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Associations between systemic omega-3 fatty acid levels with moderate to severe dry eye disease signs and symptoms at baseline in the Dry Eye Assessment and Management (DREAM) Study

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Abstract

Purpose: Omega-3 (n-3) fatty acid supplementation is used to treat systemic inflammatory diseases, but the role of n-3 in the pathophysiology and therapy of dry eye disease (DED) is not definitive. We evaluated the relationship of systemic n-3 levels with signs and symptoms at baseline in the Dry Eye Assessment and Management (DREAM) Study.

Methods: Blood samples from participants at baseline were analyzed for n-3 and n-6, measured as relative percentage by weight among all fatty acids in erythrocytes. Symptoms were evaluated using the Ocular Surface Disease Index. Signs including conjunctival staining, corneal staining, tear breakup time (TBUT), and Schirmer's test with anesthesia were also evaluated.

Results: There was no correlation between the systemic n-3 levels and DED symptoms. When the associations with signs of DED were assessed, lower DHA levels were associated with higher conjunctival staining, with mean scores of 3.31, 2.96, and 2.82 for low, medium and high levels of

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DHA, respectively, (linear trend p=0.007). None of the other signs were associated with DHA or the other measures of n-3.

Conclusion: Previous studies have found varying results on the role of n-3 supplementation with the signs and symptoms of DED. Among patients with DED enrolled in the DREAM Study, lower systemic n-3 levels were not associated with worse symptoms and most signs of DED.

Keywords

dry eye disease; omega-3 fatty acids; inflammation

Dry eye disease (DED) is a chronic, inflammatory ocular condition affecting approximately 14% of adults in the United States.[1–3] Typical treatment of DED includes artificial tears or eye drops such as cyclosporine (Restasis) and lifitegrast (Xiidra) that affect inflammatory mediators in the eye.[4,5] However, these therapies do not work for everyone. Therefore, clinicians and their DED patients continue to seek better methods to treat the signs and symptoms of DED, and are often attracted to “natural” treatments such as nutritional supplements.

Omega-3 (n-3) polyunsaturated fatty acid (PUFA) supplementation is often recommended for prevention and treatment of a variety of diseases ranging from coronary heart disease to dry eye disease (DED), in large part because of the anti-inflammatory properties of n-3.[6–13] High levels of two n-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are found in fish and other seafood.

n-3 PUFAs exhibit anti-inflammatory properties, in part, because they compete with arachidonic acid (AA) as a substrate for cyclooxygenases and 5-lipoxygenase. PUFAs derived from n-3 and n-6 compete for enzymes involved in their metabolism. From a metabolic perspective, n-3 fatty acids suppress the interconversion of n-6 essential fatty acids (EFAs) to AA. There is an overproduction of pro-inflammatory prostaglandin E2 (PGE2) and underproduction of anti-inflammatory PGE1 and PGE3 when the systemic levels of n-6/n-3 fatty acid ratio is high.[7] In addition to anti-inflammatory properties, n-3 fatty acids may also provide neuroprotective and neuroregenerative effects.[14,15]

The systemic ratio of n-6/n-3 has been widely discussed as an objective measure of maintaining ideal fatty acid balance to maximize anti-inflammatory effects of n-3. The ideal n-6/n-3 ratio in the diet is about 2/1[16], as is seen in the Mediterranean diet, rich in cold-water fish and natural oils. An unfortunate consequence of industrialization of food production may be a disturbance in the ratio of n-6/n-3 fatty acids, with a higher consumption of n-6 relative to n-3. From a teleologic perspective, humans have evolved with a diet that consisted of a 1/1 ratio of n-6/n-3 fatty acids, to current Western diets, with ratios closer to 15/1.[17] Studies suggest that increasing systemic levels of n-3 fatty acids, like EPA and DHA, by oral supplementation would help in the lowering of the n-6/n-3 ratio and hence have an anti-inflammatory effect.[7,8,17–19] When the n-6/n-3 ratio is 4/1 or lower, there is competitive inhibition of the conversion of di-homo-linolenic acid (DGLA) to AA resulting in more anti-inflammatory PGE1.[18,20] Therefore, it has been recommended that people

ingest more n-3 than n-6 to help keep that ratio low in order to enhance the anti-inflammatory effects.[8,21,22]

Some studies have shown anti-inflammatory effects of n-3 intake as measured by decreased T-cell infiltration, decreased systemic and tear inflammatory cytokines, and inhibition of ocular surface human leukocyte antigen D-related (HLA-DR), a biomarker believed to be associated with DED.[19,23–29]

Previous randomized clinical trials have shown varying results of n-3's effectiveness in decreasing the signs and symptoms of DED and have used a wide variety of doses of both n-3 and n-6.[9,27,30–32] Three of the trials, measured blood levels of fatty acids in study participants at baseline and follow-up.[9,29,32] In a highly selective study population of patients having high tear osmolarity and relatively mild symptoms, Epitropolous et al. found that 3 months of n-3 supplementation (2240 mg daily) increased the n-3 index in plasma, decreased tear osmolarity, improved tear stability, and reduced symptoms over a 3-month period. However, the association between plasma n-3 levels and signs and symptoms was not evaluated.[9]

Macsai et al. found a significant increase of n-3 and decrease of n-6/n-3 ratio in both red blood cells (RBCs) and plasma of the n-3 experimental group after one year of treating subjects with 3300 mg of n-3 daily. Improvement in signs and symptoms were variable across both the experimental and placebo group. However, no direct comparison was made between the systemic levels of n-3 and the signs and symptoms of DED.[32]

The Dry Eye Assessment and Management (DREAM) Study was a large-scale, real-world, multi-center, double-masked, placebo-controlled randomized clinical trial measuring the effects of n-3 supplementation on DED.[33] Recently reported results from the DREAM Study showed that after 1 year of supplementation, there was no difference between n-3 (3000mg of n-3 daily) and placebo (5000mg of refined olive oil daily) on both signs and symptoms of DED.[30] In this report, we evaluated the association between systemic n-3 levels at baseline with the severity of signs and symptoms in DREAM participants, both in patients taking low-dose n-3 supplements and those not taking n-3 supplements.

Methods

Overview

The Dry Eye Assessment and Management (DREAM) Study was multi-center, double-masked, placebo-controlled randomized clinical trial measuring the effects of n-3 supplementation on DED (ClinicalTrials.gov number NCT02128763). To enhance the generalizability of the study findings, the DREAM Study used minimally restrictive inclusion and exclusion criteria to capture a broad spectrum of typical DED patients seeking additional treatment for their symptoms. The study enrolled subjects with moderate to severe DED symptoms, as defined by the Ocular Surface Disease Index (OSDI; Allergan), and who also demonstrated repeatable signs of DED.[33] Eligible patients needed a score on the Ocular Surface Disease Index (OSDI) between 25 and 80, inclusive, at a screening visit and between 21 and 80, inclusive, at an eligibility confirmation visit. Scores on the OSDI range

from 0 to 100, where 0 indicates no ocular discomfort. Patients needed to have at least 1 eye with at least 2 of the following 4 signs: conjunctival lissamine green staining score ≥1 on a scale of 0–6; corneal fluorescein staining score ≥4 on a scale of 0–15; tear film break up time (TBUT) ≤ 7 seconds; and Schirmer test with anesthesia measurement ≥1 to ≤ 7 mm/5min. The same qualifying signs had to be present in the same eye on examination at both the screening and eligibility confirmation visits. Fluorescein corneal staining was scored using the National Eye Institute [NEI]/industry-recommended scoring guidelines.[34] Lissamine green conjunctival staining was scored using a modified version of the NEI/industry-recommended scoring guidelines – the entire temporal and the entire nasal section of each eye were graded on a scale of 0 to 3 (0: no staining, 3: severe staining) for a total possible score of 6 in each eye. Subjects reported usage and dosage of dietary supplements containing n-3. Subjects taking greater than 1200mg n-3 daily were excluded. In addition, blood was collected at baseline to assess systemic fatty acids levels based on lipid analysis of erythrocytes. Blood samples were shipped to the Peroxisomal Diseases Laboratory at the Kennedy Krieger Institute in Baltimore, MD within 24 hours of collection for lipid analysis.[33]

Subjects were enrolled from 27 sites across the United States which included private and academic ophthalmology and optometry practices. The DREAM Study was approved by the respective clinical center institutional review boards or a centralized institutional review board (University of Pennsylvania). The DREAM Study was in compliance with the Health Insurance Portability and Accountability Act, and adhered to the principles of the Declaration of Helsinki.

Blood Analysis

Red blood cell (RBC) total lipid fatty acid profiles were assessed at The Kennedy Krieger Institute (Baltimore, MD). Venous blood (~8 ml) was drawn into EDTA tubes and shipped overnight at room temperature for processing and analysis. RBCs were separated from plasma by centrifugation and the plasma and buffy coat removed. The RBCs were re-suspended in 4°C phosphate buffered saline (PBS, Sigma Aldrich), centrifuged and the PBS and any remaining buffy coat removed. This washing procedure was then repeated. The packed RBCs were stored under nitrogen at −80°C while awaiting analysis. RBC membrane total lipid fatty acids were derivatized to their pentafluoro-benzyl bromide fatty acid esters which were separated and identified by negative ion gas chromatography mass spectrometry using a 50-meter SP2560 column (Supelco) as previously described.[35,36] Each run was required to pass clinical laboratory quality control before the data were released. RBC fatty acids are presented as percent of total lipids.

Statistical Analysis

Associations between measures of n-3 (EPA, DHA, n-3 index (EPA+DHA), and ratio of n-6/n-3) and dry eye symptoms (OSDI) and signs (conjunctival staining, corneal staining, TBUT, and Schirmer's test) were evaluated at baseline. EPA and DHA levels were grouped into categories that placed approximately 50% of patients into the medium category and 25% into the low and high categories. The scores of dry eye symptoms and signs were compared among levels of EPA and DHA and tertiles of both EPA+DHA and ratio of

n-6/n-3 using analysis of variance with tests of linear trend. Linear regression analyses were performed with n-3 measures as continuous independent variables for predicting dry eye symptoms or signs, adjusted for age, gender, and presence of Sjogren syndrome. For the regression analyses of associations between n-3 measures and dry eye signs, the generalized estimating equations approach was used to accommodate the inter-eye correlation in signs from both eyes of a participant. All statistical analyses were performed in SAS v9.4 (SAS Institute Inc., Cary, NC). After applying a Bonferroni adjustment to accommodate the multiple comparisons of n-3 levels across the 4 signs of DED, only p-values less than 0.0125 were considered statistically significant when analyzing signs.

Results

Systemic levels of fatty acids of all DREAM participants at baseline are listed in Table 1. Subjects who were taking some form of n-3 supplementation before entering the study reported a median daily dose of 600 mg with an interquartile range of 330 to 900 mg. These subjects had significantly higher levels of EPA, DHA, and n-3 index ($p<0.0001$), and a significantly lower n-6/n-3 ratio ($p<0.0001$) than subjects not taking any n-3 supplementation before entering the study. There were no significant differences in signs or symptoms of DED between these groups after adjusting for age, gender, and presence of Sjogren Syndrome. (Table 2).

There were no significant associations between n-3 measures and dry eye symptoms assessed using OSDI (all p-values$\geq$0.15, Table 3). The association between n-3 measures and dry eye signs are reported in Table 4. After accounting for multiple comparisons, lower DHA levels were associated with higher conjunctival staining, with mean scores of 3.31, 2.96, and 2.82 for low, medium and high levels of DHA, respectively, (linear trend $p=0.007$).

The analysis of the association of n-3 measures and dry eye symptoms and signs using data excluding participants taking n-3 supplements at baseline showed results similar to those cited above for the OSDI symptom scores and most of the DED signs (Supplemental Digital Content (SDC) Tables 1 and 2). However, the inverse association of EPA level with tear break-up time (lower EPA values associated with longer (better tear break up times) that was of borderline statistical significance in Table 4 ($p=0.014$) was statistically significant when participants taking n-3 supplements were excluded ($p=0.004$). Results were similar when the concentration of the fatty acids, rather than the percent of total lipids, were used in analyses.

Discussion

The DREAM cohort at baseline is representative sample of the United States population in terms of systemic fatty acid concentrations. A study by Harris et al. defined systemic fatty acid norms of the US population by measuring the systemic fatty acid levels in erythrocytes of nearly 160,000 people.[37] The mean baseline n-3 index (EPA + DHA) of the DREAM Study population at baseline approximately corresponds to the 50th percentile found by Harris et al.[37]

Lipid analysis of erythrocytes provides the most accurate and objective measure of the systemic levels of n-3. Erythrocytes are not influenced by what has been recently ingested,

since their lifespan is approximately 4 months, and therefore will reflect a person's overall diet of the past 4 months.[38] This gives an accurate measure of how n-3 supplementation changes n-3 levels throughout the body and may give an indication of how n-3 levels correlate with signs and symptoms of disease. Measurements of fatty acids on erythrocytes are commonly performed in n-3 supplementation studies to provide objective evidence of compliance with treatment, measure the change of systemic levels in the active group of a RCT, and analyze if the change in systemic levels correlate with changes in signs and symptoms.[9,29,33,37,39]

The eligibility criteria of the DREAM Study allowed people who were taking low doses of n-3 supplements (≤ 1200 mg) to still enroll in the study.[33] Erythrocyte analysis showed that the subjects who were taking n-3 supplementation before entering the study (n=134) actually had significantly higher systemic levels of n-3 as compared to those subjects who were not taking any n-3 supplementation before entering the study (n=386) (Table 1). However, despite these significant differences in systemic levels of n-3 between the two groups, there were no statistically significant difference in severity of DED signs and symptoms (Table 2). This finding helps support the conclusion that systemic levels of n-3 are not associated with the severity of DED.

Signs and symptoms of DED were compared to several different metrics for determining systemic levels of n-3 (EPA, DHA, n-3 index (EPA+DHA), and n-6/n-3) since these metrics have been extensively studied and are believed to be behind the mechanism of n-3's anti-inflammatory effects.[7,8,17–22,37] Despite analyzing systemic levels several different ways, no correlation was found between systemic levels and symptoms of DED. In addition, the majority of DED signs did not correlate with systemic levels of n-3.

There was an association of systemic levels of EPA and TBUT that was of borderline statistical significance after adjusting for multiple comparisons and statistically significant among patients not taking n-3 supplements (Table 4 and Supplemental Digital Content (SDC) Tables 1). A lower systemic level of EPA was associated with a longer TBUT. However, the estimated decrease in TBUT with each increase of 1% of EPA was approximately 0.5 seconds, an amount with little clinical significance. Nonetheless, the effect is the opposite direction of what one would expect. It has been demonstrated that DHA and EPA are detectable and are in higher levels in the human tear film resulting from taking omega-3 supplementation, suggesting that oral intake of omega-3 supplements has sufficient bioavailability.[40] It has also been suggested that elevated levels of PUFA from consumption of essential fatty acid containing supplements may lead to more disordered and less tightly-packed tear-lipid films, with reduced water evaporation resistance, and as a result, TBUT becomes shorter.[41,42] Our results support this notion that a higher serum n-3 level was associated with shorter TBUT.

The results from this study bring into question the role of n-3 in DED. All results, whether statistically significant or not, are likely not clinically significant since the mean difference in clinical signs between the high and low systemic levels are approximately 0.5 second (TBUT), less than 1 point (corneal (out of 15) and conjunctival staining (out of 6 maximum)) or less than 1.5 mm (Schirmer's test). These results bring into question the

assertion that n-3 fatty acids are beneficial for the treatment of DED and the mechanism by which they affect DED.

The conclusions of this study are limited due to the enrollment criteria of the DREAM clinical trial.[30] Only patients suffering from DED were evaluated so it cannot be determined if a population of non-DED patients would have had higher systemic levels of n-3. However, enrolled subjects ranged from moderate to severe DED, and the mean systemic n-3 levels of these subjects was approximately equal to the 50th percentile of the population in an observational study by Harris et al.[37] Therefore, it is likely that the n-3 levels found in this population of DED patients would be similar to a population of non-DED patients. Another limitation is due to subjects being permitted to take n-3 supplements (≤1200mg) before entering the study. It cannot be inferred if these supplements were beneficial in treating their signs or symptoms of DED. However, it is still true that they are suffering from moderate to severe DED, and that despite having higher systemic levels of n-3 (Table 1), they still had similar severity of signs and symptoms of those not taking supplements (Table 2). Additionally, this study did not quantify n-3 bioavailability in the tear film.

There is still variable evidence that n-3 supplementation is beneficial with respect to other diseases such as cardiac disease. The REDUCE-IT study found that 2000 mg of ethyl eicosapentaenoic acid daily significantly lowered the risk of ischemic events, including cardiovascular death in patients with elevated triglyceride levels.[43] However, a recent meta-analysis of 10 studies involving 77,917 individuals on n-3 for treating cardiovascular disease concluded that “n-3 fatty acids had no significant association with fatal or nonfatal coronary heart disease or any major vascular events.”[44]

The cross-sectional associations provided in this report demonstrated that there was no association between systemic levels of n-3 and the severity of DED in patients in their normal lives before entering an interventional randomized clinical trial. This supports the conclusion that n-3 may not play a significant role in the cause or therapy of DED. More evidence is needed to determine if and how n-3 may affect the pathophysiology of DED.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Conflicts of Interest and Source of Funding:

Dr. Asbell reports personal fees from Santen, personal fees from Shire, grants and personal fees from Novartis, personal fees from Medscape, grants and personal fees from MC2 Therapeutics, grants, personal fees and non-financial support from Valeant, Bausch& Lomb, personal fees from Allergan, personal fees from ScientiaCME, grants and personal fees from Rtech , personal fees from Oculus , grants and personal fees from Miotech, personal fees and non-financial support from Shire , personal fees and non-financial support from Santen, personal fees and non-financial support from CLAO, personal fees from Vindico, outside the submitted work. Dr. Lin report, grants from Amorphex Therapeutics, grants from CooperVision, Inc, grants from Essilor USA, grants from GLIA LLC, grants from Johnson & Johnson, grants from Leo Lens, Inc, grants from Orinda Pharma, grants from Verily Life Science, grants from Viewpoint Pharmaceuticals, personal fees from Google X, personal fees from Health Advances, personal fees from Novartis, personal fees from Shire, during the conduct of the study. Dr. Hom reports grants from Allergan, Takeda,Tarsus Pharmaceuticals, Bausch Health, Hovine Scientia, Tear Solutions, and Kala Pharamceuticals. Mr. Kuklinski reports a grant from MC2 Therapeutics. Dr. Maguire reports personal fees from Genentech/Roche. For the remaining authors, none were declared.

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Table 1.

Baseline systemic levels of n-3 measures in DREAM participants by use of n-3 supplements at baseline

Baseline Fatty Acid Measure	Participants	Taking n-3 supplements		
	All (N=520)	No (N=386)	Yes (N=134)	P[§]
Eicosapentaenoic acid (EPA), percent of total lipids by weight				
Mean (SD)	0.60 (0.40)	0.48 (0.24)	0.96 (0.54)	<0.0001
Median (1st quartile, 3rd quartile)	0.47 (0.35, 0.70)	0.41 (0.32, 0.56)	0.81 (0.58, 1.18)	
Docosahexaenoic acid (DHA), percent of total lipids by weight				
Mean (SD)	3.89 (1.15)	3.62 (1.03)	4.65 (1.13)	<0.0001
Median (1st quartile, 3rd quartile)	3.75 (3.04, 4.61)	3.48 (2.84, 4.22)	4.62 (3.80, 5.38)	
n-3 index (EPA + DHA), percent of total lipids by weight				
Mean (SD)	4.49 (1.46)	4.10 (1.21)	5.61 (1.55)	<0.0001
Median (1st quartile, 3rd quartile)	4.24 (3.43, 5.29)	3.88 (3.23, 4.72)	5.45 (4.35, 6.57)	
Ratio of n-6:n-3				
Mean (SD)	4.77 (1.38)	5.15 (1.24)	3.69 (1.15)	<0.0001
Median (1st quartile, 3rd quartile)	4.75 (3.81, 5.74)	5.18 (4.29, 5.95)	3.55 (2.89, 4.22)	

[§]From two-sample t-test for comparison of means.

Table 2

Baseline dry eye symptoms and signs by use of n-3 supplements at baseline

Dry eye symptoms and signs at baseline	(Minimum, maximum)*	Taking n-3 supplements		P§
		No (N=386) Mean (SE)	Yes (N=134) Mean (SE)	
OSDI score	(21, 81)	42.4 (1.33)	43.0 (1.68)	0.71
Conjunctival staining score	(0, 6)	2.94 (0.07)	2.91 (0.12)	0.84
Corneal staining score	(0, 15)	3.81 (0.14)	3.88 (0.23)	0.81
TBUT (seconds)	(0, 19)	3.12 (0.08)	3.08 (0.14)	0.78
Schirmer test (mm)	(0, 36)	9.67 (0.33)	9.30 (0.55)	0.55

*Minimum and maximum scores among 520 DREAM patients

§From analysis of variance for comparison of dry eye symptoms (OSDI) measured at the person level, and from using the generalized estimating equations approach for comparison of dry eye signs measured at the eye level, adjusted by age, gender and presence of Sjogren syndrome.

Table 3.

Adjusted mean values of the Ocular Surface Disease Index (OSDI) by n-3 measures at baseline

Baseline n-3 measure	Participants (N=520)	Baseline OSDI Mean (SE)	P[§]
EPA, percent of total lipids by weight			0.39
Low (≤0.36)	136	42.5 (1.7)	
Medium	255	43.9 (1.4)	
High (>0.70)	129	40.8 (1.7)	
As continuous			
Slope (SE)		−0.69 (1.82)	0.71
DHA, percent of total lipids by weight			0.13
Low (≤3.0)	127	42.9 (1.7)	
Medium	263	44.0 (1.4)	
High (>4.6)	130	40.0 (1.6)	
As continuous			
Slope (SE)		−0.89 (0.61)	0.15
n-3 index (EPA + DHA), percent of total lipids by weight			0.31
1st tertile (≤3.7)	175	43.2 (1.6)	
2nd tertile (>3.7, ≤4.9)	173	43.2 (1.5)	
3rd tertile (>4.9)	172	41.5 (1.5)	
As continuous			
Slope (SE)		−0.60 (0.49)	0.21
Ratio of n-6:n-3			0.27
1st tertile (≤4.1)	172	41.0 (1.5)	
2nd tertile (>4.1, ≤5.4)	173	43.9 (1.6)	
3rd tertile (>5.4)	175	42.9 (1.6)	
As continuous			
Slope (SE)		0.63 (0.49)	0.19

[§]P-value from test of linear trend, adjusted by age, gender and the presence of Sjogren syndrome.

Table 4

Adjusted mean values of measurements of dry eye signs by n-3 measures at baseline

	Eyes	Conjunctival staining score		Corneal staining score		TBUT (seconds)		Schirmer test (mm)	
Baseline n-3 measure	**(N=1040)**	**Mean (SE)**	**P§**	**Mean (SE)**	**P§**	**Mean (SE)**	**P§**	**Mean (SE)**	**P§**
EPA, percent of total lipids by weight			0.11		0.34		0.03		0.52
Low (≤0.36)	272	3.23 (0.16)		3.89 (0.31)		3.35 (0.17)		10.2 (0.69)	
Medium	510	2.89 (0.13)		4.14 (0.26)		3.03 (0.14)		9.73 (0.61)	
High (>0.70)	258	2.96 (0.15)		4.20 (0.28)		2.92 (0.17)		9.63 (0.78)	
As continuous									
Slope (SE)		−0.04 (0.15)	0.81	0.001 (0.26)	0.99	−0.37 (0.15)	0.014	−0.33 (0.76)	0.66
DHA, percent of total lipids by weight			0.007		0.81		0.22		0.71
Low (≤3.0)	254	3.31 (0.16)		3.94 (0.32)		3.36 (0.17)		9.78 (0.73)	
Medium	526	2.96 (0.12)		4.32 (0.26)		2.96 (0.14)		9.66 (0.58)	
High (>4.6)	260	2.82 (0.16)		3.85 (0.29)		3.07 (0.18)		10.1 (0.79)	
As continuous									
Slope (SE)		−0.13 (0.05)	0.02	−0.03 (0.10)	0.75	−0.06 (0.07)	0.45	0.03 (0.26)	0.91
n-3 index (EPA + DHA) percent of total lipids by weight			0.02		0.54		0.38		0.95
1st tertile (≤3.7)	350	3.25 (0.14)		4.14 (0.29)		3.22 (0.15)		9.84 (0.64)	
2nd tertile (>3.7, ≤4.9)	346	2.86 (0.14)		4.20 (0.27)		2.96 (0.15)		9.71 (0.66)	
3rd tertile (>4.9)	344	2.88 (0.14)		3.94 (0.28)		3.06 (0.17)		9.89 (0.72)	
As continuous									
Slope (SE)		−0.08 (0.04)	0.04	−0.02 (0.08)	0.80	−0.06 (0.05)	0.25	−0.01 (0.21)	0.97
Ratio of n-6:n-3			0.03		0.68		0.21		0.70
1st tertile (≤4.1)	344	2.89 (0.14)		3.99 (0.28)		3.00 (0.17)		10.5 (0.74)	
2nd tertile (>4.1, ≤5.4)	346	2.89 (0.13)		4.18 (0.28)		3.00 (0.15)		9.66 (0.65)	
3rd tertile (>5.4)	350	3.23 (0.14)		4.11 (0.29)		3.26 (0.16)		9.76 (0.64)	
As continuous									
Slope (SE)		0.11 (0.05)	0.02	−0.02 (0.09)	0.79	0.10 (0.06)	0.10	0.01 (0.23)	0.99

§From test of linear trend using the generalized estimating equations approach to account for with inter-eye correlation, adjusted by age, gender and the presence of Sjogren syndrome.