Pilot, Prospective, Randomized, Double-masked, Placebo-controlled Clinical Trial of an Omega-3 Supplement for Dry Eye

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**Purpose:** To investigate the potential effect of dietary supplementation with omega-3 fatty acid on lipid composition of meibum, aqueous tear evaporation, and tear volume in patients with dry eye.

**Methods:** In a pilot, prospective, randomized, double-masked study, patients with dry eye received a daily dose of fish oil, containing 450 mg of eicosapentaenoic acid, 300 mg of docosahexaenoic acid, and 1000 mg of flaxseed oil (TheraTears Nutrition; Advanced Vision Research, Woburn, MA) for 90 days. There were 2 patient visits: baseline and final. At these visits, patients completed the ocular surface disease index to score subjective symptoms, and slit-lamp examinations, breakup time, corneal staining, Schirmer type I, fluorophotometry, evaporometry, and collection of meibomian gland secretion samples for lipid composition analysis were performed.

**Results:** A total of 36 patients with dry eye completed the study. At the end of the study, 70% of the patients became asymptomatic, whereas for the placebo group, 7% of the symptomatic patients became asymptomatic. Schirmer testing and fluorophotometry suggested that the omega-3 supplement increased tear secretion. The lipid composition of the samples collected from the omega-3 group was found to be very similar to that from the placebo group. No trends between groups were seen for other objective parameters.

**Conclusions:** Dietary supplementation with omega-3 fatty acids in dry eye showed no significant effect in meibum lipid composition or aqueous tear evaporation rate. On the other hand, the average tear production and tear volume was increased in the omega-3 group as indicated by both Schirmer testing and fluorophotometry.

**Key Words:** dry eye, omega-3 fatty acid, tear evaporation, meibum, lipid composition

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Currently, dry eye conditions are among the most common disorders treated by ophthalmologists throughout the world. The International Dry Eye Workshop (2007) concluded that “the core mechanisms of dry eye are driven by tear hyperosmolarity and tear film instability” and that “tear hyperosmolarity is regarded as the central mechanism causing ocular surface inflammation, damage, and symptoms and the initiation of compensatory events in dry eye.” Tear osmolarity can increase because of either decreased tear secretion or increased tear film evaporation.

Several studies have established an association between the dietary intake of omega-3 (n-3s) supplement with changes in the meibomian gland oils, and its implications in dry eye disease. First, the meibomian gland oils of patients with Sjögren syndrome are influenced by the dietary intake of n-3s. Second, data from 32,470 women in the Women’s Health Study found that a low dietary intake of n-3s, a high n-6:n-3 ratio, or both increase the risk of clinically diagnosed dry eye in women. And finally, Oxholm and collaborators reported that the severity of dry eye and dry mouth disease in patients with Sjögren syndrome was inversely proportional to membrane and serum levels of docosahexaenoic acid (DHA). Topical application of n-3s has been shown in dry eye induced in mice to significantly decrease corneal fluorescein staining, CD11b+ cell number, and expression of cornea interleukin (IL)-1α and corneal and conjunctival tumor necrosis factor (TNF)-α as compared with topical linoleic acid (LA). There are multiple health benefits of dietary supplementation with n-3s particularly in the area of coronary and vascular health; so if dietary n-3 supplementation is beneficial for dry eye, systemic administration of n-3s makes good sense.

There have been 2 anecdotal reports of dietary supplementation with flaxseed oil thinning meibomian gland secretions and improving dry eye symptoms. Long-chain fish oil n-3s are antiinflammatory and block the gene transcription of the proinflammatory cytokines TNF-α, IL-1α, and IL-1β, the very cytokines that block neurotransmitter release in the lacrimal and salivary glands in dry eye. Blocking TNF has restored tear secretion in an autoimmune rabbit model for dry eye. The present pilot study was performed to investigate the effect of flaxseed and fish oils dietary supplementation on the tear film and the ocular surface.
MATERIALS AND METHODS

Study Population

The study protocol, consent form, and data accumulation methods used in this randomized and double-masked study, were approved by the Institutional Review Board of the University of Texas Southwestern Medical Center. The eligibility criteria for patients entering the study were previous clinical diagnosis of dry eye. In addition, they were required to have positive vital dye staining with 1% lissamine green according to the National Eye Institute/Industry Workshop scale (1995) detected by slit-lamp examination in the absence of concurrent disease including eyelid or ocular surface inflammation beyond 1+ bulbar conjunctival injection. Exclusion criteria were patients with previous ocular surgery, alterations of the lacrimal drainage system, or eyelid abnormalities. Patients with any systemic disease that might affect the ocular surface and pregnant or lactating women were excluded as well. Likewise, patients using ophthalmic medications or systemic medication affecting tear secretion or lipid metabolism or treatment with commercially available essential fatty acids (EFA) or vitamin supplements were excluded. Thirty-six patients with dry eye, some with concomitant meibomitis or meibomian gland dysfunction, were enrolled in this pilot study.

Study Design

Patients were randomly assigned to receive a daily soft capsule in the morning containing either the active drug or placebo for 90 days. Each daily dose of the active softgels provided fish oil, containing 450 mg of eicosapentaenoic acid (EPA) and 300 mg of DHA and 1000 mg of flaxseed oil (TheraTears Nutrition; Advanced Vision Research, Woburn, MA). The placebo capsules contained wheat germ oil. Both active and placebo softgels appeared identical and were supplied in identical containers for masking purposes. Preservative-free TheraTears lubricant eyedrops were supplied to standardize patient’s topical therapy. All patients were instructed to follow their usual diet and to record changes in a diary.

There were 2 patient visits: baseline and final (after 3 months of treatment). At these visits, patients completed the ocular surface disease index (OSDI) to score subjective symptoms, and physicians performed slit-lamp examinations, measured breakup time (BUT) and corneal staining, and performed Schirmer without anesthesia, fluorophotometry, and evaporometry. Meibomian gland secretion samples were collected for lipid composition analysis. By protocol design, only the left eyes of all subjects were tested. The patients and study personnel were masked with respect to group assignment to assure accuracy. At the final visit, the capsule containers were collected and remaining capsules counted as a measure of compliance.

Clinical Assessment

At baseline and final visits (after 3 months of treatment), the OSDI questionnaire was administered by an interviewer. The following clinical signs and objective measurement parameters were evaluated at the slit lamp and recorded: eyelid margin edema and hyperemia (0/1/2/3), conjunctival injection and edema (0/1/2/3), BUT (an average of 3 repeated measurements), and 1% lissamine green staining (pattern and score). The density of each stain was recorded on a 4-point scale, with 0 being no staining and 3 being severe according to the National Eye Institute/Industry Workshop scale (1995). Schirmer without anesthesia was measured at 5 minutes. Meibomian gland expressibility was defined as the number of glands that could be expressed in the lower eyelids. Meibum appearance was scored as 0 = clear excreta or clear with small particles, 1 = turbid excreta with normal viscosity, 2 = turbid excreta with increased viscosity, 3 = secretions retain shape after expression, and 4 = no expressible glands.

Tear Volume, Flow, and Turnover

Fluorophotometry was performed using Fluorotron Master (Ocumetrics, Mountain View, CA). The left eye had a baseline fluorescein scan performed, before the instillation of 0.5 μL of 0.5% sodium fluorescein onto the ocular surface. Eight sequential measurements were taken to determine the decay of tear fluorescence after the first minute and every 3 minutes thereafter until completion at 19 minutes. The data were used to calculate tear volume, flow, and turnover.

Aqueous Tear Evaporation

Aqueous tear evaporation was measured with an evaporometer (Oxdata, Portland, OR) as previously described.

Meibum Lipid Analysis

Meibum was expressed from upper and lower eyelids of each of the volunteers by squeezing using a plastic conformer, that is placed posterior to the eyelid, and a cotton swab. The expressed meibum was collected with a polished platinum spatula. Care has been taken to avoid contamination of the meibum with aqueous tears. Medium was weighed and stored as previously described. Meibum was analyzed using atmospheric pressure ionization mass spectrometry in combination with high performance liquid chromatography (HPLC) as described earlier. The experiments were repeated at least 3 times for each of the samples. To justify structural assignments of the detected compounds, the meibum lipids were compared with authentic lipid standards as described earlier. To reduce uncontrollable variations because of equipment calibrations and tuning, samples for each patient, obtained both at baseline and at 90 days, were measured on the same day.

Statistical Analysis

Although this is a pilot study not aiming to achieve statistical significance, statistical analyses were carried out using SigmaStat 2.03 Software (Systat Software, Inc, Richmond, CA). The statistical analysis within the groups was carried out using Student’s t test. P ≤ 0.05 was considered to be statistically significant. Grubb’s test was used to find possible outliers in the evaporometry and fluorophotometry measurements.

RESULTS

A total of 36 dry eye patients with and without meibomian gland dysfunction completed the study, of which 21 were randomized to the treatment group and 15 were placed in the placebo group. The majority (20) were women and the
remaining (16) were men. They were between the ages of 29 and 84 years with a mean of 61 years. At baseline, the groups did not differ significantly in any parameter. No particular change was reported in the patients’ diet.

Subjective Symptoms

At baseline, all patients, in addition to a history of dry eye, had symptoms of dry eyes; however 47% of the treated group did not meet OSDI dry eye scores, 26% reported mild dry eye symptoms, and the remaining 27% reported moderate dry eye symptoms. At the end of the study, 70% of the patients with symptoms became asymptomatic and 30% moved from moderate into the mild group. In the placebo group, at baseline, 27% did not meet OSDI dry eye score, 26% reported mild symptoms, and 47% reported moderate symptoms. At the end of the study, 37% of the symptomatic patients became asymptomatic and 63% moved from the moderate group into the mild one.

Objective Parameters

In this pilot study, we looked for trends. No trends between groups were seen for eyelid margin edema and hyperemia, conjunctival injection and edema, BUT, 1% lissamine green, meibomian gland expressibility, meibum appearance, or tear evaporation rate. Although the improvements did not achieve statistical significance in this pilot study, both Schirmer testing and fluorophotometry suggested that the omega-3 supplement increased tear secretion. Increase in Schirmer test for active treatment averaged from 8.13 ± 5.07 to 12.33 ± 9.86 mm, whereas increase in Schirmer test for placebo averaged from 8.00 ± 6.50 to 10.52 ± 9.35 mm (Fig. 1). Average tear flow increased for active treatment from 0.13 ± 0.10 μL/min at baseline to 0.20 ± 0.22 μL/min after 90 days, whereas average tear flow decreased for placebo from 0.16 ± 0.13 to 0.13 ± 0.10 μL/min (Fig. 2). Average tear volume increased for the active group from 0.86 ± 0.55 μL at baseline to 0.98 ± 0.48 μL after 90 days, whereas it decreased for the placebo group from 1.44 ± 1.13 to 0.62 ± 0.22 μL (Fig. 3).

A typical chromatogram of a meibum sample from the control group and its corresponding mass spectrum (MS) are presented in Figure 4. The vast majority of lipids are eluted as 1 major HPLC peak, indicating that they were of very hydrophobic nature. In previous work, they were identified as a mixture of wax esters (WE), cholesterol esters, and triacylglycerols. More hydrophilic (polar) compounds (mono- and diacyl glycerols, ceramides, fatty acid amides, and the like) would be detected as HPLC peaks with much longer retention times than the hydrophobic peak with a retention time of 3.2 minutes. However, those more polar compounds were not observed in meibum above the noise level in this study.

HPLC-MS experiments revealed that meibum had an extremely complex lipid composition; hundreds of well-defined mass spectrometric signals were observed in a typical sample. Because some of the signals might have belonged to multiple isobaric compounds, the real number of meibum

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**FIGURE 1.** Schirmer strip wetting at baseline and after 90 days of treatment (active and placebo groups). There was a higher increased trend for schirmer strip wetting in the active group.

**FIGURE 2.** Tear flow measured by fluorophotometry at baseline and after 90 days of treatment (active and placebo). After treatment, an increased trend was only observed in the active group.

**FIGURE 3.** Tear volume measured by fluorophotometry at baseline and after 90 days of treatment (active and placebo). After treatment, an increased trend was only observed in the active group.
components might have been even higher. The staggering complexity of meibum lipids forced us to concentrate on the major lipid species, each of which would produce a mass spectrometric signal with the intensity of, at least, 5% or more of the intensity of the major signal in an MS of a sample. Routinely, 3 MS signals were found to be dominating the lipid pool of any given meibum sample—m/z 369, 619, and 647 (Fig. 4). Previously, these 3 signals were identified as those of cholestadiene (a product of spontaneous in situ dehydration of free cholesterol and a product ion of all cholesterol esters) and 2 C\textsubscript{18:1} oleic acid–based WE with C\textsubscript{24:0} and C\textsubscript{26:0} alcohol moieties, respectively. There were approximately 40 compounds found that overcame the 5% threshold (Figs. 4 and 5; Table 1). Some of these compounds have been already positively identified in our previous publications, whereas the others remain unknown. Thus, the only feasible approach to meibum comparison between active and placebo treatment groups, at the moment, remains lipid mapping or “fingerprinting” of the study samples.

The lipid composition of the samples collected from the omega-3 group was found to be very similar to that from the placebo group. The mass spectra indicated no changes in the apparent ratio of the major detected compounds. Phospholipids were not detected above the noise level. No change was observed in oleic acid and oleic acid–based WE, between groups. LA and ω-linolenic acid (ALA) were not visible as individual peaks in the active or placebo groups, either at baseline or at 90 days.

**DISCUSSION**

Although this pilot study was underpowered to achieve statistical significance, with an enrollment of 36 patients, 21 in the active group and 15 in the placebo group, the average tear production and tear volume was increased in the omega-3 group as indicated by both Schirmer testing and fluorophotometry. Papas and Singh\textsuperscript{26} found that the omega-3 supplement...
improved dry eye symptoms and significantly increased both stimulated and unstimulated salivary gland secretion. In a separate randomized clinical trial from Brazil, oral flaxseed oil improved ocular surface inflammation markers quantified by conjunctival impression cytology, OSDI symptoms questionnaire, Schirmer I test, and tear BUT in patients with rheumatoid arthritis or systemic lupus erythematosus associated with keratoconjunctivitis sicca and Sjögren syndrome.²⁷ Our findings are directionally consistent with these previously published studies. The present study, however, found that dietary supplementation with omega-3 fatty acids in dry eye showed no significant effect in meibum lipid composition or aqueous tear evaporation rate.

There are a number of possible explanations for the lack of positive results. Among these possibilities are the choice of active ingredients in the omega-3 dose, the dosing schedule, and the length of the trial in a population with chronic dry eye syndrome. Theoretically, omega-3 long chain metabolites, EPA, and DHA are synthesized through a series of desaturation and elongation reactions by the same set of Δ⁵ and Δ⁶ desaturases and elongases. Thus, there is a competition between LA and ALA for the enzymes that metabolize them.²⁸²⁹ Although ALA is the preferred substrate of the Δ⁶ desaturase enzyme, the excess of dietary intake of LA compared with ALA results in greater net formation of arachidonic acid (AA) and its proinflammatory derivates than EPA derivates with antiinflammatory properties. However, diets high in long chain n-3 fatty acid diet seem to influence the conversion of EPA derivates in higher proportions than AA derivates.³⁰⁻³² The Δ⁵ desaturases have a limited activity to convert DGLA to AA. Therefore, the synthesis of the antiinflammatory eicosanoids, such as prostaglandin of the 1-series (Fig. 6) derived from DGLA can overcome the effects of the proinflammatory AA-derived eicosanoids.³³ Furthermore, several cells types such as neutrophils, macrophage, and epidermal cells have shown the capability to metabolize DGLA into the 15-lipoxygenase product, 15-(S)-hydroxy-8,11,13-eicosatrienoic acid (15-HETrE), which inhibits the synthesis of AA-derived 5-lipoxygenase metabolites.³³ (Fig. 6).

The DGLA cyclooxygenated product prostaglandin of the 1-series (Fig. 6) has been shown to have antiinflammatory properties at the ocular surface and to stimulate tear production.³⁴³⁵ Previous studies have shown that patients with Sjogren syndrome had a lower dietary intake of omega-3 including EPA and DHA.³⁶ Similarly, the same relation between increased omega-3 intake and reduced clinically diagnosed dry eye in women was observed.³⁷

In a randomized clinical trial, oral flaxseed oil improved ocular surface inflammation markers quantified by conjunctival impression cytology, OSDI symptoms questionnaire, Schirmer I test, and tear BUT in patients with rheumatoid arthritis or systemic lupus erythematosus associated with keratoconjunctivitis sicca and Sjögren syndrome.²⁷ Moreover,ALA topical application in dry eye induced in mice showed a statistically significant difference in corneal fluorescein staining, CD11b+ cell number and expression of cornea IL-1α and corneal and conjunctival TFN-α as compared with topical LA.⁶ In our study, we were unable to demonstrate a significant effect of omega-3 fatty acids on tear production, but the data trends in Schirmer, tear volume, and tear flow seemed to be higher at the final visit in the treated group. It is noteworthy that some factors influence Δ⁵ and Δ⁶ desaturases activity. Interference with the metabolism
EFAs by saturated fats, glucose, insulin deficiency, viruses, alcohol, and aging reduce the formation of GLA, DGLA, EPA, and DHA and their beneficial metabolites.

EFAs are important constituents of all cell membranes. Specifically, n-3 fatty acids are incorporated into phospholipids, sphingolipids, and plasmalogens; thus, they are able to alter membrane fluidity and influence the behavior of membrane-bound enzymes and receptors. Therefore, the membrane fatty acid composition can affect cell and organ functions. Dietary supplementation with omega-3 EFA does affect serum lipid profiles as previously reported. Long chain n-3 fatty acids supplementation leads to an increase in their levels in plasma and tissues. EPA concentrations increase in a linear manner in response to dietary intake. On the other hand, dietary DHA causes a dose-dependent saturable increase in plasma phospholipids DHA concentrations with doses up to approximately 2 g/d.

It has also been reported that membrane and serum levels of DHA are inversely proportional to dry eye severity in Sjogren syndrome. Although meibomian glands are sebaceous gland, there are some differences in the morphology and chemical nature of meibum, compared with sebum chemical composition. In our previous studies, we found only extremely low amounts of phospholipids in meibum. Therefore, the difference in the accumulation and retention of DHA an EPA in meibomian gland secretion versus serum and sebum may be related to the differences in lipid metabolism in the corresponding tissues and organs. The ratio of integration of dietary omega-3 fatty acid seems to be tissue specific. The exogenously present fatty acids could influence the incorporation of dietary omega-3 depending on their bioavailability. This may partially explain our findings, whereas oral intake of omega-3 EFA does not play a role in meibum lipid metabolism.

It is evident that some gaps exist in our present understanding of how dietary supplementation with long chain omega-3 fatty acid can affect dry eye physiopathology. Further studies are needed to determine the beneficial dosage of omega-3 for dry eye treatment. This study suggests that the most promising endpoints for a larger clinical trial would include dry eye symptoms, Schirmer test results, and fluorophotometry.

REFERENCES