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Effects of dietary supplementation with fish oil on dry eye syndrome subjects: randomized controlled trial

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ABSTRACT

The purpose of this study was to evaluate the efficacy of fish oil supplementation added to usual dry eye treatment in dry eye subjects in a randomized controlled trial. Twenty-seven typical dry eye subjects were selected from 43 candidates by the diagnostic criterion for dry eye in this study. They were assigned to the randomized fish oil group (n = 15) or the placebo group (n = 12). Fish oil group ingested fish oil capsules containing eicosapentaenoic acid (EPA, 1245 mg/day) and docosahexaenoic acid (DHA, 540 mg/day) for 12 weeks. Placebo group ingested placebo capsules without EPA or DHA. A visual analog scale test estimating subjective symptoms, the Schirmer I test, tear film break-up time (BUT) measurement, fluorescein staining, and rose bengal staining were performed every 4 weeks during the 12-week supplementation period and 4-week washout period. The subjective symptom of "eye pain", BUT, and changes in rose bengal staining score of the fish oil group were significantly improved after 8–12 weeks of supplementation and/or 4 weeks of washout, compared to those of the placebo group. These results suggest that fish oil supplementation added to usual care may be effective in the treatment of dry eye.

Dry eye disease is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface (4). Based on data from the largest epidemiologic studies of dry eye to date, the Women's Health Study (11), and other studies (10, 12), it has been estimated that about 3.23 million American women 50 years and older have dry eye disease (13). A recent study estimated the prevalence of definite dry eye disease to be 10.1% in male subjects and 21.5% in female subjects among young and middle-aged Japanese office workers (17). This discrepancy by sex may be explained by the dysfunction of tear and oil secretion from the lacrimal and meibomian glands, which has been reported to be regulated by sex hormones (9, 15, 16).

Recently, there have been reports noting that nutrients from diets were involved in the pathogenesis of dry eye. Miljanović *et al.* reported that the prevalence rate of dry eye was significantly lower in people with a high n-3 polyunsaturated fatty acids (PUFA) intake compared to those with a low n-3 PUFA intake in the analysis of 32,470 healthy women from 45 to 84 years old (5). Moreover, they reported that the diagnosis of dry eye was significantly lower in the population with a high intake of tuna containing n-3 PUFA, and the diagnosis of dry eye was significantly higher in the population with a

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high ratio of n-6 PUFA/n-3 PUFA. Furthermore, other groups reported that the composition of the lipid layer of tear fluid was involved in the tear film break-up time (BUT) and the rate of tear evaporation (8) and that n-3 PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were delivered to the lacrimal gland and retina from diets containing n-3 PUFA in rats (14). Thus, we hypothesized that supplementation with fish oil containing n-3 PUFA such as EPA and DHA improves dry eye syndrome by affecting the tear lipid layer and enhancing tear stability.

Subjective symptoms including dryness and irritations were added in diagnosis criteria of dry eye disease from 2006 in Japan (18). Therefore, tear stability, fluorescein staining, rose bengal (RB) staining, and subjective symptoms of dry eye were selected as the primary evaluation item for this study. In this study, we evaluated the efficacy of fish oil supplementation for dry eye patients.

MATERIALS AND METHODS

Experimental supplements. For the fish oil group, we used a commercially available fish oil supplement that contains EPA and DHA in the form of a soft gel capsule (Nippon Suisan Kaisha, Ltd., Tokyo). For the placebo group, we made a placebo supplement that did not contain EPA or DHA. The placebo supplement contained mainly middle chain triglycerides as edible oil. The fatty acid composition of capsules and the daily dose of EPA and DHA of experimental supplements are shown in Table 1.

Table 1 *Fatty acid composition (weight percent of total fatty acids) of dietary supplement and the daily dose of EPA and DHA*

			Fish oil	Placebo
Fatty acid				
8:0		(%)	0.3	83.3
10:0		(%)	0.3	15.5
12:0		(%)	0.2	0.2
14:0		(%)	4.6	ND
15:0		(%)	0.2	ND
16:0		(%)	5.7	ND
16:1		(%)	8.2	ND
16:2		(%)	1.7	ND
16:3		(%)	3.1	ND
16:4		(%)	6.0	ND
17:0		(%)	0.2	ND
17:1		(%)	0.2	ND
18:0		(%)	0.4	ND
18:1		(%)	8.3	0.7
18:2n-6		(%)	1.1	0.3
18-3n-3		(%)	0.8	ND
18-4n-3		(%)	4.8	ND
20:1		(%)	0.3	ND
20:2n-6		(%)	0.3	ND
20:4n-6		(%)	1.0	ND
20:4n-3		(%)	1.0	ND
20:5n-3	EPA	(%)	29.6	ND
21:5n-3		(%)	1.2	ND
22:5n-6		(%)	0.4	ND
22:5n-3		(%)	2.6	ND
22:6n-3	DHA	(%)	12.9	ND
24:1		(%)	0.2	ND
unidentified		(%)	4.4	0.0
EPA		mg/day	1245	0
DHA		mg/day	540	0

*ND, not detected.

Experimental design. This study was a double-blind, randomized, parallel-group, and placebo-controlled trial. The supplementation period was 12 weeks, and the washout period was 4 weeks. Measurements were carried out before supplementation and after 4, 8, and 12 weeks of supplementation and after 4 weeks of washout. Subjects took five capsules (460 mg/capsule) at breakfast, lunch, and dinner every day (15 capsules/day). We evaluated the changes in the objective and subjective symptoms of dry eye by the visual analog scale (VAS) test, tear BUT measurement, Schirmer I test, fluorescein staining score, and RB staining score. This study was performed according to the Helsinki Declaration and was approved by the institutional review boards at Keio University School of Medicine (Tokyo).

Subjects. Subjects were males and females over 40 years old, and informed consent for study participation was obtained from all subjects before the screening test. Dry eye subjects were selected by the following inclusion and exclusion criteria. In the supplemental period, taking of supplements other than the experimental supplements was prohibited. Subjects were instructed to maintain their normal food and exercise habits. All eligible subjects were randomly allocated to the fish oil or placebo group by using a random number table. All usual dry eye treatments such as eye-drops, but not supplementation, were maintained during this experiment in all subjects. These treatments were different among individuals.

Inclusion criteria. Both new patients and previous dry eye patients were included in this study. The di-

agnosis of dry eye was made by the following Japanese criteria: 1) presence of symptoms of dry eye, 2) abnormality of tear production as determined by the Schirmer I test (<5 mm after 5 min) or presence of tear film instability as determined by tear film BUT (<5 s), and 3) positive ocular surface RB (>3 points) or fluorescein vital staining (>3 points).

Exclusion criteria. Subjects with a severe disorder of the ocular surface such as Stevens-Johnson syndrome or ocular pemphigoid, with a systemic illness such as diabetes, hypertension, or autoimmune disease, taking supplements containing EPA or DHA, with hemophilia, gastrointestinal ulceration, urinary tract hemorrhage, hemoptysis, vitreous hemorrhage, or hemorrhagic tendency, treated by an ophthalmologic surgery within the previous 6 months, treated by punctal plug within the previous 1 month, or wearing contact lenses were excluded.

Measurement of subjective symptoms. We examined the changes of the subjective symptoms of eye pain and dry sensation before and after the supplementation period by a VAS test. In the case of eye pain, we used the form written as "I have no eye pain" (0) and "I feel extreme eye pain" (100) on the extreme ends of a 100-mm line.

Tear function tests and ocular surface vital staining. The standard tear film BUT measurement was performed. One percent fluorescein dye was instilled into the conjunctival sac as previously reported (3, 6). The interval between the last complete blink and the appearance of the first corneal black spot in the stained tear film was measured three times, and the mean value of the measurements was calculated. This was followed by staining with 1% RB solution. Fluorescein and RB stainings of the ocular surface were noted and scored. Both fluorescein and RB staining scores ranged between 0 and 9 points. Any score above 3 points was regarded as abnormal. For further evaluation of tears, the Schirmer I test without anesthesia was performed. The standardized strips of filter paper (ZONE-QUICK; Showa Yakuhin Kako Co., Ltd., Tokyo) were placed in the lateral canthus away from the cornea and left in place for five minutes with the eyes closed (6). Readings were recorded in millimeters of wetting for five min. A reading of less than 5 mm was referred to as an aqueous deficiency.

Statistical analysis. Data are expressed as the means ± the standard error (SE). The effects of time, treatment, and time × treatment were evaluated by two-way repeated-measures ANOVA. To compare the groups at certain times, we used a Mann-Whitney U test. Statistics were calculated using the StatView software package (Windows Version J 5.0, Abacus Concepts, Berkeley, CA). The significance level was set at $P < 0.05$.

RESULTS

Analyzed subject number

Twenty-seven dry eye subjects were selected from 43 candidates by the inclusion and exclusion criteria. During the supplementation period, one subject dropped out because of the reason other than the present experiment, and final data were calculated from 26 subjects (20 females and 6 males). Thirty eyes from 15 subjects in the fish oil group (10 females, 5 males; mean age: 52.5 ± 2.5 years) and 22 eyes in the placebo group (10 females, 1 male; mean age 51.9 ± 2.2 years) were evaluated in this study.

Subjective symptoms

The VAS score of eye pain after 12 weeks of supplementation in the fish oil group was significantly improved compared with the placebo group (Fig. 1a). In the dry sensation score, there were no significant differences between the two groups (Fig. 1b).

Tear film break-up time

In the BUT score, significant differences were observed at 12 weeks after supplementation and after the 4-week washout period between the two groups (Fig. 2a).

Schirmer test

There were no significant differences between the two groups in the Schirmer I test results (Fig. 2b).

Fluorescein staining score

In the fluorescein staining score, there was no significant difference between the two groups (Fig. 3a). Changes in the fluorescein staining score were also not significantly different between the two groups (Fig. 3b).

Rose bengal staining score

In the RB staining score, there was no significant difference between the two groups (Fig. 3c). Changes in the RB staining score after 8 and 12 weeks of supplementation in the fish oil group were significantly improved compared to those in the placebo group (Fig. 3d).

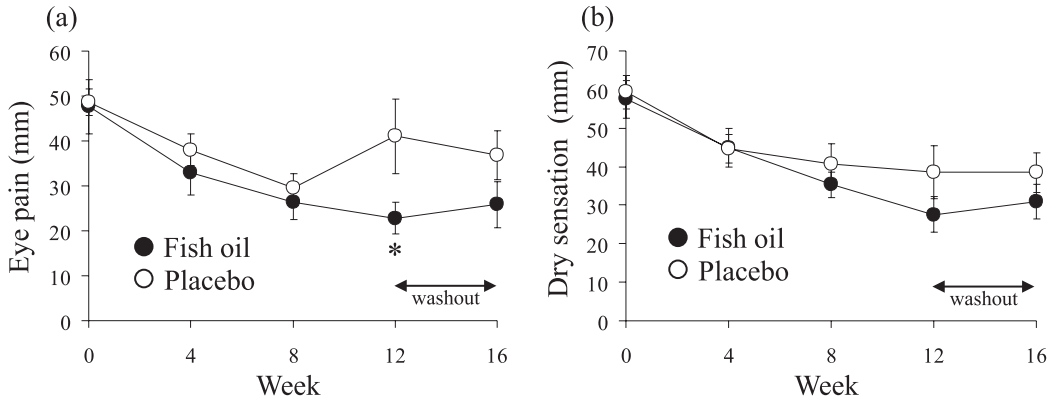

Fig. 1 Subjective symptoms of (a) "eye pain" and (b) "dry sensation" before and after supplementation for 12 weeks and after 4 weeks of washout in the fish oil group (n = 15) or the placebo group (n = 11). Each value is the mean ± SE. *$P <$ 0.05 *vs.* placebo group by Mann-Whitney U test. There were no significant differences between the two groups overall by two-way ANOVA.

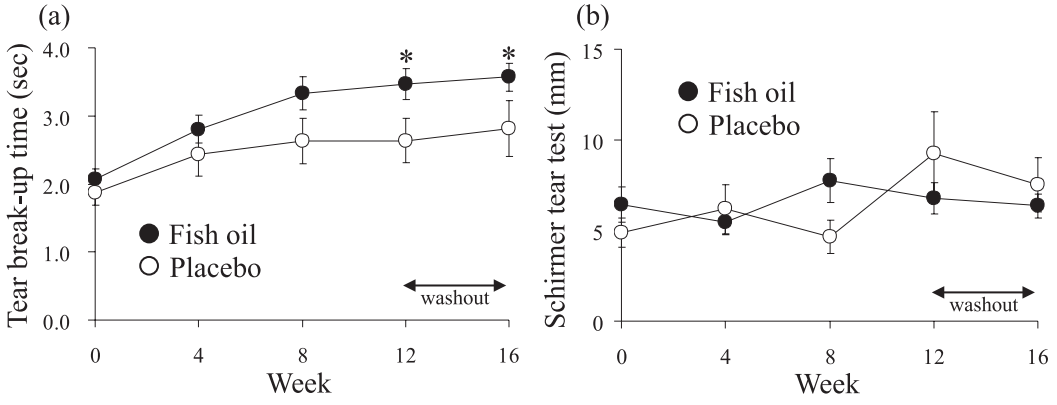

Fig. 2 (a) Tear break-up time and (b) Schirmer I test scores before and after supplementation for 12 weeks and after 4 weeks of washout in the fish oil group (n = 30 eyes) or the placebo group (n = 22 eyes). Each value is the mean ± SE. *$P <$ 0.05 *vs.* placebo group by Mann-Whitney U test. There were no significant differences between the two groups overall by two-way ANOVA.

DISCUSSION

In the present study, supplementation with fish oil containing EPA and DHA appeared to improve dry eye symptoms in the patients. We examined the effectiveness of fish oil supplementation for dry eye patients alongside usual dry eye treatment such as eye-drops in this study. Although the symptoms of the placebo group were also improved because of the usual treatment and/or placebo effects, it was suggested that fish oil supplementation accelerates recovery from dry eye symptoms compared to the recovery time with the usual treatment in this study. Furthermore, the improvement of the BUT in the fish oil group was also observed after the 4-week washout period. This result suggests that the treatment effect of fish oil continues for 4 weeks after the washout of fish oil supplementation. Moreover, side effects and dropouts caused by supplementation were not observed in this study. These results suggest that fish oil supplementation is a safe and worthwhile additional treatment for dry eye.

Wojtowicz *et al*. recently reported that average tear production and tear volume were increased in a group of patients taking an n-3 PUFA supplement containing 450 mg EPA, 300 mg DHA, and 1000 mg flaxseed oil for 90 days (19). However, since these improvements did not achieve statistical significance, the efficacy of n-3 PUFA for dry eye was not clear in that study. In the present study, we showed the efficacy of n-3 PUFA supplementation for the improvements of subjective symptoms, BUT, and RB staining score in the dry eye patients.

In the Schirmer I test, although there were no sig-

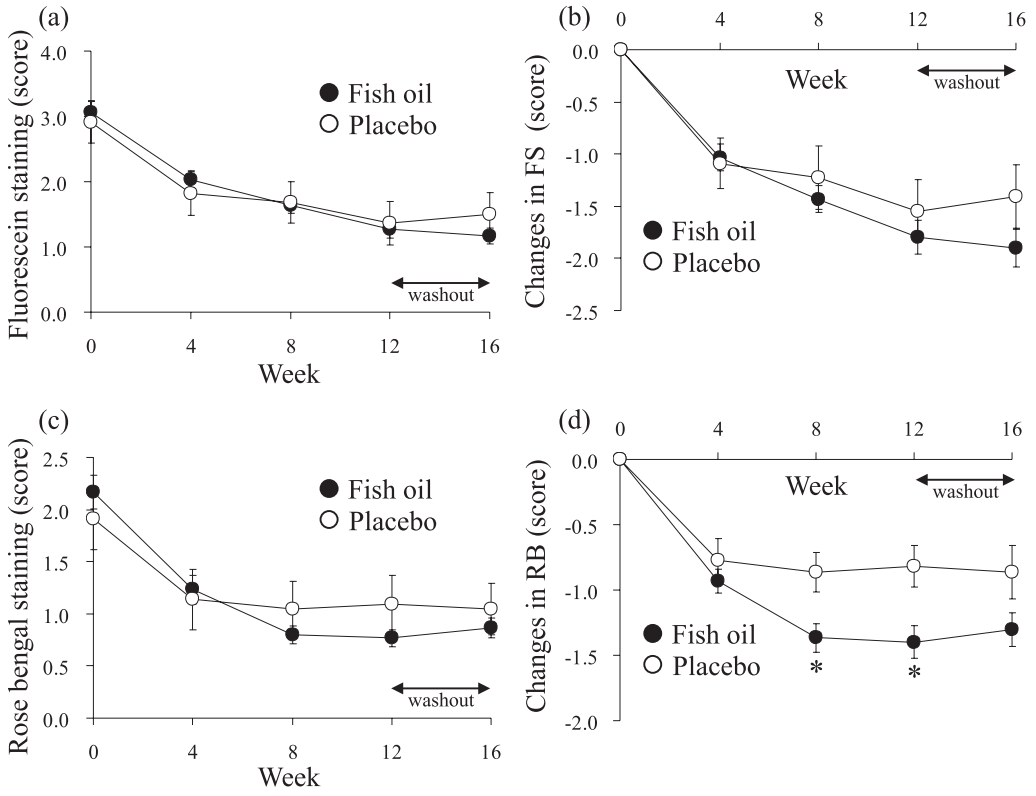

Fig. 3 (a) Fluorescein staining scores, (b) changes in fluorescein (FS) staining scores, (c) rose bengal staining (RB) scores, and (d) changes in RB staining scores before and after supplementation for 12 weeks and after 4 weeks of washout in the fish oil group (n = 30 eyes) or the placebo group (n = 22 eyes). Each value is the mean ± SE. *$P < 0.05$ *vs.* placebo group by Mann-Whitney U test. There were no significant differences between the two groups overall by two-way ANOVA (a, b, c). There was a significant difference between the two groups overall by two-way ANOVA (d).

nificant differences between the two groups, the BUT score in the fish oil group was significantly extended compared with the placebo group. These results suggest that tear stability was improved by fish oil supplementation, although tear volume was not affected in the present experimental condition. Wojtowicz *et al*. reported that tear stability was not changed by the n-3 PUFA supplementation for 90 days (EPA 450 mg/day, DHA 300 mg/day, and flaxseed oil 1000 mg/day) (19), and Craig *et al*. reported that tear stability was enhanced by increasing the thickness of tear lipid layer (2). Thus, the lipid layer of tears may have been thickened by the present fish oil dose (EPA 1245 mg/day and DHA 540 mg/day), because the tear stability was improved in this study. We are planning the study to examine whether fish oil supplementation thickens tear lipid layer in mice. Furthermore, since the patients with poor lipid secretion from the meibomian gland exhibited increased tear evaporation and decreased BUT (7), the improvement of BUT in the fish oil group in this study may have been achieved by enhancing lipid secretion from the meibomian gland. We are also planning the study to elucidate whether fish oil supplementation is effective for meibomian gland dysfunction patients.

In the present study, the improvement of BUT in the fish oil group after 12 weeks of supplementation did not reach the normal level (>5 s). Further research is needed to determine whether fish oil supplementation for longer than 12 weeks can accelerate the treatment effects and improve the BUT score to a normal level. However, since additive effects for usual treatments of dry eye were confirmed in this study, clinical value of fish oil supplementation is thought to be high in dry eye patients. Although there are some treatment approaches for dry eye such as artificial tears, punctal plug therapy, and autologous serum application (1), fish oil supplementation may have additive effects for these dry eye treatment approaches.

The changes in RB staining score in the fish oil group were significantly improved compared with those in the placebo group. This result suggests that

fish oil supplementation improves mucin disorders of the keratoconjunctival epithelium. The improvement mechanism is thought to be the result of the improvement of tear stability observed in the BUT score changing. Moreover, it is possible that fish oil supplementation directly affects conjunctival goblet cells secreting mucin. We are studying the precise mechanisms of the improvement of mucin layer by fish oil supplementation in experimental animals.

In summary, our results suggested that daily oral administration of EPA 1245 mg and DHA 540 mg for 12 weeks improved "eye pain" subjective symptom, BUT score, and discontinuity of the mucin layer assessed by RB staining in the dry eye patients. The improvement of the "eye pain" subjective symptom was presumed to be induced by the enhancement of tear stability and the improvement of the mucin disorders. We believe fish oil supplementation is a clinically valuable additional treatment for dry eye patients.

REFERENCES

1. Calonge M (2001) The treatment of dry eye. *Surv Ophthalmol* **45**, S227-S239.
2. Craig JP and Tomlinson A (1997) Importance of the lipid layer in human tear film stability and evaporation. *Optom Vis Sci* **74**, 8–13.
3. Kaido M, Ishida R, Dogru M, Tamaoki T and Tsubota K (2008) Efficacy of punctum plug treatment in short break-up time dry eye. *Optom Vis Sci* **85**, 758–763.
4. Lemp MA and Foulks GN (2007) The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf* **5**, 75–92.
5. Miljanović B, Trivedi KA, Dana MR, Gilbard JP, Buring JE and Schaumberg DA (2005) Relation between dietary n-3 and n-6 fatty acids and clinically diagnosed dry eye syndrome in women. *Am J Clin Nutr* **82**, 887–893.
6. Miyawaki S (2000) Revised Japan criteria for Sjögren syndrome. *Ryumachi* **40**, 48–53.
7. Nichols KK, Foulks GN, Bron AJ, Glasgow BJ, Dogru M, Tsubota K, Lemp MA and Sullivan DA (2011) The international workshop on meibomian gland dysfunction: executive summary. *Invest Ophthalmol Vis Sci* **52**, 1922–1929.
8. Ohashi Y, Dogru M and Tsubota K (2006) Laboratory findings in tear fluid analysis. *Clin Chim Acta* **369**, 17–28.
9. Oprea L, Tiberghien A, Creuzot-Garcher C and Baudouin C (2004) Hormonal regulatory influence in tear film. *J Fr Ophtalmol* **27**, 933–941.
10. Schaumberg DA, Dana R, Buring JE and Sullivan DA (2009) Prevalence of dry eye disease among US men: estimates from the Physicians' Health Studies. *Arch Ophthalmol* **127**, 763–768.
11. Schaumberg DA, Sullivan DA, Buring JE and Dana MR (2003) Prevalence of dry eye syndrome among US women. *Am J Ophthalmol* **136**, 318–326.
12. Schein OD, Hochberg MC, Muñoz B, Tielsch JM, Bandeen-Roche K, Provost T, Anhalt GJ and West S (1999) Dry eye and dry mouth in the elderly: a population-based assessment. *Arch Intern Med* **159**, 1359–1363.
13. Schein OD, Muñoz B, Tielsch JM, Bandeen-Roche K and West S (1997) Prevalence of dry eye among the elderly. *Am J Ophthalmol* **124**, 723–728.
14. Schnebelen C, Viau S, Grégoire S, Joffre C, Creuzot-Garcher CP, Bron AM, Bretillon L and Acar N (2009) Nutrition for the eye: different susceptibility of the retina and the lacrimal gland to dietary omega-6 and omega-3 polyunsaturated fatty acid incorporation. *Ophthalmic Res* **41**, 216–224.
15. Sullivan DA (2004) Tearful relationships? Sex, hormones, the lacrimal gland, and aqueous-deficient dry eye. *Ocul Surf* **2**, 92–123.
16. Sullivan DA, Wickham LA, Rocha EM, Krenzer KL, Sullivan BD, Steagall R, Cermak JM, Dana MR, Ullman MD, Sato EH, Gao J, Rocha FJ, Ono M, Silveira LA, Lambert RW, Kelleher RS, Tolls DB and Toda I (1999) Androgens and dry eye in Sjögren's syndrome. *Ann N Y Acad Sci* **876**, 312–324.
17. Uchino M, Schaumberg DA, Dogru M, Uchino Y, Fukagawa K, Shimmura S, Satoh T, Takebayashi T and Tsubota K (2008) Prevalence of dry eye disease among Japanese visual display terminal users. *Ophthalmology* **115**, 1982–1988.
18. Uchino Y, Uchino M, Dogru M, Ward S, Yokoi N and Tsubota K (2012) Changes in dry eye diagnostic status following implementation of revised Japanese dry eye diagnostic criteria. *Jpn J Ophthalmol* **56**, 8–13.
19. Wojtowicz JC, Butovich I, Uchiyama E, Aronowicz J, Agee S and McCulley JP (2011) Pilot, prospective, randomized, double-masked, placebo-controlled clinical trial of an omega-3 supplement for dry eye. *Cornea* **30**, 308–314.