Dietary Omega 3 Fatty Acids Decrease Intraocular Pressure with Age by Increasing Aqueous Outflow

Christine T. O. Nguyen,1 Bang V. Bui,3 Andrew J. Sinclair,2 and Algis J. Vingrys1

PURPOSE. To determine whether there is an association between dietary omega-3 (ω-3) fatty acid intake, age, and intraocular pressure (IOP) caused by altered aqueous outflow.

METHODS. Sprague-Dawley rats were fed either ω-3-sufficient (ω-3−) or ω-3-deficient (ω-3−) diets from conception. The diets had 7% lipid content. The ω-3− diet contained safflower, flaxseed, and tuna oils (5.5:1.0:0.5), and the ω-3− diet contained safflower oil only. Intraocular pressure was measured at 5 to 40 weeks of age under light anesthesia (ω-3−, n = 39; ω-3−, n = 48). Aqueous outflow was determined at 45 weeks in a subgroup of animals (ω-3−, n = 15; ω-3−, n = 22) using pulsed infusion. Ciliary body tissues (n = 6 per group) were assayed for fatty acid content by thin-layer and gas-liquid chromatography in both diet groups.

RESULTS. Animals raised on ω-3− diets had a 13% decrease in IOP at 40 weeks of age (13.48 ± 0.32 mm Hg vs. 15.46 ± 0.29 mm Hg; P < 0.01). When considered as a change in IOP relative to 5 weeks of age, the ω-3− group showed a 23% decrease (P < 0.001). This lower IOP in the ω-3− diet group was associated with a significant increase (+56%; P < 0.001) in outflow facility and a decrease in ocular rigidity (−59%; P < 0.001). The ω-3− group showed a 3.3 times increase in ciliary body docosahexaenoic acid (P < 0.001).

CONCLUSIONS. Increasing dietary ω-3 reduces IOP with age because of increased outflow facility, likely resulting from an increase in docosanoids. This indicates that dietary manipulation may provide a modificable factor for IOP regulation. However, further studies are needed to consider whether this can modify the risk for glaucoma and can play a role in treatment of the disease. (Invest Ophthalmol Vis Sci. 2007;48:756–762) DOI:10.1167/iovs.06-0588

The omega-3 (ω-3) and omega-6 (ω-6) families of polyunsaturated fatty acids (PUFAs) are “essential” fats because they are needed to sustain health, and their 18-carbon chain precursors cannot be synthesized by mammals but must be ingested in the diet.1 Once consumed, ω-3 and ω-6 PUFAs undergo elongation and desaturation to form longer chain metabolites. These long-chain products can become incorporated into cell membranes, thereby influencing membrane-bound protein activity, or they can undergo oxygenation to form eicosanoid and docosanoid metabolites, which are short-acting local hormones. To form these longer chain metabolites, ω-3 and ω-6 PUFAs compete for enzymes. Therefore, it is the balance between these two families rather than their absolute dietary intake that is central to normal cellular function and that can ameliorate the effects of disease.2–4

The Western diet is relatively deficient in ω-3 PUFAs because of a higher intake of ω-6–rich oils (e.g., many common vegetable oils), margarines, and meats,5 in contrast to the traditional Japanese diet, which is high in ω-3–rich fish.6 Western populations have a higher prevalence of many systemic diseases, such as cardiovascular disease, than the traditional Japanese population, and an association has been suggested between disease prevalence and dietary intake.7 SanGiovanni and Chew8 recently reviewed evidence suggesting that ω-3 deprivation may predispose to ocular disease later in life. One ocular disease that shows increased prevalence with age is glaucoma. However, despite its being the second leading cause of vision loss in the world,9 the association between dietary PUFAs and glaucoma has been poorly investigated.

This study focuses on the major modificable risk factor in glaucoma—elevated intraocular pressure (IOP).9 Although the diagnosis of glaucoma is made independently of IOP, lowering pressure is beneficial. Modulating diet and physical activity are recognized by the World Health Organization as important in reducing the incidence and associated risk factors of disease,10 and recently the benefit of these actions has been shown for IOP in glaucoma.11 It is notable that IOP increases with age in Western populations12–15 but decreases with age in the traditional Japanese population.14,15 Variations in dietary fat intake may underlie the differences in these age-related IOP changes. Indeed, ω-3 supplementation decreases IOP in rabbits,10 though they have since been shown to be poor models of glaucoma,16 and in a rat model of hypertension.17 Therefore, it is of interest to consider the time course of ω-3 PUFA dietary modulation on the IOP of normotensive rats.

Intraocular pressure is determined by the balance between aqueous humor production and outflow. Aqueous production involves the action of membrane-bound ionic pumps and receptors.18,19 It is established that ω-3 PUFA deficiency can affect membrane-bound protein activity20–22 and, hence, may have an impact on aqueous production. If this is so, the literature suggests that a deficiency in ω-3 should lead to down-regulation of ionic pump activity and then to reduced aqueous production and decreased IOP, contrary to epidemiological data. Alternatively, ω-3 PUFA deficiency may affect aqueous outflow. Trabecular and uveoscleral outflow pathways can be influenced by long-chain ω-3 and ω-6 metabolites, particularly the eicosanoid and docosanoid fatty acid metabolites.23,24 Hence, if reduced dietary intake of ω-3 PUFA leads to a relative IOP increase with age, it is likely to involve a reduction in outflow.

The aim of this study was to consider the hypothesis that diets low in ω-3 PUFA lead to age-related increases in IOP. If this is so, diet may afford a simple modificable factor for the control of elevated IOP. In addition, we considered whether the change in IOP resulted from compromised aqueous outflow.

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**METHODS**

All experimental procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes set out by the National Health and Medical Research Council of Australia and conforms to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Animals**

Sprague-Dawley rats (Rattus norvegicus) were housed at 21°C with a 12-hour light/12-hour dark cycle (on at 8 am). Animal position relative to the ceiling light source was regularly rotated to maintain ambient light levels at an average of 50 lux to minimize the potential for light damage. Two doses of anesthesia were used, one for IOP (light sedation) and the other for aqueous outflow measurements and tissue fatty acid analysis (deep sedation). Light sedation resulted from intramuscular injection of 30.25 mg/kg ketamine/xylazine (Ketamid 100 mg/mL, Xylazil 100 mg/mL; Troy Laboratories, Smithfield, Australia), whereas deep sedation was achieved with 60.5 mg/kg ketamine/xylazine (Ketamid 100 mg/mL, Xylazil 100 mg/mL; Troy Laboratories). One drop of proxymetacaine hydrochloride provided corneal anesthesia (Ophthalmic 5 mg/mL; Allergan, Frenchs Forest, Australia). During IOP and aqueous outflow measurements, body temperature was maintained at 37°C by passive heating.

**Dietary Manipulations**

Dams were placed on relevant diets from the day of impregnation to ensure maximal tissue changes in fatty acid profiles. Pups were weaned at postnatal day 21 and were maintained on maternal diets to 45 weeks of age. Experimental groups balanced sex, weight, and familial lineage to minimize selection and assignment bias. Diets were either ω-3 sufficient (ω-3⁺) or ω-3 deficient (ω-3⁻), as prepared (Glen Forest Stockfeeders, Perth, Australia) from common macronutrients (Table 1), and complied with the guidelines of the Nutrition Research Council. The lipid component comprised 7% of the total diet, with the ω-3⁺ diet containing safflower, flaxseed, and tuna oils (5.1:1:0.5), whereas the ω-3⁻ diet contained safflower oil only. The fatty acid composition of the diets is detailed in Table 1. To minimize oxidation, diets were nitrogen vacuum-sealed and stored at -4°C, and each animal was supplied with enough food for a maximum of 2 days ad libitum consumption, which ensured no discoloration.

In IOP experiments the entire cohort of animals was used (n = 39; ω-3⁺ n = 48), whereas a random subset was used to evaluate aqueous outflow (ω-3⁺ n = 15; ω-3⁻ n = 22) and provide ciliary body tissue for fatty acid analysis (ω-3⁺ n = 5; ω-3⁻ n = 6).

**Ciliary Body Tissue Fatty Acid Analysis**

Eyeballs were excised from deeply anesthetized animals at 45 weeks of age. An eyecup was created by cutting circumferentially around the cornea, approximately 1 mm in front of the limbus. The lens and vitreous were removed, and four relaxing incisions were made (at the site of corneoscleral junction) to yield a flat mount, then the retina was removed using filter paper. This allowed the ciliary body to be viewed by retro-illumination with a 1×4 magnification light microscope (VMZ, Olympus Optical Co. Ltd., Tokyo, Japan) for extraction with tweezers. Six ciliary bodies per sample were stored in 2.5 mL chloroform-methanol (2:1, vol/vol) containing butyraldehyde and toluene (10 mg/mL) as an antioxidant. These samples then underwent phospholipid extraction by thin-layer and gas-liquid chromatography, as previously reported by our group.

**Intraocular Pressure**

It has been established that the tonometer (Tono-Pen XL; Medtronic Solan) allows repeatable noninvasive measurement of IOP in rats. To minimize the effects of anesthesia, a light dosage was used, and readings were taken within 5 minutes of injection. The IOP reported in this article represents the average of 12 valid individual readings made with a tonometer (Tono-Pen XL; Medtronic Solan). Pilot trials show that the measurement error of the mean using this technique was ±7.8%. Measurements were conducted between 10 am and 3:30 pm to control for diurnal variation. Intraocular pressure readings were taken under normal room lighting (500 lux) at 5, 10, 20, and 40 weeks of age.

**Aqueous Outflow Facility**

Aqueous outflow facility was assessed in rats at 45 weeks of age using the pulsed-infusion method described by Zhang et al. We modified this method for rats and derived indices of aqueous outflow facility, resting IOP, and ocular rigidity. For this purpose, we used a Hanks balanced salt solution (JRH Biosciences, Kansas City, KS), which approximates aqueous for key salt and ionic concentrations ([Na⁺] = 140 mEq/L; [Cl⁻] = 140 mEq/L; [HCO₃⁻] = 25 mEq/L; [Ca²⁺] = 1.25 mEq/L; [Mg²⁺] = 1.125 mEq/L) and provides ciliary body tissue fatty acid analysis (Ophthalmic 5 mg/mL; Allergan, Frenchs Forest, Australia).}

**Table 1. Dietary Composition of ω-3⁺–Sufficient and ω-3⁻–Deficient Diets**

<table>
<thead>
<tr>
<th>Nutrient composition*</th>
<th>ω-3⁺</th>
<th>ω-3⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>10.69</td>
<td>10.69</td>
</tr>
<tr>
<td>Casein</td>
<td>20.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Starch</td>
<td>39.75</td>
<td>39.75</td>
</tr>
<tr>
<td>Dextrinised starch</td>
<td>13.20</td>
<td>13.20</td>
</tr>
<tr>
<td>dl methionine</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Trace minerals</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>1.31</td>
<td>1.31</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>0.69</td>
<td>0.69</td>
</tr>
<tr>
<td>Potassium sulphate</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Potassium citrate</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamins</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Choline chloride 50%</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Lipid source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safflower oil (high linoleic)</td>
<td>5.50</td>
<td>7.00</td>
</tr>
<tr>
<td>Flaxseed oil</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>Tuna oil</td>
<td>0.50</td>
<td>—</td>
</tr>
</tbody>
</table>

* Diets contain common macronutrients (data from Glen Forest Stockfeeders, Perth, Aust) and differ only in their lipid source, which comprised 7% of dietary components. Data expressed as grams fatty acid per 100 grams.

† The ω-6:ω-3 ratio was calculated from the ratio of the summed ω-6 and ω-3 species. Where fatty acid species are not shown these were found in trace amounts that failed to return values above baseline noise. Data expressed as grams fatty acid per 100 grams total fatty acid. LA, linoleic acid; LNA, α-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
ene tubing (0.38-mm inner diameter) connected the cannula to one side of a calibrated pressure transducer (Transpac IV; Abbott Critical Care Systems, Sligo, Ireland), whose output was captured for processing (Powerlab 8/SP, Powerlab, Castle Hill, NSW, Australia). The other side of the pressure transducer was attached to a 100-μL syringe (100F; SGE, Ringwood, Australia) driven by a syringe pump (serial #28974; Harvard Apparatus, Cambridge, MA) that allowed short pulses (0.6 seconds) of either 1 or 2 μL fluid. During the procedure, corneas were kept hydrated with topical application of methyl cellulose (Celluvisc, Allergan, CA) every 15 minutes.

A schematic IOP profile after three successive pulses is shown in Figure 1. The increase in IOP with bolus introduction provides a measure of ocular rigidity (mm Hg/μL), and the recovery gives instantaneous outflow (μL/min). Ocular rigidity was constant below resting IOP and hence was determined from the average of these measurements. Resting IOP (mm Hg) and aqueous outflow facility (μL/min/mm Hg) were determined by plotting the instantaneous outflow versus initial pressures after each pulse. Instantaneous outflow (Vo/n, μL/min) was calculated as follows.

Given a fixed infusion volume (X = 1 or 2 μL), the ratio of IOP recovery rate (pressure out, Po/n, mm Hg/min) to IOP increase (pressure in, Pi/n, mm Hg) can be equated to the volume out/rate infused (Vo/n, μL/min/Vi/n, μL), as shown in equation 1:

\[ \frac{P_0/n}{P_i/n} = \frac{V_o/n}{V_i/n} \]  

Instantaneous outflow (Vo/n) at each given pressure can then be derived, where Vi/n represents the cumulative volume of successive infusions (Vi/n = \( \sum (V_{r1}, V_{r2} \ldots V_{r n} + X_n) \)), Pi/n is the cumulative IOP (pi/n = \( \sum (P_{r1}, P_{r2} \ldots P_{rn} + X_n) \)), and Po/n represents the slope of the curve after a given infusion.

At pressures lower than resting IOP, instantaneous outflow increases at a slow rate with increasing IOP, reflecting the normal drainage and filling of the eye. At pressures greater than resting IOP, instantaneous outflow increases at a faster rate because outflow facility is a function of pressure. Therefore, resting IOP is the intersection of a two-line fit to the data (see Fig. 4B).

Aqueous outflow facility was calculated from baseline-corrected instantaneous outflow (see Fig. 5A) between resting IOP and 40 mm Hg, where the response begins to saturate (see Fig. 4A). Baseline correction is necessary to account for leakage after cannula insertion.

Baseline-corrected instantaneous outflow is then plotted against resting IOP and hence was determined from the average of these measurements. Baseline-corrected instantaneous outflow was calculated as follows.

\[ P_0/n = V_0/n \times \frac{V_i/n}{P_i/n} \]  

Statistical Analysis

Group data are expressed as mean ± SE. Data underwent Komologrov-Smirnov testing (GraphPad Prism, version 4; GraphPad Software, San Diego, CA), and homogeneity was evaluated with a variance ratio (\( \sigma^2 \) maximum/\( \sigma^2 \) minimum). Repeated-measures ANOVA with Geisser Greenhouse correction was used to compare IOP measurements between diet groups at 5, 10, 20, and 40 weeks of age, and post hoc comparisons were performed with Bonferroni correction. Paired t tests were used to compare IOP within each diet group and between different ages. Unpaired t tests were used to compare aqueous outflow facility and tissue fatty acid profiles between diet groups at 45 weeks of age. For all analyses except heterogeneous data (aqueous outflow facility), \( \alpha = 0.05 \) was applied; for heterogeneous data, a more stringent criterion (\( \alpha = 0.01 \)) was adopted to protect against type 2 errors.33 A Grubbs test was used to trim up to 1 outlier from any data set. Because our correlations had variable sets, Demming regression and Spearman correlation were adopted to determine associations.

RESULTS

Ciliary Body Tissue Fatty Acid Analysis

Figure 2 shows the relative fatty acid content of the ciliary body. Of the prostaglandin/docosanoid–producing compounds (docosahexaenoic acid [DHA] and arachidonic acid [AA]), the \( \omega-3 \) group returned a 3.3× increase in DHA but an insignificant change in AA, compared with the \( \omega-3 \) group. In addition, \( \omega-3 \) animals returned significant (\( P < 0.05 \)) increases in 18:1\(-9\) and 22:4\(-6\) (LA) and decreases in 22:4\(-6\) and 22:5\(-6\), culminating in a change of \( \omega-6: \omega-3 \) ratio from \( \omega-5^-3 = 6.4:1 \) to \( \omega-5^-3 = 23.9:1 \).

Intraocular Pressure

Figure 3 shows the average tonometer (Tono-Pen; Medtronic Solan) readings for the two diet groups from 5 to 40 weeks of age. The raw data (Fig. 3A) show that the \( \omega-5^-3 \) group had a higher starting IOP (5 and 10 weeks) than the \( \omega-5^-3 \) group (Bonferroni posttest; \( P < 0.05 \)). However, with age (40 weeks) this trend was reversed, and the \( \omega-5^-3 \) group exhibited a lower IOP (1.98 mm Hg) than the \( \omega-5^-3 \) group (\( F_{1,3} = 17.5; P < 0.001 \)).
However, at 20 weeks, the $\omega^3$ group showed a significantly greater reduction in IOP than the $\omega^5$ group (Bonferroni posttest; $P < 0.05$). This significant difference was also found at 40 weeks (Bonferroni posttest; $P < 0.001$); in fact, the aging trends between diets further diverged. The IOP reduction was sustained in the $\omega^5$ group, but IOP increased in the $\omega^3$ group. At 40 weeks of age, the $\omega^3$ group showed a decrease of $-1.95$ mm Hg and the $\omega^5$ group showed an increase in IOP of $+1.58$ mm Hg relative to their starting levels. Hence, at 40 weeks, the $\omega^3$ and $\omega^5$ diets showed an absolute difference of approximately 15% ($-1.98$ mm Hg; Fig. 3A) and a relative change of 23% ($-3.55$ mm Hg; Fig. 3B). This difference might have been mediated by changes in aqueous outflow (see Introduction), and we considered this issue by measuring aqueous outflow in 45-week-old animals.

**Aqueous Outflow Facility**

Figure 4A shows IOP traces during pulsed-infusion challenge for a representative animal from each diet group. With each bolus infusion, IOP abruptly increased and then gradually decreased because of aqueous outflow over the minute before the next pulse. As can be seen from the representative traces, the IOP increase in response to infusion was lower in $\omega^3$ animals than in $\omega^5$ animals ($2.95 \pm 0.10$ mm Hg/$\mu$L vs. $7.15 \pm 0.13$ mm Hg/$\mu$L; $P < 0.001$); therefore, ocular rigidity was 59% lower in the $\omega^3$ animal. The instantaneous outflow for these representative animals is shown in Figure 4B, which demonstrates the two phases of the response. The intersection of the two-line fit gives the resting IOP and indicates that baseline IOP was lower in the $\omega^3$ group than in the $\omega^5$ group ($-4.22$ mm Hg; $\sim$33%; Fig. 4C), consistent with that found at 40 weeks with a tonometer (Tono-Pen; Medtronic Solan). Indeed, the correlation ($P < 0.05$) between pulsed method resting IOP and tonometer (Tono-Pen; Medtronic Solan) IOP taken at 45 weeks of age was significantly positive ($\gamma = 0.81x + 3.07$; $r_s = 0.34$; Fig. 4D, thick line). Our relationship has 95% confidence limits that encompass values given in the literature for rat tonometer (Tono-Pen; BioRad) calibrations with manometrically set IOP ($\gamma = 0.79x + 4.65$; Fig. 4D; thin line).32

**Figure 3.** The effect of altering $\omega^3$ fatty acid content on IOP. (A) Mean IOP (±SEM) values for animals fed $\omega^3$ diets (filled, $n = 39$) and $\omega^5$ (unfilled, $n = 48$). (B) Aging IOP trends are expressed as a difference from values obtained at 5 weeks of age. Between diets, $^aP < 0.05$. Between ages, $^bP < 0.05$ ($\omega^3$, $\omega^5$, respectively).

**Figure 4.** Pulsed-infusion method determination of IOP. (A) Representative profile of pulsed outflow. IOP spikes represent the rapid introduction of $1 \mu$L fluid and provide a measure of ocular rigidity, whereas the slower recovery in IOP is a measure of aqueous outflow. Outflow capabilities of the $\omega^5$ animal (thick) coped with the IOP challenge better than those of the $\omega^3$ animal (thin). (B) Representative instantaneous outflow with increasing pressure ($\omega^3$ [filled circles]; $\omega^5$ [unfilled circles]). Intersection point of two-line fit determines resting IOP ($\omega^3$ [thick arrow]; $\omega^5$ [thin arrow]) and is also indicated in (A). (C) $\omega^3$ Group (filled) has a lower initial IOP than the $\omega^5$ group (unfilled); $^aP < 0.05$. (D) Correlation between resting IOP derived by the pulsed method and tonometer (Tono-Pen; Medtronic Solan) (thick line) is statistically similar to that reported in the literature for rat eye tonometer (Tono-Pen; BioRad, Santa Ana, CA) calibrations with manometrically set IOP (thin line).32 ($\omega^3$ [filled circles]; $\omega^5$ [unfilled circles]).
Aqueous outflow facility was 56% greater in the ω-3 group than in the ω-5 group (0.053 ± 0.003 vs. 0.034 ± 0.001 μL/min per mm Hg; P < 0.001), as illustrated by the different slopes of the linear fits in Figure 5A and as summarized in Figure 5B. Resting IOP had a significant negative correlation (P < 0.05) with aqueous outflow facility (γ = -0.0050x + 0.12; r = -0.66; Fig. 5C). Therefore, the increased IOP in ω-5 animals was strongly associated with reduced aqueous outflow facility.

**DISCUSSION**

We show a direct association between ω-3 dietary intake and IOP reduction with age. IOP increased with age in rats fed the ω-5 diet but was relatively decreased in those fed the ω-5 diet. Given that the incidence of glaucoma increases with age, the differences in IOP at 40 and 45 weeks are of greatest interest. A relative difference of 23% to 33% (tonometer [Tono-Pen; Medtronic Solan]; ω-5:ω-3 ratio, 2:1 to 5:16 vs 10:1 to 25:15). This implies that increased aqueous outflow facility accounted for the reduction in IOP. Given that the incidence of glaucoma increases with age, further studies will have to determine the dose response for ω-3 dietary intake on IOP.

In our model the general trend in IOP at younger ages was parallel in both diets, with an increase in IOP from 5 to 10 weeks and a decrease by 20 weeks. Similar age-related profiles have been found in other studies of rodent fluid control (e.g., blood pressure) and appears to be a common pattern in rat physiology. One interesting observation was that the ω-3 group had higher IOP than the ω-5 group at early ages. This unexpected finding is difficult to reconcile given that ω-3 PUFA dietary effects generally increase with age. One possibility is that a selection bias occurred even though we attempted to minimize this by careful group allocation (see Methods).

It is unlikely that aqueous production drove the IOP reduction because increasing ω-3 is expected to improve the efficiency of ciliary body membrane-bound ionic pumps, thereby elevating production and IOP. One well-established, ω-3-sensitive G-protein–coupled system is phototransduction, measurable by the a-wave of the electroretinogram. Hence, we assayed the role that G-protein dysfunction (using methods common to our laboratory) can have on aqueous production by correlating a-wave parameters against IOP. We found no significant relationship (IOP vs. phototransduction amplitude: r = 0.02, P = 0.92; IOP vs. phototransduction sensitivity: r = -0.30, P = 0.20) in our animals (n = 32). Consequently, it is unlikely that the IOP change found in ω-3 dietary manipulation involves any G-protein–derived change to aqueous production.

We showed that the ω-3 group had a 59% (P < 0.001) decrease in ocular rigidity and a 56% (P < 0.001) increase in ocular outflow facility compared with the ω-5 group. Both factors have significant implications for IOP regulation. Reduced ocular rigidity (ω-3) means that aqueous volume changes will result in smaller increases in IOP. In addition, the increased aqueous outflow facility accounted for the reduction in resting IOP in the ω-3 group. Both ω-6 and ω-3 produce metabolites that increase outflow. In particular, the prostaglandins (PGs) and the docosanoids are known to modulate fluid dynamics and the inflammatory response. These metabolites are formed by the oxygenation of fatty acids through cyclooxygenase (COX) and lipooxygenase (LOX) enzymes. The source of these metabolites is likely to be the ciliary body because the anterior uvea has a greater capacity to synthesize cyclooxygenase products than the cornea, lens, or retina. In addition, these hormones are locally active, consistent with the close association between the uvea and the outflow pathways. We found no difference in the ciliary body precursors of PGs (Fig. 2, AA); hence, these metabolites cannot account for the divergence in aging IOP between our ω-3 and ω-5 groups. In contrast, a 3.3-fold increase in the docosanoid precursor (DHA) was observed in the ω-3 group compared with the ω-5 counterparts. The docosanoid unoprostone isopropyl (Rescula 0.15%; CIBA, Duluth, MN) has ocular hypotensive properties and appears to act at the trabecular meshwork by relaxing the smooth muscle-like tissue, though a uveoscleral contribution cannot be excluded. Although a significant increase in linoleic acid (LA) was found in the ω-3 group, perhaps because of effective competition by α-linolenic acid (ALA; 18:3ω-3), it is not known to produce PG metabolites and, hence, probably does not influence aqueous outflow.

**FIGURE 5.** The effect of altering ω-3 fatty acid on aqueous outflow determined by pulsed infusion. (A) Aqueous outflow facility determined from averaged baseline-corrected instantaneous outflow linear regression parameters (ω-3 [thick line]; ω-5 [thin line]). Variance illustrated in 5 mm Hg–binned data ± SEM (ω-3 [filled circles]; ω-5 [unfilled circles]). (B) The ω-3 group (filled) had higher aqueous outflow facility than the ω-5 group (unfilled). *P < 0.05. (C) IOP has a significant negative correlation with aqueous outflow facility; r = -0.66 (ω-3 [filled circles]; ω-5 [unfilled circles]).
Two factors may explain our findings of increased aqueous outflow facility in the ω-3⁺ group. First, higher levels of docosanoids (DHA metabolites in the ω-3⁺ group lower IOP. Second, the shift toward anti-inflammatory ω-3 products and against pro-inflammatory ω-6 eicosanoids act in concert to reduce any IOP elevating inflammatory tendency. Nevertheless, it would be ideal to assay the aqueous humor itself to determine the specific compounds acting in this medium.

Although elevated IOP is a major risk factor in glaucoma, it is not a defining factor. The studies that evaluate ω-3 effects on glaucoma are sparse and provide conflicting outcomes. Japanese and Western populations have a lower prevalence of high-tension glaucoma than Western populations; but Japanese populations do have an increased prevalence of low-tension glaucoma. Prospective studies have been inconclusive. Ren et al. found decreased ω-3 PUFA levels in glaucoma patients compared with their healthy siblings, whereas Kang et al. found that a lower ω-6/ω-3 consumption ratio was associated with increased glaucoma prevalence. Further studies are needed to clarify this relationship.

In summary, this study demonstrates that increasing ω-3 PUFA consumption leads to decreased IOP with age through increased aqueous outflow facility. It is likely that these changes are docosanoid driven. Further studies are needed to consider whether manipulation of dietary omega-3 fatty acids may be important in modifying the risk for chronic eye diseases such as glaucoma.

Acknowledgment

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References