–59)	41.75 ± 9.39 (22–57)	42.95 ± 6.74 (33–59)	.388†
Men (no., percentage)	21 (52.5%)	10 (50.0%)	11 (55.0%)	.500‡
Women (no., percentage)	19 (47.5%)	10 (50.0%)	9 (45.0%)	
Dry eye diagnosis*	12:22:5	5:12:2	7:10:3	.708§

* Data presented as the ratio of confirmed dry eye:probable dry eye:non-dry eye.
† Mann-Whitney *U* test.
‡ Fisher exact test.
§ χ^2 test.
No.=number.

standard deviation was 5.[10] Assuming 1:1 randomization, alpha was set at 0.05 and power was 80%. Then, the estimated sample size in each group was 17. The severity of ocular disease in each subject was analyzed. Data were compared with the paired *t* test for continuous variables, the Wilcoxon signed rank test for keratoconjunctival epithelial damage, and the Wilcoxon matched-pairs test for subjective symptoms. The baseline characteristics of the supplementation and placebo groups were compared with the Mann–Whitney *U* test for age, the Fisher exact test for gender, and the χ^2 test for dry eye diagnosis. The unpaired *t* test (continuous variables) and the Mann–Whitney *U* test (categorical variables) were used to assess the significance of differences in each parameter between the two groups. Statistical significance was indicated at $P<.05$. StatLight software (Yukms Co. Ltd., Tokyo, Japan) was used for statistical analysis.

III. RESULTS

A. Animal Study

Consistent with our previous study, tear production gradually decreased to below baseline in the vehicle group after the rats were exposed to the dry eye condition.[9] The mixed supplement components suppressed the decrease in tear production in a dose-dependent manner. Significant suppression was observed on day 2 at both the 10 mg/kg

Table 3. Effect of supplementation on dry eye, as assessed by ophthalmic examination

Variable	Time point	Supplement group		Placebo group		*P* value†
		Mean ± SD	*P* value*	Mean ± SD	*P* value*	
TFBUT (seconds)	Baseline	3.23 ± 2.44	-	3.15 ± 1.79	-	.353
	week 4	4.26 ± 3.08	**.038**‡	3.85 ± 2.20	**.018**‡	.239
	week 8	4.31 ± 2.41	**.012**‡	4.50 ± 2.65	**.001**‡	.412
Fluorescein staining score (points)	Baseline	1.37 ± 1.38	-	1.95 ± 1.67	-	.146
	week 4	1.16 ± 1.26	.107	1.60 ± 1.39	.055	.155
	week 8	0.95 ± 0.97	**.036**‡	1.25 ± 1.25	**.003**‡	.253
Schirmer test (mm)	Baseline	11.61 ± 8.86	-	17.40 ± 10.67	-	**.039**§
	week 4	14.44 ± 12.06	.178	18.00 ± 11.99	.370	.201
	week 8	16.28 ± 10.12	.065	19.85 ± 12.79	.141	.175

* The within-group score comparisons between baseline and week 4 and week 8 were performed using the paired *t* test (‡$P<.05$).
† The between-group score comparisons at each time-point (baseline, week 4, week 8) were performed using the Student *t* test (§$P < .05$) for the BUT and Schirmer test and the Wilcoxon rank sum test for the fluorescein staining score.
TFBUT = tear film breakup time.
All values are expressed as the mean ± standard deviation (SD).
Significant values are expressed in bold.

and 50 mg/kg dosages, as compared with the tear production in the vehicle group (Figure 1A). The mixed supplement components also dose-dependently suppressed ROS formation from the LG. Significant suppression was observed at the 50 mg/kg dose as compared with the vehicle (Figure 1B).

B. Human Study

1. Baseline Subject Characteristics

Forty participants were enrolled in the intervention study and assigned to either the placebo or supplementation group (n=20 per group). Twenty subjects in the placebo group (100%) and 19 in supplementation group (95%) were included in the efficacy analyses. One subject in the supplementation group was excluded from the efficacy analysis because of a lack of data.

Table 2 shows the baseline characteristics of the subjects in both groups. No participants had meibomian dysfunction. There were no significant differences in any variable between the placebo and supplementation groups.

2. Effect of Supplementation on Objective Dry Eye Parameters

The measured dry eye objective parameters are summarized in Table 3. The TFBUT increased at week 4 and week 8 from baseline in both the placebo and supplementation groups, with no significant difference between the two groups. The fluorescein staining score was significantly decreased from baseline at week 8 in both groups, with no significant difference between the two groups. The Schirmer test value was increased from baseline and differed significantly between the two groups. We further analyzed the rate of increase in the Schirmer value and found that the trend was greater in the supplementation group, although there was no significant difference between two groups (week 4, P=.08; week 8, P=.06; Figure 2).

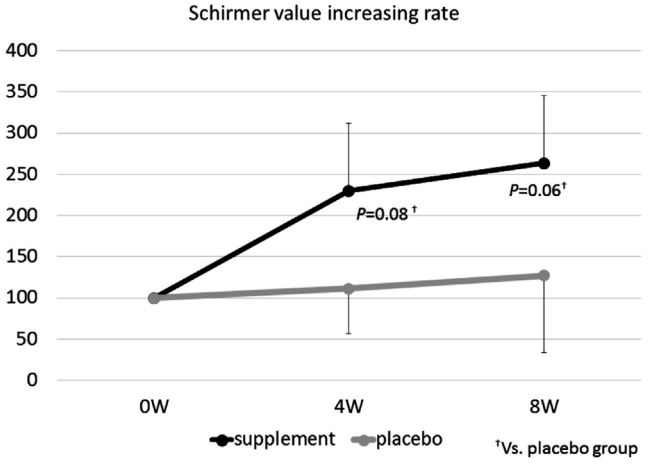

Figure 2. Rate of increase in the Schirmer test value in human subjects in the supplement and placebo groups. Data are presented as the mean ± standard deviation. †Indicates a statistically significant difference versus the placebo group.

3. Effects of Supplementation on Subjective Dry Eye Symptoms

Figure 3 shows the subjective symptom scores at baseline, week 4, and week 8. The scores were compared within each group between baseline, week 4, and week 8 and between the two groups at week 4 and week 8. The DEQS total score was significantly lower at week 4 and week 8 than at baseline in both groups (P<.05; Figure 3A). When the participants with confirmed dry eye (n=12) were analyzed, the decrease in the DEQS total score was significantly greater in the supplementation group (n=5) than in the placebo group (n=7) at week 4 and week 8 (P=.00 and P=.02, respectively, Figure 3E).

Among the ocular symptom items in the DEQS, foreign body sensation, dry eye sensation, and ocular fatigue improved following treatment (Figure 3B–D and F–H). The improvement was more apparent in the confirmed dry eye participants, especially in ocular fatigue, and was significantly greater in the supplementation group than in the placebo group (Figure 3H).

4. Safety and Tolerability

Table 4 summarizes the biochemical analysis results. None of the 40 subjects (0.0%) in either group reported any adverse events or side effects.

IV. DISCUSSION

We found that the mixed supplement components significantly maintained tear production in a rat dry eye model. In a subsequent double-blind, randomized controlled study of humans with dry eye symptoms, we also observed that both objective parameters and subjectively quantified dry eye symptoms were alleviated after 8 weeks of daily supplementation. The dry eye symptom score was especially improved significantly in participants with confirmed dry eye, as compared with the score in the placebo group. These results indicate that supplementation mitigated the effects of dry eye.

Concerning tear production, the increased rate of production was generally higher in the supplementation group than in the placebo group. These results were supported by those in the initial dry eye rat model. We previously found that the oxidation of protein, fat, and DNA at the ocular surface was accelerated in the rat swing model, which was the dry eye model used in this study.[9,12] Suppression of blink activity exposes the ocular surface to ambient oxygen and induces direct oxidation of cellular components. Based on these results, oxidative stress may be involved in the deterioration of ocular surface cells and dysfunction of the LG. It has also been reported that increased free radicals induce lacrimal dysfunction in superoxide dismutase-1 knockout mice.[13] Tobacco smoking, which is a risk factor for dry eye, reportedly induces ROS formation at the ocular surface and LG in both rats and humans.[14-16] These results suggest that dry eye is associated with oxidative stress.

The combined dietary supplement in this study was designed to reduce oxidative stress. Vitamins C and E,

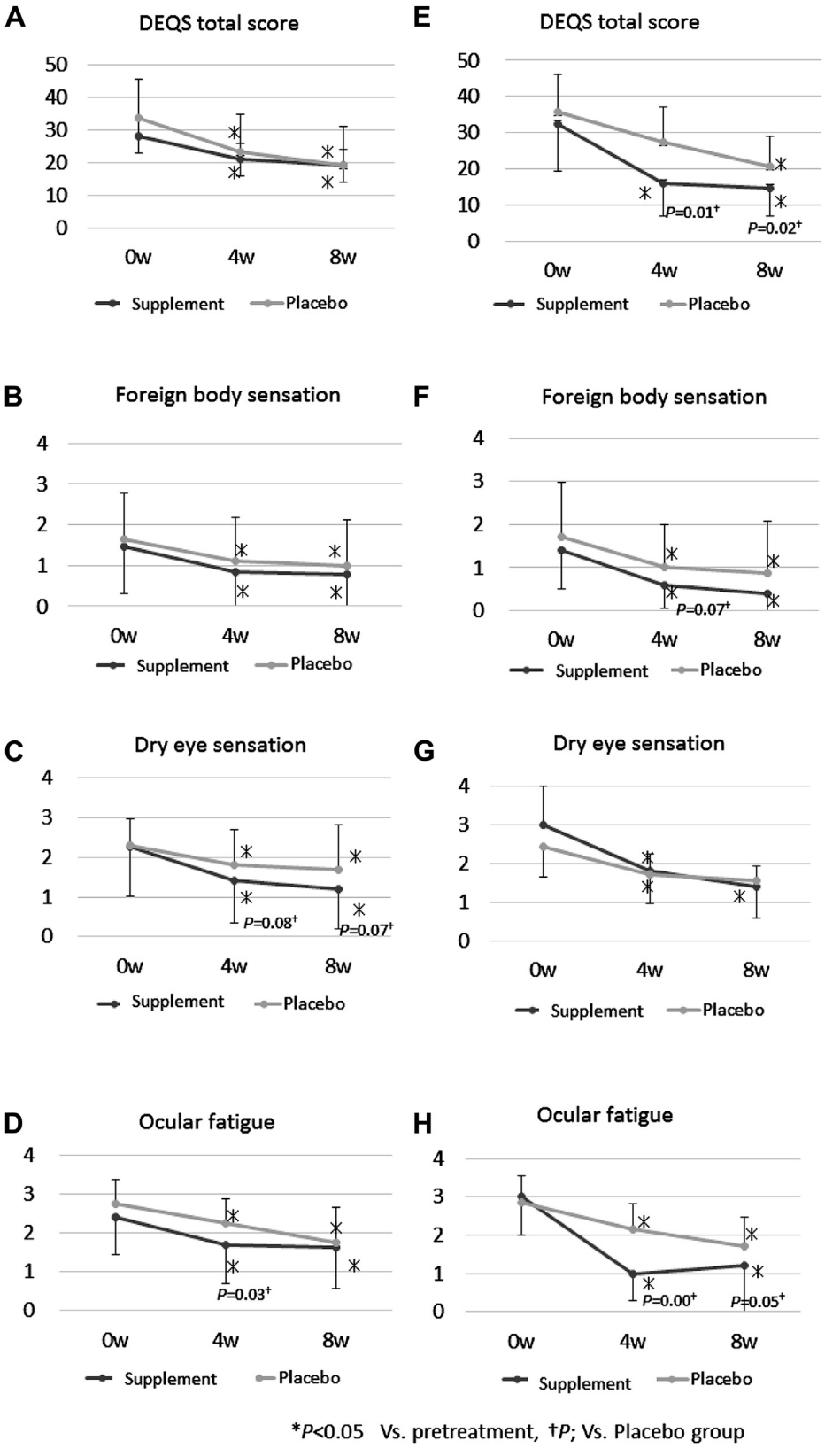

Figure 3. Dry Eye-Related Quality-of-Life Symptom (DEQS) questionnaire results in supplement and placebo groups. A—D. DEQS scores in all subjects (A. total score, (B) foreign body sensation, (C) dry eye sensation, (D) ocular fatigue). E—H. DEQS scores in subjects with confirmed dry eye (E. total score, (F) foreign body sensation, (G) dry eye sensation, (H) ocular fatigue).

zinc, lutein, and EPA/DHA were the major components of the supplement in this study and likely act as anti-oxidant and anti-inflammatory agents.[17] The selection of these constituents was based on previous reports of their efficacy in alleviating dry eye. Lactic acid bacteria were also included in the supplement because they are known to reduce

Table 4. Biochemical analysis

Variable	Time point	Supplement group		Placebo group		*P* value†
		Mean ± SD	*P* value*	Mean ± SD	*P* value*	
Total protein	Baseline	7.42 ± 0.39	-	7.32 ± 0.44	-	.225
	week 8	7.22 ± 0.38	.005‡	7.16 ± 0.37	.014‡	.307
AST	Baseline	19.75 ± 6.99	-	19.35 ± 6.34	-	.425
	week 8	20.55 ± 6.55	.131	20.40 ± 9.21	.229	.476
ALT	Baseline	21.35 ± 13.60	-	22.00 ± 13.6	-	.441
	week 8	23.35 ± 11.90	.158	23.80 ± 19.28	.221	.465
LD	Baseline	188.00 ± 30.95	-	182.25 ± 28.29	-	.272
	week 8	177.80 ± 24.34	.003	178.45 ± 32.39	.164	.472
ALP	Baseline	186.95 ± 62.21	-	190.15 ± 56.74	-	.433
	week 8	180.35 ± 50.23	.127	195.25 ± 53.29	.169	.184
γ-GT	Baseline	31.40 ± 28.52	-	33.65 ± 27.72	-	.401
	week 8	32.45 ± 32.29	.226	33.60 ± 31.53	.493	.455
UN	Baseline	13.18 ± 4.55	-	12.18 ± 3.34	-	.217
	week 8	12.26 ± 2.52	.090	11.29 ± 2.37	.170	.109
UA (men)	Baseline	6.00 ± 1.19	-	6.46 ± 1.12	-	.186
	week 8	5.96 ± 1.34	.185	6.54 ± 0.91	.151	.136
UA (women)	Baseline	4.30 ± 1.06	-	3.99 ± 0.85	-	.247
	week 8	4.24 ± 1.10	.278	4.19 ± 1.11	.059	.460
Total Chol	Baseline	192.90 ± 34.14	-	200.50 ± 36.44	-	.250
	week 8	189.70 ± 30.13	.293	200.55 ± 31.23	.495	.135
LDL-C	Baseline	107.75 ± 31.38	-	118.40 ± 31.98	-	.147
	week 8	103.35 ± 29.81	.203	119.75 ± 28.96	.350	.043§
HDL-C (men)	Baseline	59.30 ± 15.93	-	54.27 ± 9.52	-	.193
	week 8	59.20 ± 17.26	.472	57.00 ± 7.55	.091	.358
HDL-C (women)	Baseline	70.40 ± 15.60	-	64.56 ± 10.51	-	.179
	week 8	70.50 ± 17.83	.484	63.11 ± 13.48	.136	.163
TG	Baseline	117.85 ± 90.13	-	128.25 ± 70.49	-	.345
	week 8	117.80 ± 107.33	.498	124.95 ± 65.11	.414	.401
Glu	Baseline	95.00 ± 30.91	-	89.05 ± 13.28	-	.218
	week 8	90.00 ± 17.37	.259	92.80 ± 18.55	.246	.313

* The within-group score comparisons between baseline and week 8 were performed using the paired *t* test (‡$P < .05$).

† The between-group score differences at each time-point (baseline, week 4, week 8) were assessed using the Student *t* test or the Aspin−Welch *t* test (§$P<.05$).

AST, aspartate aminotransferase; ALT, alanine transaminase; LD, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GT, gamma-glutamyl transferase; UN, urea nitrogen; UA, urinalysis; Chol, cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol, TG, triglycerides; Glu, glucose.

All data are expressed as the mean ± standard deviation (SD).

oxidative stress.[18,19] The *E. faecium* WB2000 used in this study has been administered in traditional Japanese medicine (Strong Wakamoto®) to treat gastrointestinal discomfort for more than 50 years and generally has few recognized virulence determinants. As a result, this strain is considered safe for use in food. In one study, EPA/DHA consumption was associated with a decreased incidence of dry eye,[20] and EPA administration was beneficial in treating dry eye. Oral lactoferrin consumption reportedly may increase the tear volume and improve dry eye symptoms.[21,22] The current study results may be caused by a reduction in oxidative stress through supplementation, a mechanism supported by the animal results (Figure 1B); the molecular mechanisms are currently being investigated and will be described in a future report.

This study has several limitations. The follow-up period was only 2 months. The positive effects during this short period potentially support the use of supplementation to reduce dry eye symptoms and increase tear production, but long-term data are required to determine its clinical utility. And the sample size of this study was relatively small. Also, no patients with meibomian gland disease (MGD) were included in this study. We note that the relatively young ages of the participants might have had an effect on the incidence of MGD in our study population. Supplementation may also affect MGD; therefore, in a future multicenter and larger clinical study, we will consider MGD.

V. CONCLUSION

Findings of this study indicate that supplementation is a promising and safe therapy for alleviating dry eye symptoms and objective ocular changes, although additional data are required to validate the use of supplementation as a common intervention for dry eye.

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