

# $\omega$ -3 Tear Film Lipids Correlate With Clinical Measures of Dry Eye

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**PURPOSE.**  $\omega$ -3 and  $\omega$ -6 polyunsaturated fatty acids modulate inflammatory processes throughout the body through distinct classes of lipid mediators that possess both proinflammatory and proresolving properties. The purpose of this cross-sectional study was to explore the relationship between lipid profiles in human tears and dry eye (DE) symptoms and signs.

**METHODS.** Forty-one patients with normal eyelid and corneal anatomy were prospectively recruited from a Veterans Administration Hospital over 18 months. Symptoms and signs of DE were assessed, and tear samples were analyzed by mass spectrometry-based lipidomics. Statistical analyses comparing the relationship between tear film lipids and DE included Pearson/Spearman correlations and *t*-tests.

**RESULTS.** Arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) were present in more than 90% of tear film samples. The ratio of  $\omega$ -6 (AA) to  $\omega$ -3 (DHA+EPA) fatty acids was correlated with multiple measures of tear film dysfunction (tear breakup time, Schirmer 2 scores, and corneal staining; all *P* < 0.05). Arachidonic acid-derived prostaglandin E<sub>2</sub> was detected in the majority of samples and correlated with low tear osmolarity, meibomian gland plugging, and corneal staining.

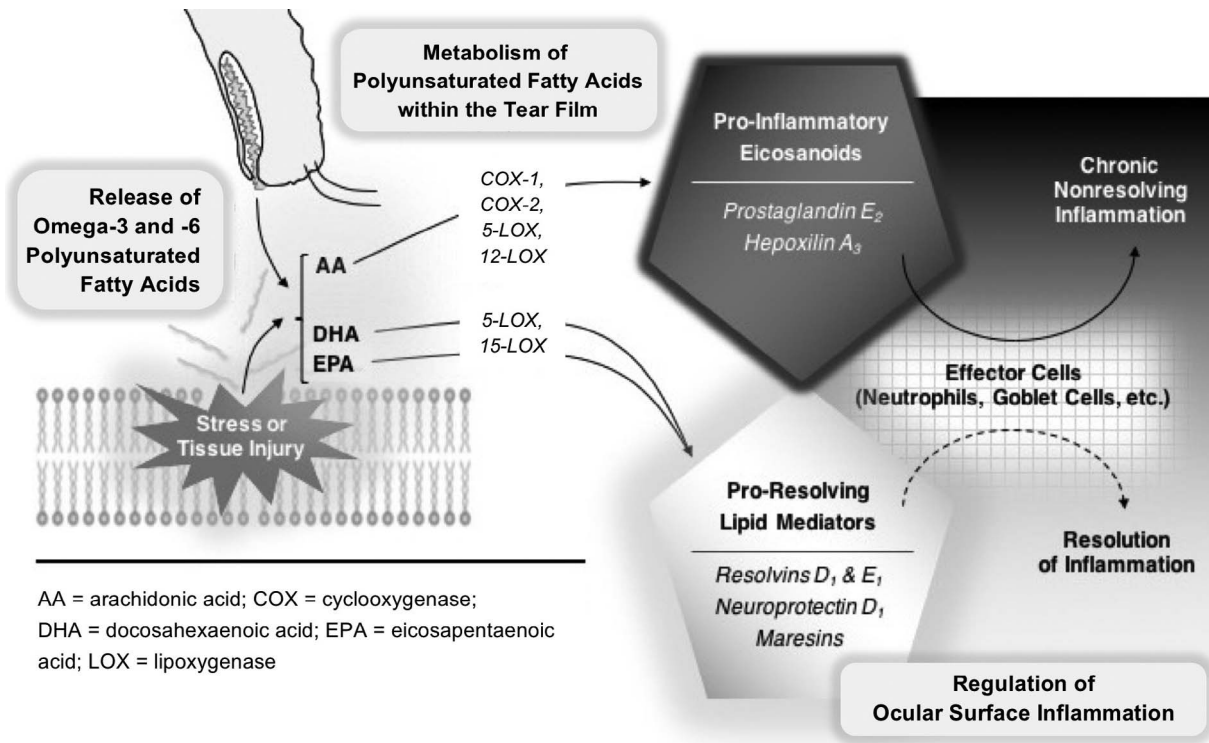
**CONCLUSIONS.** Both  $\omega$ -3 and  $\omega$ -6 lipid circuits are activated in the human tear film. The ratio of  $\omega$ -6: $\omega$ -3 tear lipids is elevated in DE patients in proportion to the degree of tear film dysfunction and corneal staining. Metabolic deficiency of  $\omega$ -3 tear film lipids may be a driver of chronic ocular surface inflammation in DE.

Keywords: dry eyes, tears, fatty acids, prostaglandins, nutrition

Dry eye (DE) is a multifactorial disorder characterized by symptoms of ocular pain and visual disturbance and a myriad of signs including decreased tear production, increased evaporation, hyperosmolarity, and damage to the ocular surface.<sup>1</sup> Dry eye symptoms range in quality, severity, and chronicity and affect tens of millions of Americans.<sup>2,3</sup> Inflammation is a well-described component of DE,<sup>4,5</sup> and various proinflammatory mediators, including innate and adaptive immune cells (infiltrating macrophages and monocytes,  $\gamma\delta$ , and regulatory T cells, intraepithelial lymphocytes, and natural killer cells<sup>5</sup>), cytokines (IL-1 $\beta$  and -6, IFN- $\gamma$ , TNF- $\alpha$ <sup>6</sup>), chemokines (CCL3-5, CXCL9-11, CXCR3<sup>7</sup>), and prostaglandins (PGE<sub>2</sub> and PGD<sub>2</sub><sup>8</sup>) have been found at elevated levels in the tears of DE patients compared with controls. Prostaglandins belong to a diverse class of inflammatory lipid mediators called eicosanoids, which are derived from the oxygenation of arachidonic acid (AA), an  $\omega$ -6 polyunsaturated fatty acid (PUFA), that is enzymatically released from cell membranes of activated cells in

response to environmental stress. The release of AA and subsequent generation of eicosanoid lipid mediators is responsible for triggering the acute inflammatory response to corneal injury.<sup>9</sup>

A less well-described aspect of the inflammatory process is its active resolution, mediated by proresolving lipids, such as resolvins, protectins, and maresins. In experimental models of acute, self-resolving inflammation, the early metabolism of AA into proinflammatory eicosanoids is superseded by a resolving phase, in which proresolving lipid mediators predominate.<sup>10</sup> Resolution of inflammation is thus an active process, and chronic nonresolving inflammation may result from underactivation of the resolving phase mediators.<sup>11</sup> The interplay between proinflammatory and proresolving lipid mediators is an emerging framework for understanding the pathogenesis of chronic inflammatory diseases,<sup>12,13</sup> including DE.<sup>14-20</sup> Most proresolving lipid mediators are derived from  $\omega$ -3 PUFA precursors, such as



**FIGURE 1.** Hypothesized model of chronic ocular surface inflammation in dry eye.  $\omega$ -3 and  $\omega$ -6 polyunsaturated fatty acids are released into the tear film as part of meibum and/or from the ocular surface epithelium in response to injury from desiccating stress. Cyclooxygenases and lipoxygenases are expressed on the ocular surface by corneal epithelial cells and resident polymorphonuclear leukocytes. The  $\omega$ -6 species, AA, is a substrate for cyclooxygenases and lipoxygenases and is converted to several classes of proinflammatory eicosanoids. In contrast, the  $\omega$ -3 species, DHA and EPA, are also substrates for 15-lipoxygenase and 5-lipoxygenase, but are converted to several classes of proresolving, anti-inflammatory, and neuroprotective mediators. The formation of proinflammatory and proresolving lipid mediators regulates activation of effector cells on the ocular surface. In healthy eyes, proresolving lipid mediators counteract the eicosanoids and promote speedy resolution of inflammation. In dry eye, metabolic deficiency of  $\omega$ -3 species leads to underproduction of proresolving lipid mediators and a state of chronic nonresolving inflammation on the ocular surface.

docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These proresolving lipid mediators have essential roles in controlling epithelial wound healing, inflammatory cell migration, and nerve regeneration.<sup>21-23</sup> Thus, the bioavailability of  $\omega$ -3 and  $\omega$ -6 PUFAs may influence the initiation, duration, and resolution of the inflammatory response to injury on the ocular surface (Fig. 1).

We hypothesize that DE is a metabolic disorder characterized by an imbalance of  $\omega$ -3 and  $\omega$ -6 PUFAs, leading to underproduction of proresolving lipid mediators, which promotes nonresolving inflammation on the ocular surface. Our hypothesis is informed by several lines of epidemiologic, clinical, and experimental evidence. Epidemiologic studies suggest that individuals with higher dietary intake of DHA and EPA are protected against DE.<sup>24</sup> Several randomized clinical trials have also shown that dietary  $\omega$ -3 supplementation (DHA+EPA) has favorable effects on DE signs and symptoms.<sup>25,26</sup> The ocular surface highly expresses lipoxygenase (LOX) and cyclooxygenase (COX) enzymes that metabolize PUFAs into lipid mediators, which regulate inflammatory, immune, and wound-healing responses.<sup>17,23,27-31</sup> Experimental evidence from cell culture and animal models of DE suggests that DHA-derived neuroprotectin D<sub>1</sub> (NPD<sub>1</sub>), resolvins D<sub>1</sub> (RvD<sub>1</sub>), and EPA-derived resolvins E<sub>1</sub> (RvE<sub>1</sub>) are proresolving lipid mediators that are particularly relevant in maintaining ocular surface health and tear film function.<sup>14,15,17,18,32</sup> Here, for the first time, we evaluate whether  $\omega$ -3 and  $\omega$ -6 lipid profiles can be detected in human tears and whether these measures correlate with DE disease severity in human subjects.

## METHODS

### Study Population

The Miami Veterans Administration eye clinic serves veterans in South Florida and evaluates patients with a variety of ophthalmic conditions including refractive issues, cataracts, glaucoma, and retinal pathologies in addition to performing screening for eye pathology in patients with systemic conditions (diabetes, hypertension). Patients were prospectively recruited from the eye clinic between November 2013 and April 2015. Informed consent was obtained from each patient, and protected health information was accessed by the study team in a Health Insurance Portability and Accountability Act (HIPAA)-compliant manner. Patients underwent a complete ocular surface examination, and those without notable abnormalities of their eyelids or ocular surface were included. As we wished to study “idiopathic” DE, that is DE symptoms not associated with well-established ocular or systemic conditions, patients were excluded from the study if they had concomitant ocular or systemic processes that could confound their clinical presentation, such as anatomic abnormalities of their eyelids (e.g., ectropion), conjunctiva (e.g., pterygium), and/or cornea (e.g., edema); history of glaucoma, refractive, or retinal surgery; an active external ocular process; cataract surgery within the last 6 months; use of contact lenses or ocular medications with the exception of artificial tears; HIV; sarcoidosis; graft-versus host disease; multiple sclerosis; stroke; or collagen vascular disease. Patients were asked not to use any artificial tears within 2 hours of

testing. This study was conducted with adherence to the tenets of the Declaration of Helsinki and with approval from the Miami Veterans Administration Institutional Review Board.

### Data Collection

Demographic information was collected for each patient, including age, sex, race, ethnicity, smoking status, medications, and medical history.

### Dry Eye Symptoms and Ocular Pain

Dry eye symptoms were assessed via the ocular surface disease index (OSDI), which assesses the impact of DE on visual function, and the Dry Eye Questionnaire Score 5 (DEQ5), which assesses specific discomforts (tearing, dryness, etc.) independent of visual function.<sup>33,34</sup> A numerical rating scale (NRS; score 0–10) was used to assess the “average intensity of eye pain during the past week.”

### Dry Eye Signs

Further ocular surface examination for DE signs included, in the order performed, measurement of tear osmolarity, corneal sensitivity, tear breakup time (TBUT), corneal staining, Schirmer score with anesthesia, and eyelid assessment. Tear osmolarity was measured once in each eye (TearLAB, San Diego, CA, USA). Fluorescein dye was instilled, and TBUT was measured three times in each eye and averaged. Corneal staining was assessed in five areas of the cornea and scored 0–3 in each area (National Eye Institute [NEI] scale). A Schirmer 2 score was recorded as millimeters of wetting at 5 minutes. Eyelid vascularity and meibomian gland plugging were graded on a scale of 0–3 (0 = none; 1 = mild; 2 = moderate; 3 = severe).<sup>35</sup> Meibomian gland drop out was measured via noncontact meibography (a technique that uses transillumination to evaluate degree of area loss of glands according to the Meiboscale). Finally, meibum quality was graded on a scale of 0–4 (0 = clear consistency; 1 = cloudy consistency; 2 = granular consistency; 3 = toothpaste; 4 = no meibum expressed<sup>16</sup> using digital pressure).

### Determination of Corneal Sensitivity

Mechanical detection and pain thresholds of the central cornea were assessed with a modified Belmonte noncontact aesthesiometer, which was developed based on the original Belmonte instrument.<sup>36</sup> The tip of the aesthesiometer (0.5 mm in diameter) was placed perpendicular to and 4 mm from the surface of the cornea of the right eye. Stimulation consisted of pulses of air at room temperature (approximately 23°C–26°C)<sup>37</sup> applied to the corneal surface. The method of limits, using ascending series only, was used to measure threshold.

For corneal detection threshold measurements, subjects were presented with a stimulus immediately following a blink and asked to indicate whether they felt the stimulus by pressing a button. The initial flow rate was set at a level below threshold (50 mL/min for most individuals) and increased by 10 mL/min (with 15-second intervals between stimuli) until the subject stated that they felt the stimulus or the maximum allowable flow rate (400 mL/min) was reached. Two ascending series were conducted, and detection threshold was defined as the arithmetic mean of the value at which the subject pressed the button across the two series. To estimate ocular pain threshold, the flow rate was further increased beyond the detection threshold in 10 mL/min increments until the subject reported the stimulus as painful or the maximum allowable flow rate (400 mL/min) was reached. Two ascending series were conducted in this way, and pain threshold was defined as

a mean of the two series. All threshold measures were performed during the morning hours by the same operator (ALM). Corneal sensitivity was evaluated after tear osmolarity followed by a 2-hour break, after which time the remaining dry eye testing was performed.

### Tear Collection

Fifty microliters of sterile saline was instilled by a pipette to the inferior cul-de-sac of each eye prior to assessment of meibum quality. Tears were immediately collected by capillary action using a 1- $\mu$ L microcaps pipette (Drummond Scientific Company, Broomall, PA, USA) applied gently to the temporal margin of the lower eyelid. Each study eye was sampled once. A minimum of 50  $\mu$ L tears (approximately six disposable micropipettes) was collected and released by bulb dispenser into 1.5-mL Nalgene polypropylene cryogenic vials (Sigma-Aldrich Corp., St Louis, MO, USA). These vials were labeled with de-identified subject codes and immediately placed in a  $-80^{\circ}$ C freezer.

### Laboratory Methodology

Inflammatory and proresolving lipid mediators and specific  $\omega$ -6 PUFA and  $\omega$ -3 PUFA pathway markers were identified and quantified by liquid chromatography (LC)-tandem mass spectrometry (MS/MS). In brief, 400 pg class-specific deuterated (-d) internal standards (AA-d8, DHA-d5, PGE<sub>2</sub>-d4, lipoxin A<sub>4</sub>-d5, leukotriene B<sub>4</sub>-d4, 15-hydroxyicosatetraenoic acid-d8) were added to each tear sample prior to processing to calculate the recovery of specific classes of PUFA, LOX, and COX metabolites. Collected tears (30–50  $\mu$ L) containing internal standards were combined with 2 mL methanol, dried under a gentle stream of nitrogen, immediately resuspended in high-performance LC mobile, and placed in a refrigerated autosampler for lipidomic analysis. Eicosanoids and docosanoids were identified and quantified by LC-MS/MS-based lipidomics based on published methods.<sup>30,38</sup> Processed tear samples were analyzed by a triple-quadrupole linear ion trap LC-MS/MS system (MDS SCIEX 3200 QTRAP; Applied Biosystems, Foster City, CA, USA) equipped with a Kinetex C18 mini-bore column (Phenomenex, Torrance, CA, USA). The mobile phase was a gradient of water/acetonitrile/acetic acid (72:28:0.01, vol:vol:vol) and isopropanol/acetonitrile (60:40, vol:vol) with a 450- $\mu$ L/min flow rate. Tandem MS/MS analyses were performed in negative ion mode, and prominent fatty acid metabolites were quantified in multiple reaction monitoring mode using established and specific transitions as previously described.<sup>30,31,38–41</sup> Calibration curves (1–1000 pg) and specific LC retention times for each compound were established with synthetic standards (Cayman Chemical, Ann Arbor, MI, USA). Structures were confirmed for selected autacoids by MS/MS analyses using enhanced product ion mode with appropriate selection of the parent ion in quadrupole 1.

Tear samples were analyzed by LC-MS/MS in two batches. In the first batch ( $N = 21$  patients), tear samples from both eyes were analyzed separately, and the results were averaged. In the second batch ( $N = 20$  distinct patients), tear samples from both eyes were pooled and analyzed together.  $\omega$ -6: $\omega$ -3 ratios and PGE<sub>2</sub> levels were statistically comparable between the two batches.

### Statistical Analysis

All statistical analyses were performed using SPSS Version 22 (SPSS, Inc., Chicago, IL, USA) statistical package. Analyses included comparison of means ( $t$ -test), medians (Mann-



**TABLE 1.** Demographic and Clinical Information of Study Population

Characteristic	Number	Percent
Sex, male	38	93%
Race, white*	18	44%
Ethnicity, Hispanic	8	20%
Comorbidities		
Hypertension	29	71%
Hypercholesterolemia	26	63%
Diabetes	10	24%
Posttraumatic stress disorder	13	32%
Depression	22	54%
Osteoarthritis	15	37%
Sleep apnea	7	17%
Benign prostatic hyperplasia	5	12%
Medications		
Antidepressants	15	37%
Antianxiolytics	16	39%
Analgesics	26	63%
Antihistamine	6	15%
Oral NSAIDs†	23	56%
$\omega$ -3 supplement‡	8	20%
Artificial tears	30	73%
Characteristic	Mean	SD
Dry eye symptoms and ocular pain		
DEQ5	13	5.1
OSDI	36	23
NRS for ocular pain	4.1	2.7
Dry eye signs		
Osmolarity,§ mOsm/L	304	19
TBUT,§ s	8.9	3.9
Corneal staining§	2.5	3.1
Schirmer score,§ mm	13	7.3
Eyelid vascularity§	0.5	0.7
Meibomian gland plugging§	2.0	0.7
Meibomian gland atrophy§	1.6	1.2
Meibum quality§	1.9	1.3
Detection thresholds, mL/min	108	60
Pain thresholds, mL/min	212	91

\* Nonwhite study participants included 22 who self-identified as black and 1 as other.

† Ibuprofen 600–800 mg, aspirin 81 mg, naproxen 250 mg; variable dosing strategies.

‡ Fish oil 1000 mg (DHA 200 mg/EPA 300 mg) dosed between one and four times daily.

§ Value in more severely affected eye.

Whitney *U*), and correlations (Pearson and Spearman); *P* < 0.05 was considered statistically significant.

## RESULTS

### Study Population

The study group (*N* = 41) comprised a racially and ethnically diverse population of late middle-aged and elderly, predominantly male, subjects (mean age, 62 years; SD, 11; range, 27–83 years), as described in Table 1. Seventy-one percent of subjects were actively using artificial tears, on average  $3.0 \pm 1.4$  times daily, for an average duration of  $36 \pm 38$  months. Fifty-six percent of patients were taking nonsteroidal anti-inflammatory drugs (NSAIDs), and 20% were taking an  $\omega$ -3 supplement. Subjects displayed a wide range of symptoms and signs of DE,

ranging from none to severe (93% with DEQ5  $\geq 6$ , 81% with OSDI  $\geq 20$ ; 56% with NRS  $\geq 4$ ). The majority of subjects had one or more eyelid abnormalities including meibomian gland plugging (78%), a score of 2 or greater on meibum quality (57%), meibomian gland atrophy (44%), and increased eyelid vascularity (10%). A majority of subjects also had evidence of tear dysfunction with TBUT  $\leq 8$  seconds in 56%, Schirmer < 8 mm in 29%, and osmolarity  $\geq 308$  mOsm in 28%.

### Mass Spectroscopy

Five principal species, AA, DHA, EPA, PGE<sub>2</sub>, and hepoxilin A<sub>3</sub> (HxA<sub>3</sub>), were detected in the majority of samples. Arachidonic acid, DHA, EPA, and PGE<sub>2</sub> were detected in >90% of samples and HxA<sub>3</sub> in 81%.

### Correlations Between Lipid Species

The five principal lipid species were positively correlated with one another (Supplementary Table S1). Associations were strongest between DHA and AA and less strong for the other pairwise comparisons.

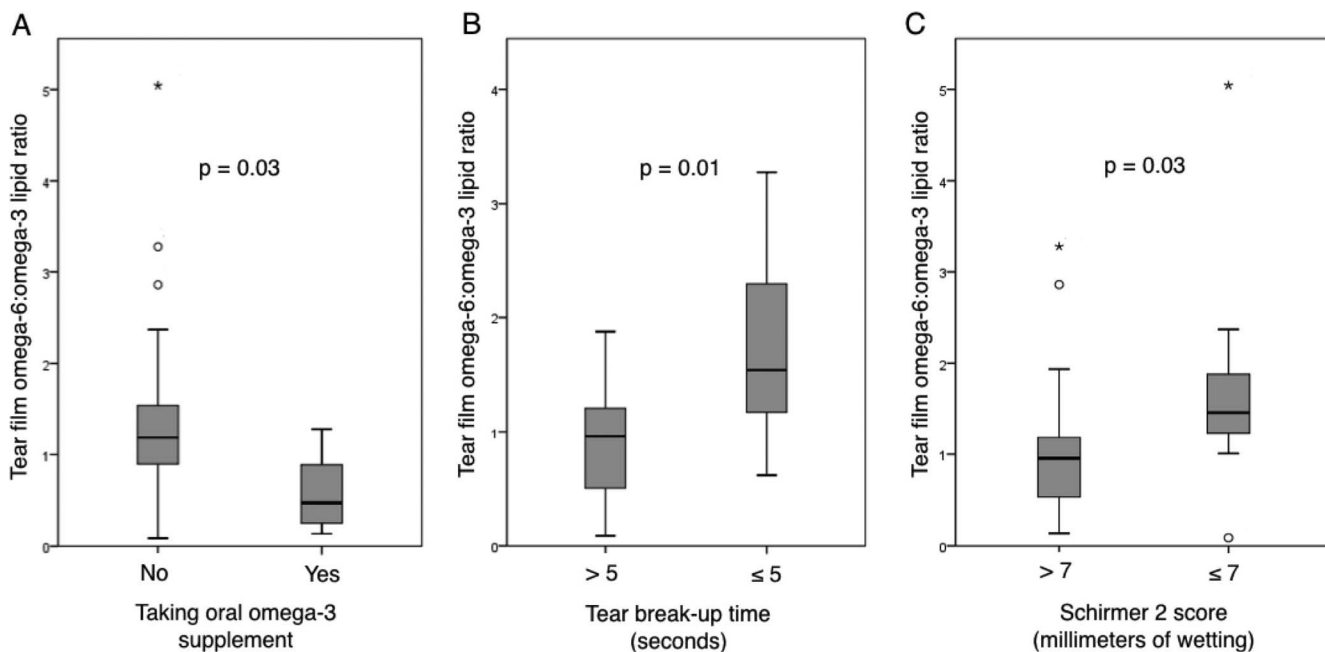
### Clinico-Pathologic Correlation

The  $\omega$ -6: $\omega$ -3 ratio (proinflammatory/anti-inflammatory) and PGE<sub>2</sub> levels were evaluated for correlations with demographic characteristics and symptoms and signs of DE. There was no significant correlation of either measure with age or sex in our study population. White patients demonstrated a lower  $\omega$ -6: $\omega$ -3 ratio compared with blacks ( $0.82 \pm 0.61$  vs.  $1.6 \pm 1.1$ ; *P* = 0.01) and lower PGE<sub>2</sub> levels ( $11.3 \pm 2.7$  vs.  $13.2 \pm 3.2$ ; *P* = 0.05), suggesting a less inflammatory tear lipid profile. Patients with osteoarthritis had a higher  $\omega$ -6: $\omega$ -3 ratio compared with those without arthritis ( $1.7 \pm 1.2$  vs.  $0.95 \pm 0.64$ ; *P* = 0.02) but similar PGE<sub>2</sub> levels, suggesting a more proinflammatory tear lipid profile. Differences were also seen between groups with respect to medication use. Those taking NSAIDs had lower levels of all lipid mediators compared with those not taking NSAIDs, with a significant difference in PGE<sub>2</sub> levels between the groups ( $11.3 \pm 2.6$  vs.  $13.7 \pm 3.1$ ; *P* = 0.01; Supplementary Fig. S1). Those taking  $\omega$ -3 supplements had modestly higher tear film levels of all lipid mediators compared with those not taking  $\omega$ -3 and a significantly lower  $\omega$ -6: $\omega$ -3 ratio ( $0.58 \pm 0.43$  vs.  $1.37 \pm 0.96$ ; *P* = 0.03; Fig. 2A), suggesting a less inflammatory lipid profile. Means were not significantly different by the other demographics, comorbidities, and medications listed in Table 1.

Dry eye symptoms and ocular pain were not significantly correlated with  $\omega$ -6: $\omega$ -3 ratio or PGE<sub>2</sub>, nor were metrics of corneal sensitivity (stimulus detection and pain thresholds). However, several clinically important signs of ocular surface disease were correlated with inflammatory tear lipids (Table 2). Higher levels of PGE<sub>2</sub> were correlated with lower tear osmolarity, more meibomian gland plugging, and more corneal staining. Less healthy tear parameters including shorter TBUT, lower Schirmer scores, and more corneal staining correlated with higher  $\omega$ -6: $\omega$ -3 ratios (Figs. 2B, 2C).

## DISCUSSION

This is the first study to demonstrate that the major biologically relevant  $\omega$ -3 PUFAs (DHA and EPA) are detectable in the human tear film, suggesting activation of these proresolving lipid circuits in DE. The anti-inflammatory potential of  $\omega$ -3 series proresolving lipid mediators has been established by multiple animal and cell culture models of DE.<sup>14,16–19,32,42</sup> Changes in



**FIGURE 2.** Box-and-whisker plots comparing tear film ω-6:ω-3 lipid ratios in patients with and without (A) use of ω-3 supplements ( $0.58 \pm 0.43$  vs.  $1.37 \pm 0.96$ ;  $P = 0.03$ ), (B) evaporative deficiency defined by TBUT  $\leq 5$  seconds ( $1.89 \pm 1.24$  vs.  $0.87 \pm 0.47$ ;  $P = 0.01$ ), and (C) aqueous tear deficiency defined by Schirmer 2 score  $\leq 7$  mm ( $1.70 \pm 1.20$  vs.  $1.02 \pm 0.74$ ;  $P = 0.03$ ). Gray boxes represent the interquartile range between the 25th to 75th percentile, middle line represents the median, and vertical line extends from the minimum to the maximum value, excluding outliers (open circles represent values larger than the upper quartile plus 1.5 times the interquartile range; asterisks represent values larger than the upper quartile plus 3 times the interquartile range).

**TABLE 2.** Correlations Between Lipid Species, Demographics, Comorbidities, and Dry Eye Metrics

Clinical Features	ω-6:ω-3 (Pearson/ Spearman)	PGE <sub>2</sub> (Pearson/ Spearman)
<b>Demographics</b>		
Age	-0.22/-0.22	-0.08/-0.14
<b>DE symptoms</b>		
DEQ5	0.14/0.19	-0.06/-0.06
OSDI	0.16/0.17	0.03/0.02
<b>Ocular pain</b>		
Numerical rating scale (average ocular pain intensity in last week)	-0.03/0.05	-0.13/-0.14
<b>Corneal sensitivity</b>		
Detection thresholds (Belmonte)	-0.10/-0.05	-0.05/-0.02
Pain thresholds (Belmonte)	-0.08/-0.008	-0.02/-0.003
<b>Ocular signs</b>		
Osmolarity	0.19/-0.04	-0.32*/-0.40*
TBUT	-0.36*/-0.37*	-0.18/-0.20
Corneal staining	0.18/0.31*	0.25/0.35*
Schirmer score	-0.26/-0.38*	-0.11/-0.10
Eyelid vascularity	-0.28/-0.27	0.14/0.11
Meibomian gland plugging	0.29/0.17	0.38*/0.40*
Meibomian gland atrophy	0.11/0.15	0.20/0.13
Meibum quality	0.21/0.11	0.17/0.13

\*  $P < 0.05$ .

tear volume have been shown to correlate with dietary ω-3 intake in mice,<sup>42</sup> and here we demonstrated a significant correlation between the tear film ω-6:ω-3 ratio and tear volume (Schirmer score), as well as tear stability (TBUT) and corneal staining. Additionally, human studies have shown that ω-3 (DHA+EPA) supplementation has beneficial effects on the signs and symptoms of DE.<sup>25,26</sup> Although our study was noninterventional, we found that patients taking ω-3 supplements had higher tear film PUFA levels and a lower, less inflammatory ω-6:ω-3 ratio. This suggests that oral intake of ω-3 supplements has sufficient bioavailability to directly impact inflammatory lipid expression in the human tear film.

Previous investigations have measured AA<sup>43</sup> and related ω-6 series eicosanoids including 12-HETE<sup>44</sup> and PGE<sub>2</sub><sup>8</sup> in the tear film of human DE subjects. Here we detected AA, PGE<sub>2</sub>, and, for the first time in human tears, the nonclassical eicosanoid HxA<sub>3</sub>, in the majority of subjects. Although previous investigations reported differences in DE tears compared with healthy controls, we have gone further to show that inflammatory lipid profiles correlate with phenotypic variations in ocular surface disease severity among patients.

The role of eicosanoids in the regulation of corneal inflammation was first recognized by Srinivasan and Kulkarni, who showed in various models of corneal injury that polymorphonuclear leukocytes (PMNs) were recruited from conjunctival vessels through the tear film, ultimately attaching to injured corneal epithelium in a AA- and PGE<sub>2</sub>-dependent manner.<sup>9</sup> Prostaglandin E<sub>2</sub> is synthesized from AA by COX and PGE synthase, and both these enzymes are up-regulated on the ocular surface of mice placed in a DE environmental chamber.<sup>8</sup> Chronic corneal injury results in recruitment 5-LOX expressing PMNs and expression of COX in corneal epithelial cells, inducing formation and release of proinflammatory and proangiogenic mediators (PGE<sub>2</sub> and leukotriene B<sub>4</sub>) that drive and amplify ocular surface inflammation.<sup>31</sup>

The role of  $\omega$ -3 fatty acids in down-regulating inflammation is an emerging paradigm for understanding the pathogenesis of chronic inflammatory diseases,<sup>12,13</sup> including DE.<sup>14–20</sup> DHA and EPA are conditionally essential fatty acids, which can be produced from  $\alpha$ -linolenic acid, but the rate of conversion is generally insufficient to replace metabolic consumption of DHA and EPA.<sup>45</sup> In a survey of dietary fat intake across 28 countries, 20 failed to meet minimum World Health Organization recommended levels of DHA and EPA intake, including the United States and the majority of European nations.<sup>46</sup> DHA and EPA are metabolic precursors to proresolving lipid mediators such as NPD<sub>1</sub>, RvD<sub>1</sub>, and RvE<sub>1</sub>, which counteract the proinflammatory actions of PGE<sub>2</sub>, HxA<sub>3</sub>, and other eicosanoids.<sup>10,12,13,18,47</sup> Corneal epithelial cells and resident regulatory PMNs in the corneal limbus and lacrimal gland highly express 15-LOX, a key enzyme for generating and releasing specialized proresolving mediators (lipoxins, resolvins, and neuroprotectins) that are critical for controlling ocular surface immune and wound healing responses).<sup>17,23,27–30</sup> Lipoxin A<sub>4</sub> promotes corneal epithelial wound healing,<sup>30</sup> inhibits pathologic angiogenesis and proinflammatory cytokine expression,<sup>28,29</sup> and controls effector T-cell activation.<sup>27</sup> Neuroprotectin D<sub>1</sub> is implicated in epithelial cell survival,<sup>48</sup> recovery from oxidative stress,<sup>48</sup> and wound healing.<sup>14</sup> Neuroprotectin D<sub>1</sub> has also been shown to promote corneal nerve regeneration and return of corneal sensitivity.<sup>14,17</sup> Resolvin E<sub>1</sub> exerts proresolving effects directly through G-protein-coupled receptors<sup>10</sup> and indirectly through negative feedback on COX-2 expression.<sup>20</sup> In murine models of DE, RvD<sub>1</sub> and RvE<sub>1</sub> play a role in tear film homeostasis by enhancing tear production, as well as goblet cell survival and secretion in response to desiccating stress.<sup>16,18,20</sup> These data underscore the biologic significance of  $\omega$ -3 proresolving lipid mediators in the tear film and lend support to the hypothesis that DE has a metabolic basis. Nutritional or metabolic deficiency of DHA and EPA may lead to underproduction of proresolving lipid mediators in tears, ultimately resulting in nonresolving inflammation on the ocular surface.

As with all studies, our findings need to be considered with our study limitations, which include: a cross-sectional study design, a predominantly male DE population, a small sample size, and specific metrics used to capture DE features. Because we studied tears, we cannot discern whether the lipid mediators originated from meibum, the ocular surface epithelium, or both. In addition, more work needs to be done to optimize tear collection and lipid identification in human tears, as many of the naturally occurring  $\omega$ -3 metabolites are unstable and short-lived species.<sup>49</sup> One strength of our study was the ability to calculate a standardized  $\omega$ -6: $\omega$ -3 ratio, which helps to mitigate some of the variation in lipid concentrations between individual samples. However, the  $\omega$ -6: $\omega$ -3 ratio may be an overly simplistic metric, as some  $\omega$ -6-derived eicosanoids (e.g., PGE<sub>1</sub>, Lipoxin A<sub>4</sub>) can be anti-inflammatory. Future investigation will be needed to characterize the active metabolites of the proresolving lipid mediators DHA and EPA in the human tear film (e.g., DHA-derived NPD<sub>1</sub> and RvD<sub>1</sub> and EPA-derived RvE<sub>1</sub>).<sup>16–19,32</sup>

To summarize, DE has been previously described as a chronic inflammatory disorder of the ocular surface. In addition to known proinflammatory lipids (e.g., AA, PGE<sub>2</sub>), we have shown for the first time that proresolving lipid biomarkers (DHA, EPA) can be simultaneously detected in the tear film of human subjects with DE. The ratio of proinflammatory to proresolving lipid pathway markers in the tear film was clinically significant as a biomarker for tear film dysfunction. Our findings support the hypothesis that a higher  $\omega$ -6 (proinflammatory) to  $\omega$ -3 (proresolving) ratio of lipid mediators supports nonresolving inflammation on the ocular

surface. This theoretical framework may help to devise new, more targeted therapies toward lipid pathways in DE.

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