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A Randomized, Double-Masked, Placebo-Controlled Clinical Trial of Two Forms of Omega-3 Supplements for Treating Dry Eye Disease

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Purpose: To assess the efficacy of 2 forms of oral long-chain omega-3 (ω-3) essential fatty acid (EFA) supplements, phospholipid (krill oil) and triacylglyceride (fish oil), for treating dry eye disease (DED).

Design: Randomized, double-masked, placebo-controlled clinical trial.

Participants: This study was conducted at a single site and involved 60 participants with mild to moderate DED who were randomized (1:1:1) to 1 of 3 groups: placebo (olive oil), krill oil, or fish oil supplements.

Methods: Participants received 1 of the 3 interventions: placebo (olive oil 1500 mg/day), krill oil (945 mg/day eicosapentaenoic acid [EPA], + 510 mg/day docosahexaenoic acid [DHA]), or fish oil (1000 mg/day EPA + 500 mg/day DHA) for 90 days, with monthly study visits.

Main Outcome Measures: Primary outcome measures were mean change in (1) tear osmolarity and (2) DED symptoms (Ocular Surface Disease Index [OSDI] score) between days 1 and 90. Secondary outcomes included mean change in key clinical signs (tear stability, tear production, ocular surface staining, bulbar and limbal redness, tear volume, anterior blepharitis, meibomian gland capping) and tear inflammatory cytokine levels.

Results: In total, 54 participants completed the study. At day 90, tear osmolarity was reduced from baseline with both krill oil (mean ± standard error of the mean: −18.6±4.5 mOsmol/l; n = 18; $P < 0.001$) and fish oil (−19.8±3.9 mOsmol/l; n = 19; $P < 0.001$) supplements, compared with placebo (−1.5±4.4 mOsmol/l; n = 17). OSDI score was significantly reduced at day 90 relative to baseline in the krill oil group only, compared with placebo (−18.6±2.4 vs. −10.5±3.3; $P = 0.02$). At day 90, there were also relative improvements in tear breakup time and ocular bulbar redness, compared with placebo, for both forms of ω-3 EFAs. Basal tear levels of the proinflammatory cytokine interleukin 17A were significantly reduced in the krill oil group, compared with placebo, at day 90 (−27.1±10.9 vs. 46.5±30.4 pg/ml; $P = 0.02$).

Conclusions: A moderate daily dose of both forms of long-chain ω-3 EFAs, for 3 months, resulted in reduced tear osmolarity and increased tear stability in people with DED. Omega-3 EFAs in a predominantly phospholipid form (krill oil) may confer additional therapeutic benefit, with improvements in DED symptoms and lower basal tear levels of interleukin 17A, relative to placebo. *Ophthalmology 2016;■:1–10 © 2016 by the American Academy of Ophthalmology*

Supplemental material is available at www.aaojournal.org.

Dry eye disease (DED) is a highly prevalent, multifactorial disease of the tear film and ocular surface that results in eye pain and impaired vision.[1] The mainstay of DED therapy, involving instillation of lubricant eye drops to provide temporary symptomatic relief, is supportive rather than therapeutic. Inflammation is a core mechanism in the pathogenesis of DED.[2] Various factors, including tear hyperosmolarity and instability,[2] contribute to the inflammatory response, which underwrites the chronic irritation and pain experienced by DED sufferers.[3]

Two main classes of topical anti-inflammatory agents, corticosteroids and immunomodulators, are currently used for DED management.[4] Corticosteroids target ocular surface inflammation,[5] but their potential long-term adverse effects, including cataract and steroid-induced glaucoma, limit their use to the acute control of exacerbations. Topical cyclosporin A, a fungal antimetabolite that inhibits interleukin (IL)-2–induced activation of lymphocytes,[6] is established to be safe, but there remains a need for large randomized controlled trials to clarify its clinical efficacy.[7]

Another avenue for altering the inflammatory status of the eye is through modulating systemic cytokine production with dietary intervention, in particular with omega-3 (ω-3) essential fatty acids (EFAs).[8] EFAs, which are essential to human health, must be ingested from dietary sources. The ratio of consumed ω-3 to omega-6 (ω-6) EFAs is a

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determinant of the inflammatory status of the body.[9] Whereas most eicosanoids derived from the ω-6 fatty acid pathway are proinflammatory, ω-3 EFAs bias prostaglandin metabolism toward the production of anti-inflammatory eicosanoids, which limit and resolve inflammation. In modern Western diets, the ratio of ω-6 to ω-3 intake is typically 15:1, whereas an ideal ratio is considered 4:1.[10] Increasing systemic ω-3 EFA levels through dietary intervention, to lower the ω-6 to ω-3 ratio, can therefore yield systemic anti-inflammatory benefits.

The Women's Health Study, involving over 32 000 women, reported an association between a low dietary intake of ω-3 EFAs and DED in women.[11] A 30% reduction in the risk of DED was found with each additional gram of ω-3 EFAs consumed per day.[11] Fish oil is a well-known source of long-chain ω-3 EFAs (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]). As summarized in a recent systematic review and meta-analysis of this topic,[12] oral supplementation with fish oil has undergone preliminary investigation for treating DED[13–16]; however, further evidence is required to substantiate these findings. Most clinical trials to date have been categorized with a high risk of bias, as they were not double-masked and/or had study design limitations.[12] Furthermore, the effect of krill oil supplementation for treating human ocular disease has not been investigated.

Krill oil derives from Antarctic krill, a zooplankton crustacean. Unlike fish oil, where the ω-3 EFAs are stored as triacylglycerides, in krill oil a major component of the EPA and DHA are esterified in phospholipid form; this potentially influences their tissue distribution and bioavailability.[17] Krill oil also contains the carotenoid antioxidant astaxanthin, which improves its stability.[18]

The major aim of this randomized, double-masked, placebo-controlled clinical trial was to assess the efficacy of 2 forms of long-chain ω-3 EFA supplements, phospholipid (krill oil) and triacylglyceride (fish oil), for treating DED over a 3-month intervention period.

Methods

This research project was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the University of Melbourne Human Research Ethics Committee (Health Sciences subcommittee). All participants provided written informed consent to participate. The clinical trial was prospectively registered on the Australian New Zealand Clinical Trials Registry (ACTRN12614001019695). Both eyes were tested for all clinical parameters; however, analyses are based on the eye with higher baseline (day 1) osmolarity.

Participants

The study was conducted at the University of Melbourne eye care clinic (Victoria, Australia), a community-based optometry clinic. Participant inclusion and exclusion criteria (Table S1, available at www.aaojournal.org) were designed to recruit adults with clinical signs and symptoms of mild to moderate DED. Enrollment commenced on October 29, 2014, and was completed on August 18, 2015. The trial was completed on November 24, 2015, when the final participant completed his or her day 90 visit. Participants using topical lubricant eye drops at baseline (day 1) were allowed to continue using these throughout the study. At each study visit, the investigator questioned participants with regard to how frequently they had used lubricant eye drops over the past month. There was no significant change to the frequency of eye drop utilization, compared with baseline, in any of the intervention arms over the study duration (data not shown).

Study Design

This was a randomized, double-masked, placebo-controlled clinical trial. Participants were assigned to 1 of 3 intervention arms: placebo (olive oil, 1500 mg/day), krill oil (945 mg/day EPA + 510 mg/day DHA), or fish oil (1000 mg/day EPA + 500 mg/day DHA) for 3 months. To achieve these doses, participants were instructed to consume 5 capsules per day, with or without food, at least 2 hours before or after any other medications.

The olive oil supplement was manufactured for the study (BJP Laboratories, Queensland, Australia). Olive oil is an appropriate inert control for ω-3 EFA investigations[19]; its main constituent (oleic acid, an ω-9 monounsaturated fatty acid) does not affect the levels of polyunsaturated EFAs incorporated within the body. The ω-3 EFA supplements are commercially available products (krill oil: Nutra-Life OceanClean red krill oil, Auckland, New Zealand; fish oil: Caruso's Natural Health UltraMAX fish oil, Sydney, New South Wales, Australia).

Participants attended 4 visits (day 1, day 30±7, day 60±7, and day 90±7) and were instructed to maintain their current dietary habits. The approximate dietary intake of ω-3 EFAs was determined by asking participants about their consumption of ω-3–rich foods over the preceding month. Participants were asked to quantify the approximate serving size (25, 50, 100, 150 g) and frequency of consumption of foods (including fish, oils, nuts, seeds, and spreads) containing greater than 1000 mg of combined EPA, DHA, docosapentaenoic acid, and α-linolenic acid per 100-g edible portion (Australia New Zealand Food Authority, 2011; Nutrient Data Laboratory and Beltsville Human Nutrition Research Centre, 2011). At subsequent visits, participants were questioned about changes to their diet or medications and about compliance with taking the study supplements.

Compliance was assessed by participants returning supplement containers with unused capsules at each follow-up visit for counting by an independent researcher. Acceptable participant compliance (for data inclusion) was defined, as in other nutraceutical trials,[20] as ≥75% capsule consumption, based on capsule counts.

Sample Size Calculation

A target sample size of 16 participants per arm was calculated based on having 90% power, at a 2-tailed significance level of 0.05, to detect at 25% change from baseline in Ocular Surface Disease Index (OSDI) score, assuming a standard deviation of 30%. An additional 4 participants per group were included to allow for up to 20% participant attrition, giving a recruitment target of 20 per group (60 participants in total).

Randomization

A 1:1:1 allocation ratio was used to randomize participants to 1 of the 3 intervention arms. An independent data manager generated a participant randomization sequence using a random number generator in Microsoft Excel (2007; Microsoft Corporation, Redmond, WA). This randomization schedule was provided to an independent compounding pharmacist (Dartnell's Pharmacy, Victoria, Australia), who repackaged the study supplements into identical, opaque containers using the randomization schedule. Supplement containers were labeled with the appropriate participant randomization code (from 001 to 060). Eligible participants

were sequentially enrolled by a masked research optometrist (L.A.D.), who dispensed the investigational product labeled with the appropriate code.

Masking

Participants were masked to treatment allocation, as achieved by all investigational products being dispensed in identical opaque containers; all participants also consumed the same number of capsules per day. All study personnel, including the principal investigator (L.E.D.), clinical outcome assessor (L.A.D.), laboratory outcome assessors (C.Y.W., D.C.J.), and co-investigators (H.R.C., A.J.V.), were masked to participant allocation. Following completion of all participant visits, data were analyzed only with knowledge of the simple randomization code (i.e., group A, B, or C allocation). Full unmasking of treatment allocation by the independent data manager only occurred after statistical analyses were complete.

Procedures

Unless otherwise specified, all procedures were performed at each study visit. The full schedule of assessments, including the order of testing, is provided in Table S2 (available at www.aaojournal.org).

Symptom Assessment. Dry eye symptoms were quantified on a scale from 0 (none) to 100 (maximum), using the validated, 12-item OSDI (Allergan, Inc, Irvine, CA),[21] which assesses symptoms of ocular discomfort, effects on visual function, and the impact of environmental triggers.

Visual Acuity. Noncycloplegic subjective spherocylindrical refraction was performed and monocular distance best-corrected visual acuity (BCVA) was reported as a logarithm of the minimal angle of resolution.

Tear Osmolarity. Tear osmolarity was measured bilaterally from the inferior lateral tear meniscus using the TearLab system (TearLab Corp, San Diego, CA). The instrument was calibrated according to the manufacturer's instructions. Room temperature was maintained between 20°C and 24°C.[22] Participants using lubricant eye drops were instructed not to instill these for at least 2 hours before examination; adherence to this instruction was confirmed before taking measurements. The same diagnostic pen was used for all assessments. Right eyes were measured first.

Meniscometry. Tear volume was quantified using tear meniscus height (TMH), measured both *en face* (using anterior eye photography) and in cross section (using optical coherence tomography). Three repeat measures were recorded and averaged per eye.

The Keratograph 5M (K5; Oculusoptikgerate GmbH, Wetzlar, Germany) was used to capture 3 photographs of the anterior eye under infrared illumination. ImageJ software (version 1.47v; National Institutes of Health, Bethesda, MD) was used to measure the *en face* TMH, defined as the length of a vertical line spanning from the top of the inferior tear meniscus to the eyelid margin. Measurements were taken either side of any eyelid abnormalities and as close to the center of the eyelid as possible.

An image of the inferior, central tear meniscus was captured using a 6.0-mm vertical B-scan with a 3-dimensional optical coherence tomography (Topcon Corporation, Itabashi-Ku, Japan). Cross-sectional TMH was measured as the length of a line drawn through the tear meniscus edge, beginning at the cornea and ending at the eyelid margin, using ImageJ software.

Basal Tear Collection and Cytokine Analyses. Basal tears, ~4 to 5 μl/eye, were collected by capillary flow from the inferior lateral meniscus using microcapillary tubes (MicroCaps; Drummond Scientific Company, Broomall, PA), as previously described.[23] Flow rate was monitored to exclude potential dilution effects resulting from reflex tearing. Only samples with a flow rate <2 μL/minute were used. Tear samples were stored at −80°C for subsequent analysis of cytokine concentrations. Levels of IL-2, IL-4, IL-6, IL-10, IL-17A, interferon (IFN)-γ, and tumor necrosis factor α were determined using a Cytometric Bead Array Human Th1/Th2/Th17 kit (BD Biosciences, San Jose, CA), according to the manufacturer's instructions, with the exception that samples were diluted 1 in 8.333 in a final volume of 25 μl, and a total of 1 μl of each capture bead was used in 25 μl of tear sample. Samples were analyzed on a Becton Dickinson FACSCanto II flow cytometer (Franklin Lakes, NJ) and data analyzed using Becton Dickinson FCAP Array software.

Tear Stability. Noninvasive tear breakup time (NITBUT) was quantified using the tear film surface quality breakup time parameter of the E300 corneal topographer (Medmont Pty Ltd, Victoria, Australia). We previously showed this measure to have high repeatability (coefficient of variation: 9.4%), as well as high sensitivity and specificity for diagnosing DED (82% and 94%, respectively), when using the shortest tear film surface quality breakup time of 3 repeat measures.[24]

Sodium fluorescein (NaFl) tear breakup time (TBUT) was measured using Dry Eye test strips (Amcon Laboratories, St. Louis, MO). The strip was moistened using nonpreserved saline and applied to the superior bulbar conjunctiva. One minute after instillation, NaFl TBUT was measured at the slit-lamp biomicroscope under cobalt blue illumination and a Wratten 12 yellow-barrier filter. Participants were asked to gently blink twice and then to hold their eyes open for as long as possible. The time between the second blink and the appearance of the first dark spot in the precorneal film was noted. Three NaFl TBUTs were recorded for each eye and averaged.

Slit-Lamp Examination. The anterior eye was examined at the slit-lamp biomicroscope for the degree of anterior blepharitis, meibomian gland (MG) capping, bulbar redness, and limbal redness, using the validated Efron scales.[25] Each parameter was assigned a grade, from 0.0 to 4.0, using increments of 0.1. For bulbar and limbal redness, the nasal and temporal regions were graded separately and averaged.

Corneal and conjunctival staining were assessed using an established methodology.[26] Corneal NaFl staining was examined at the slit lamp using cobalt blue illumination and a Wratten 12 yellow-barrier filter, 2 minutes after instillation of NaFl. The degree of corneal staining was assessed in each eye with the 5-point Oxford scale,[26] using grading increments of 0.1. Nasal and temporal conjunctival lissamine green (LG) staining was assessed using the Oxford scale, 1 minute after instillation of LG (GreenGlo; Sigma Pharmaceuticals, North Liberty, IA).

Tear Production. The Schirmer test was performed, with topical anesthesia, as a measure of basal tear production. One drop of 0.5% proxymetacaine hydrochloride (Alcon Laboratories, New South Wales, Australia) was instilled into the lower conjunctival sac. After 4 minutes, the folded end of a sterile Schirmer strip (Optitech Eyecare, Allahabad, India) was placed between the middle and lateral third of the inferior eyelid margin. Participants were instructed to close their eyes in a dimly lit room, and the strip wetting (in millimeters) was recorded after 5 minutes.

Intraocular Pressure. Intraocular pressure (IOP) was measured using a Perkins applanation tonometer (Clement Clarke, Harlow, United Kingdom) immediately following instillation of 1 drop of 0.5% proxymetacaine hydrochloride and NaFl dye.

Pregnancy Testing. For female subjects of self-reported childbearing potential, a 20-ml urine sample was tested with an Instalert pregnancy test (Innovacon, Inc, San Diego, CA) at days 1 and 90.

Outcome Measures

Prespecified primary outcome measures were mean change between days 1 and 90 for (1) symptoms, quantified using OSDI

score; and (2) tear osmolarity in the eye having higher osmolarity at baseline.

Secondary outcomes were mean change from baseline (day 1) at days 30, 60, and 90 for tear film stability (measured using NaFl TBUT and NITBUT), bulbar and limbal redness, ocular surface staining, tear production, tear volume, anterior blepharitis, MG capping, and basal tear levels of inflammatory cytokines.

Safety was assessed by documenting adverse events. In addition, the safety end points were mean change in BCVA and IOP between days 1 and 90.

Statistical Analyses

Data relating to participant demographics and baseline clinical characteristics were analyzed using GraphPad Prism (version 6.01; GraphPad Software, San Diego, CA) with the Mann-Whitney, Kruskal-Wallis, χ^2, 1-way analysis of variance, or unpaired *t* tests with Welch correction, as appropriate. Analyses were performed on the eye having highest tear osmolarity at the day 1 visit, as an established objective indicator of disease severity.[27] Data normality was assessed using the D'Agostino & Pearson omnibus test. Where there was a statistically significant difference between groups, Tukey or Dunn post hoc analyses were performed.

Intergroup comparisons were analyzed using GenStat (16th edition; VSN International, Hemel Hempstead, United Kingdom). Repeated measures analysis of covariance was used to assess for differences in the post-treatment means between groups (i.e., the main effect of group) at each time point (days 30, 60, and 90), with the baseline (day 1) values used as a covariate. Where there was a statistically significant main effect, a post hoc analysis was undertaken using the Fisher least significant difference for comparison between group means. For all analyses, an α level of 0.05 was adopted for statistical significance. Unless otherwise specified, data are expressed as mean ± standard error of the mean.

A post hoc analysis was also performed, combining the 2 active intervention arms (fish oil and krill oil) into a single group (a long-chain ω-3 EFA treatment group; n = 37) and comparing changes in outcome measures with placebo (n = 17) at day 90. Repeated measures analysis of covariance was used to assess for differences in the post-treatment means at day 90, with baseline values used as a covariate.

Results

Participants

Of 108 potential participants screened for eligibility, 60 met the eligibility criteria and enrolled in the clinical trial; 54 completed the study (i.e., 90% participant retention). Of the 6 participants who did not complete the study, 3 were from the placebo group; 1 discontinued after day 1 as her employment situation changed and 2 participants were lost to follow-up after day 30. One participant withdrew from the krill oil group after day 1, following self-reported surgical intervention by an ophthalmologist overseas, and another participant was lost to follow-up after day 1. One participant in the fish oil arm was lost to follow-up after day 1.

Baseline participant demographics and clinical data for each study group are provided in Table 3. At day 1, there was no significant difference between groups for age, gender, ethnicity, or the primary outcome measures of OSDI score and tear osmolarity. Baseline values for secondary clinical outcome measures were also similar between the study groups, except for NaFl TBUT, which was relatively shorter in the krill oil group (Table 3). The combined EPA + DHA dietary intake of participants at baseline was similar across all intervention groups (placebo: 135.0±34.2 mg/day, fish oil: 160.3±32.7 mg/day, krill oil: 178.9±44.7 mg/day). None of the participants reported major changes to their intake of foods containing high levels of ω-3 fatty acids throughout the study.

There was a high level of treatment fidelity, as measured by returned capsule counts. Compliance was 94%±1%, 93%±2%, and 93%±2% for the placebo, fish oil, and krill oil groups, respectively. All participants exceeded the prespecified, minimum level of compliance; no data were excluded from analysis on these grounds.

Primary Outcome Measures

Tear Osmolarity. As shown in Figure 1A, at the primary end point (day 90), tear osmolarity was reduced from day 1 with both fish oil (−19.8±4.5 mOsmol/l, n = 19; $P < 0.001$) and krill oil (−18.6±3.9 mOsmol/l, n = 18; $P < 0.001$) supplementation, compared with placebo (−1.5±4.4 mOsmol/l; n = 17). The fish oil group also showed a significant reduction in tear osmolarity compared with placebo at day 60 (−18.4±5.1 mOsmol/l, n = 19 vs. −4.5±4.6 mOsmol/l, n = 17; $P = 0.006$).

Ocular Surface Disease Index. Figure 1B shows changes to OSDI score. At day 90, OSDI was reduced, relative to day 1, in the krill oil group, compared with placebo (−18.6±2.4, n = 18 vs. −10.5±3.3, n = 17; $P = 0.02$). Although the fish oil group showed a trend toward reduced symptoms compared with placebo over the course of the study, this did not reach statistical significance at any time point.

Secondary Outcome Measures

Tear Film Stability. Figure 2 shows data relating to NaFl TBUT. The first significant intergroup difference was evident at day 60, with increases relative to day 1 in both the fish oil (3.7±1.6 seconds, n = 19; $P < 0.004$) and krill oil (4.0±1.5 seconds, n = 18; $P = 0.02$) groups, compared with placebo (−2.8±1.3 seconds, n = 17); this effect was maintained in both groups at day 90 (Fig 2A). The mean NITBUT did not alter significantly between groups, over the study duration.

Ocular Redness. Both forms of ω-3 EFA supplements significantly reduced bulbar redness (Fig 3). Relative to day 1, both the fish oil (−0.2±0.1 units, n = 19; $P = 0.005$) and krill oil (−0.2±0.1 units, n = 18; $P = 0.009$) groups had significantly less bulbar redness than the placebo group (0.2±0.1 units; n = 17) at day 30. At day 60, the krill oil group continued to show relatively decreased bulbar redness compared with the placebo group (−0.6±0.1 units, n = 18 vs. 0.0±0.2 units, n = 17; $P = 0.01$). Day 90 findings were similar to those at day 30, with both the fish oil (−0.3±0.2 units, n = 19; $P = 0.009$) and krill oil (−0.5±0.2 units, n = 18; $P = 0.004$) groups having less redness relative to day 1, compared with placebo (0.2±0.2 units, n = 17). Limbal redness did not alter significantly between groups, or within groups, over the study duration.

Ocular Surface Staining. The degree of nasal LG conjunctival staining was reduced in both the fish oil (−0.1±0.1 units, n = 19; $P = 0.04$) and krill oil (−0.2±0.1 units, n = 18; $P = 0.01$) groups compared with placebo (0.2±0.2 units, n = 17) at day 60. This reduction seemed transient, with no difference between groups by day 90. There were no significant changes to either NaFl

Table 3. Baseline Participant Demographics and Clinical Characteristics of the Study Groups

	Placebo (n = 17)	Fish Oil (n = 19)	Krill Oil (n = 18)	*P* Value
Demographics				
Age (yrs)	46.2±4.5	39.4±3.4	42.3±3.8	0.5
Gender (% female)	82	47	72	0.1
Ethnicity (% white)	94	84	72	0.2
Primary outcome measures				
Tear osmolarity (mOsmol/l)	327.1±5.0	325.6±2.6	324.8±1.7	0.4
OSDI (score/100)	31.7±2.6	36±3.4	30.5±2.4	0.3
Secondary clinical outcome measures				
NaFl TBUT (sec)	8.3±1.4	8.1±1.1	5.1±0.5*	**0.02**
NITBUT (sec)	6.4±1.6	9.2±1.7	6.6±1.4	0.5
Bulbar redness (Efron score)	2.8±0.2	2.6±0.2	2.7±0.2	0.8
Limbal redness (Efron score)	1.2±0.1	1.2±0.1	1.1±0.1	0.9
TMH – *en face* (μm)	204.6±23.9	189.8±16.1	182.4±14.5	0.7
TMH – cross section (μm)	209.9±19.6	218.2±14.7	188.6±16.1	0.7
NaFl corneal staining (Oxford score)	0.6±0.2	0.5±0.1	0.7±0.2	0.8
LG conjunctival staining – nasal (Oxford score)	0.3±0.1	0.2±0.1	0.5±0.2	0.6
LG conjunctival staining – temporal (Oxford score)	0.3±0.2	0.2±0.1	0.4±0.1	0.4
Anterior blepharitis (Efron score)	0.2±0.1	0.5±0.2	0.6±0.2	0.2
Meibomian gland capping (Efron score)	0.5±0.1	0.6±0.1	0.9±0.1	0.3
Schirmer test (mm/5 min)	14.2±2.4	10.1±2.2	10.3±2.0	0.3
BCVA (logMAR)	−0.1±0.0	−0.1±0.0	−0.1±0.0	0.8
IOP (mm Hg)	13.2±0.7	13.9±0.5	13.5±0.5	0.4

BCVA = best-corrected visual acuity; IOP = intraocular pressure; LG = lissamine green; logMAR = logarithm of the minimum angle of resolution; NaFl = sodium fluorescein; NITBUT = noninvasive tear breakup time; OSDI = Ocular Surface Disease Index; TMH = tear meniscus height.
Data are expressed as mean ± standard error of the mean. *P* values represent the main group effect.
*Denotes a statistically significant difference ($P < 0.05$).

corneal staining or temporal LG conjunctival staining between groups, or within groups, over the study duration.

Other Clinical Parameters. Tear volume (quantified using both *en face* and cross-sectional TMH), Schirmer test score, and the degree of both anterior blepharitis and MG capping were unchanged between groups, and within groups, throughout the study.

Post hoc analyses were performed, combining the 2 active intervention arms (fish oil and krill oil) into a single group (a long-chain ω-3 EFA treatment group; n = 37) and comparing changes with outcome measures with placebo (n = 17) at day 90. These analyses (Fig S4, available at www.aaojournal.org) showed additional significant effects of supplementation with ω-3 EFAs compared with analyses involving the individual fish oil and krill oil arms. At day 90, ω-3 EFA supplementation resulted in significant relative reductions in limbal redness (−0.2 ± 0.1 units, n = 37 vs. 0.0±0.1 units, n = 17; $P = 0.04$; Fig S4A, available at www.aaojournal.org), NaFl corneal staining (−0.3±0.2 units, n = 37 vs. 0.0±0.1 units, n = 17; $P = 0.03$; Fig S4B, available at www.aaojournal.org), and MG capping (−0.5±0.1 units, n = 37 vs. 0.1±0.3 units, n = 17; $P = 0.04$; Fig S4C, available at www.aaojournal.org).

Tear Inflammatory Cytokines. As shown in Figure 5, basal tear concentrations of the proinflammatory cytokine IL-17A were reduced at day 90 relative to day 1 in the krill oil group compared with placebo (−27.1±10.9 pg/ml, n = 18 vs. 46.5±30.4 pg/ml; $P = 0.02$). There were no significant intergroup differences for any of the other cytokines (IL-2, IL-4, IL-6, IL-10, IFN-γ, or tumor necrosis factor α) over the study duration.

Safety Outcomes

At day 90, there were no significant changes to BCVA or IOP in any of the study groups (Table S4, available at www.aaojournal.org). Table S5 (available at www.aaojournal.org) summarizes adverse

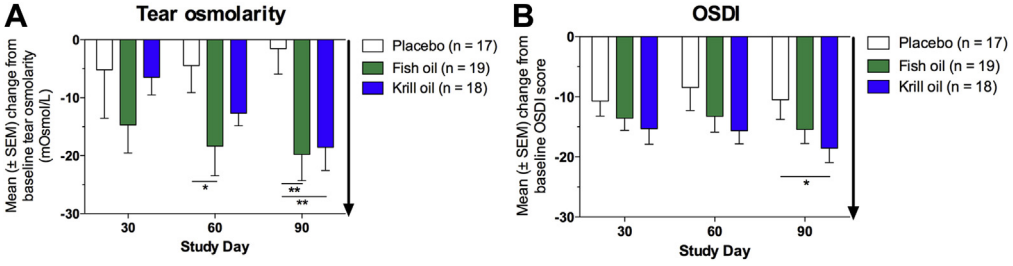

Figure 1. Primary outcome measures. Change in tear osmolarity (**A**) and Ocular Surface Disease Index (OSDI) score (**B**) from baseline (day 1) at days 30, 60, and 90 in each of the study groups. Data are expressed as mean ± standard error of the mean (SEM). Asterisks show statistically significant intergroup differences (*$P < 0.05$, **$P < 0.01$). Arrow indicates direction of improvement.

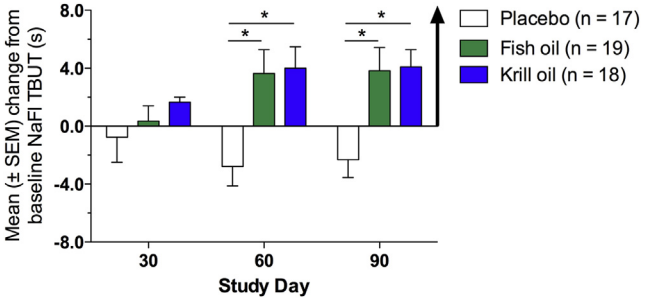

Figure 2. Tear stability. Change in sodium fluorescein (NaFl) tear breakup time (TBUT) from baseline (day 1) at days 30, 60, and 90 in each of the study groups. Data are expressed as mean ± standard error of the mean (SEM). Asterisks show statistically significant intergroup differences (*$P < 0.05$). Arrow indicates direction of improvement.

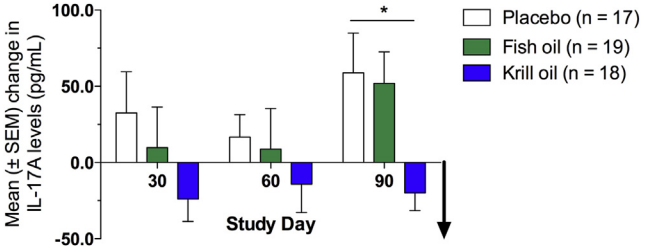

Figure 5. Tear interleukin (IL)-17A concentrations. Change from baseline in basal tear IL-17A levels (picograms per milliliter) at days 30, 60, and 90 in each of the study groups. Data are expressed as mean ± standard error of the mean (SEM). Asterisks show statistically significant intergroup differences (*$P < 0.05$). Arrow indicates direction of improvement.

events. There were no documented pretreatment adverse events. The 3 interventions were generally well tolerated. Of the 53 adverse events noted, 51 were considered mild (i.e., awareness of symptoms or signs but well tolerated) and 2 were graded as moderate (i.e., discomfort interfering with normal activity). There were no serious adverse events.

The most frequently reported adverse events were colds (24.5%), sore throat (11.3%), headache/migraine (11.3%), and gastrointestinal events (e.g., nausea, abdominal discomfort, bloating, stomach cramps, heartburn, and gastroenteritis; 13.2%). Based on the principal investigator's (L.E.D.'s) assessment, 72% were deemed to have no potential association with the study supplements and 28% of the adverse events were considered to be potentially related to the study supplements.

Discussion

This randomized, double-masked, controlled clinical trial sought to assess the efficacy of krill oil (predominantly phospholipid ω-3 EFAs) and fish oil (triacylglyceride ω-3 EFAs) supplementation for treating DED over a 3-month period. Moderate (~1000 mg EPA + ~500 mg DHA) daily doses of both forms of long-chain ω-3 EFAs were found to significantly reduce tear osmolarity, improve tear stability, and reduce ocular bulbar redness. Notably, we report novel data relating to the efficacy of krill oil supplementation as an anti-inflammatory intervention for DED. Furthermore, our findings suggest that krill oil may confer additional therapeutic benefits over fish oil, with improvements in DED symptoms (OSDI score) and lower basal tear levels of IL-17A relative to placebo at the study end point.

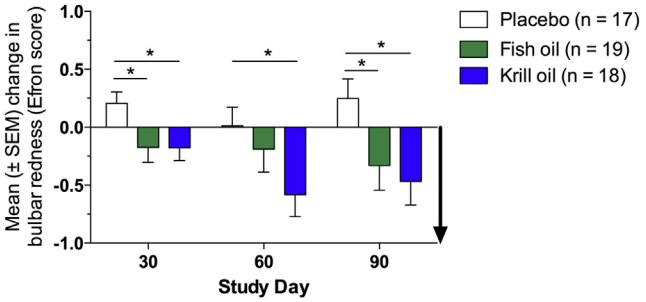

Figure 3. Ocular bulbar redness. Change in ocular bulbar redness score, measured using the Efron grading scale, from baseline (day 1) at days 30, 60, and 90 in each of the study groups. Data are expressed as mean ± standard error of the mean (SEM). Asterisks show statistically significant intergroup differences (*$P < 0.05$). Arrow indicates direction of improvement.

The effect sizes for both primary outcomes reported in this trial are highly clinically significant. This study demonstrates the beneficial effects of ω-3 EFA supplementation for reducing tear hyperosmolarity in DED. Our findings are similar to those recently reported by Epitropoulos et al,[28] over a 12-week intervention period in people with MG dysfunction, with the use of a significantly higher daily dose (1680 mg EPA + 560 mg DHA) of re-esterified ω-3 EFA supplements, in the context of having an ω-6–based oil as a control. Attenuation of a season-dependent elevation in tear osmolarity has also been described with the consumption of sea buckthorn oil, containing both ω-3 and ω-6 EFAs, over 3 months in women having DED.[29]

Tear hyperosmolarity is a well-established feature of DED, being associated with ocular surface inflammation[30] and leading to epithelial and/or goblet cell loss.[31,32] At baseline, the mean ± standard error of the mean tear osmolarity of the fish oil and krill oil groups was 326±3 and 325±2 mOsmol/l, respectively, consistent with moderate DED. At day 90, tear osmolarity was reduced from baseline with fish oil and krill oil, compared with placebo, by an average of 20 and 19 mOsmol/l, respectively. A post-treatment tear osmolarity of 306 mOsmol/l in both groups represents a profound normalization of tear tonicity (normal values[27] <308 mOsmol/l) that is markedly below the 316 mOsmol/l threshold for DED diagnosis.[33] The reduction in tear osmolarity with omega-3 supplementation shows a linear trend over the study duration, which was not asymptotic at day 90 (Fig 1A). This finding suggests the potential for further improvement with a longer treatment period.

The profound reduction in tear osmolarity was associated with clinically meaningful improvements in DED symptoms, which occurred by day 90, approximately 1 month after the normalization of tear tonicity. The finding of reduced tear osmolarity preceding symptomatic improvement in patients with DED corroborates previous reports[34,35] and supports the utility of tear osmolarity as a

responsive parameter for monitoring therapeutic efficacy in DED clinical trials. We attribute the observed lack of synchrony between changes to dry eye clinical signs and symptoms to the need for physiological improvements in key parameters underpinning the DED response (in particular, the amelioration of tear hyperosmolarity and improvement in tear stability, which relate to the degree of ocular surface inflammation) before subjective improvement is evident.

The observed attenuation of dry eye symptoms with short-term, long-chain ω-3 EFA supplementation is in agreement with results from previous clinical studies.[14–16,28] At day 90, OSDI score was reduced by an average of 19 units from baseline with krill oil supplementation, being statistically different from the placebo group (which improved on average by 10.5 units). The level of symptomatic improvement substantially exceeds the OSDI minimal clinically important difference of 7.3 units for moderate DED,[36] even in the placebo group, which did not demonstrate any significant change in the objective outcome parameter of tear osmolarity. These findings emphasize the importance of placebo intervention arms in trials investigating therapies for DED, to control for the "clinical trial effect," particularly if subjective outcome measures are adopted.

Both forms of ω-3 EFA supplements also conferred significant improvements in tear film stability. Because tear film instability is a key pathogenic factor in DED,[2] it follows that improving tear stability is an important strategy for managing DED. NaFl TBUT was increased in both the fish oil and krill oil groups, reaching statistical significance at days 60 and 90, compared with placebo. On average, NaFl TBUT improved from 8.1±1.1 and 5.1±0.5 seconds at baseline to 11.9±2.0 and 9.2±0.9 seconds at the study end point in the fish oil and krill oil groups, respectively. Given that normal NaFl TBUT is 10 seconds or longer,[37] this degree of improvement is of major clinical significance and exceeds the criteria for a clinically important difference in NaFl TBUT (change of at least 30%[38]).

A post hoc statistical analysis, combining the 2 active intervention arms (fish oil and krill oil) into a single intervention group (a long-chain ω-3 EFA treatment group; n = 37), showed additional significant improvements in clinical outcome measures with ω-3 EFAs compared with placebo (n = 17). Specifically, reductions in the severity of limbal redness, NaFl corneal staining, and MG capping were evident at day 90. The reduction in corneal staining most likely relates to the normalization of tear tonicity with ω-3 EFA supplementation, leading to a reduction in hyperosmolarity-induced ocular surface epithelial cell apoptosis.[39] In contrast to our results, several previous clinical trials found no significant effect on corneal staining with the use of ω-3 EFA supplements.[28,40,41] Divergent findings are not unexpected, given that corneal fluorescein staining is a relatively late-stage manifestation of DED[42] and differences in the investigated study populations likely account for the apparent variations in outcomes. Our findings are similar to those described with the use of the immunomodulatory agent cyclosporin A, which was found to reduce conjunctival epithelial cell apoptosis and protect against a loss of conjunctival goblet cells in an experimental murine model of DED.[43]

The observed reduction in MG capping with ω-3 EFA supplementation is also not unanticipated. The level of dietary ω-3 EFA intake is associated with differences in the polar lipid pattern of MG secretions in women with Sjögren syndrome.[44] Supplementation with short-chain omega-3 EFAs has been shown to alter the composition of MG secretions, with corresponding improvements in gland blockage and tear stability over a 12-month treatment duration.[40] A reduction in lid margin inflammation and improved MG expressibility has also been reported with the use of triacylglyceride ω-3 EFAs, in association with lid hygiene and ocular lubricants, in patients with MG dysfunction.[15]

Significant reductions in ocular redness, being a marker of ocular inflammation, were imparted by both fish oil and krill oil. The beneficial effects of ω-3 EFAs on reducing bulbar hyperemia were first apparent at day 30 and were maintained at day 90. At the study end point, bulbar redness was reduced, on average, by 0.3±0.2 and 0.5±0.2 Efron grading units in the fish oil and krill oil groups, respectively. Although this reduction was statistically significant in both groups, only the krill oil group had a clinically significant improvement (defined as ≥0.50 Efron grading units[45]). Though no previous studies have reported reductions in bulbar redness with ω-3 EFA supplementation alone, a randomized controlled study found a significant decrease in conjunctival hyperemia after 6 months of combined ω-6 and ω-3 EFA supplementation.[46]

It is interesting that bulbar hyperemia was the first clinical sign to demonstrate improvement with ω-3 EFA supplementation, with a significant reduction evident at day 30 compared with placebo. The observed decrease in ocular redness may relate to ω-3 EFA supplementation imparting ocular anti-inflammatory effects. Indeed, topical loteprednol 0.5%, a corticosteroid with potent anti-inflammatory properties, reduces clinical markers of inflammation, including conjunctival injection, in patients with DED over a 2-week treatment period.[47] Another possibility is that ω-3 EFAs directly affect vascular reactivity within the conjunctival and/or episcleral plexi. Dietary supplementation with either krill oil or fish oil for 2 weeks has been shown to have beneficial effects on cerebral blood flow autoregulation in rodents.[48] Further in support of this hypothesis is the finding of an inverse relationship between brachial artery diameter and the red blood cell membrane ratio of polyunsaturated to saturated fatty acid in patients with systemic hypertension.[49]

We also report that krill oil supplementation significantly decreased tear levels of the proinflammatory cytokine IL-17A. This is a notable effect that has not been previously reported with ω-3 EFA supplementation in humans. The finding is, however, supported by evidence in a mouse transgenic model that endogenously converts ω-6 into ω-3 polyunsaturated fatty acids (the fat-1 mouse), in which it has been shown that ω-3 EFAs modulate T-helper 17 cells to produce lower levels of inflammatory factors, including IL-17, which provides protection against psoriasis-like

inflammation.[50] It is intriguing that we did not observe this effect with fish oil supplementation, suggesting that it may relate to unique properties of krill oil, in particular its phospholipid form and/or the effects of the antioxidant astaxanthin. Topical application of astaxanthin has been shown to ameliorate ultraviolet-induced corneal epithelial damage in an acute murine model of photokeratitis.[51] In relation to bioavailability, there is evidence for differences in DHA tissue accretion within the eye, depending on the form of consumed fatty acid (i.e., phospholipid or triacylglyceride). In a study involving 10-week-old rats, incorporation of DHA into the anterior uvea, kidney, and brain was approximately 3-fold higher with a phospholipid formulation compared with a triacylglyceride form of supplementation.[52] Furthermore, in a double-masked crossover trial involving healthy participants, krill oil was found to be more effective than either fish oil or placebo at decreasing the ω-6-to-ω-3 fatty acid ratio and increasing the percentage of EPA and DHA incorporated into erythrocyte membranes.[53] Together, these findings suggest that the form of ω-3 EFA supplementation may differentially modulate ocular surface inflammation.

IL-17A, which is implicated in autoimmune disease and tissue inflammation,[54] has been suggested to play a role in mediating corneal epithelial barrier disruption in DED.[55] The observed reduction in tear IL-17A levels with krill oil supplementation parallels the immunomodulatory effects imparted by topical cyclosporin A (0.01%), which decreases conjunctival IL-17A and IFN-γ expression in experimental models of DED.[55] Therapeutic agents aimed at specifically modulating T-helper 17 cells and/or IL-17 production have been proposed as novel strategies for dry eye therapy.[56] In this respect, krill oil supplementation is arguably a compelling anti-inflammatory intervention for DED, which improves clinical signs and symptoms of DED in concert with reducing tear IL-17A, being a key proinflammatory cytokine involved in the pathogenesis of the condition.

In this study, we did not detect any significant changes to the basal levels of other tear cytokines, including IL-6 and IL-10, between the study groups. This finding contrasts with an open-label study conducted by Pinazo-Durán et al,[57] which reported decreased levels of IL-1ß, IL-6, and IL-10 in reflex tears relative to baseline, following a 3-month intervention with long-chain ω-3 EFAs combined with antioxidants. The observed differences in findings may, at least in part, relate to the use of divergent tear collection methods. In this clinical trial, we quantified cytokine levels from basal tears, whereas the study by Pinazo-Durán et al[57] assayed reflex tear samples obtained by forced stimulation. Tear collection method, as well as the type of tear (i.e., basal vs. reflex) collected, influences tear composition and hence may underlie the apparent variation in findings. Differences in the study supplement formulations, in particular the additional incorporation of a range of antioxidants, including vitamins A, C, and E; zinc; and glutathione, in the supplement utilized by Pinazo-Durán et al,[57] may also have contributed to a differential modulation of ocular surface inflammation.

We acknowledge the limitations of this proof-of-principle study, being its sample size (n = 60) and its undertaking at a single site. Nevertheless, the trial was undertaken in a robust manner, in strict accordance with the CONSORT statement,[58] thus minimizing all major potential sources of bias.

In conclusion, this study demonstrates the beneficial effect of long-chain ω-3 EFA supplementation for reducing key clinical signs and symptoms of mild to moderate DED over a 3-month treatment duration. Our findings suggest that the krill oil supplementation may confer additional benefits over fish oil. Further investigation of the therapeutic potential of krill oil for treating DED is warranted, with a larger, multicenter clinical trial over a longer treatment duration, to confirm its long-term efficacy and safety.

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Footnotes and Financial Disclosures

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Analysis and interpretation: Deinema, Vingrys, Jackson, Chinnery, Downie

Obtained funding: Downie

Overall responsibility: Deinema, Vingrys, Wong, Jackson, Chinnery, Downie

Abbreviations and Acronyms:
BCVA = best-corrected visual acuity; **DED** = dry eye disease; **DHA** = docosahexaenoic acid; **EFA** = essential fatty acid; **EPA** = eicosapentaenoic acid; **IFN** = interferon; **IL** = interleukin; **IOP** = intraocular pressure; **LG** = lissamine green; **MG** = meibomian gland; **NaFl** = sodium fluorescein; **NITBUT** = noninvasive tear breakup time; **OSDI** = Ocular Surface Disease Index; **TBUT** = tear breakup time; **TMH** = tear meniscus height.

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