Biological Safety Assessment of Docosahexaenoic Acid Supplementation in a Randomized Clinical Trial for X-Linked Retinitis Pigmentosa

Dianna H. Wheaton, MS; Dennis R. Hoffman, PhD; Kirsten G. Locke, CRA; Reginald B. Watkins, BS; David G. Birch, PhD

Background: In a 4-year placebo-controlled trial to elevate blood docosahexaenoic acid levels in patients with X-linked retinitis pigmentosa (XLRP), the goal was to assess the potential benefit of docosahexaenoic acid supplementation in altering disease progression. However, docosahexaenoic acid (22:6\(\text{H}_{9275}\)3) is a highly unsaturated fatty acid and considered a target molecule for free-radical oxidative damage. Thus, nutritional provision of docosahexaenoic acid might lead to an increase in antioxidant stress. Additional concerns, such as decreased platelet aggregation, increased bleeding time, and alterations in lipoprotein cholesterol levels, have been reported in supplementation studies with long-chain polyunsaturates.

Objective: To assess the biological safety of long-term docosahexaenoic acid supplementation.

Design: Forty-four male patients (mean age, 16 years) enrolled in a randomized, double-masked, clinical trial and received docosahexaenoic acid, 400 mg/d, or placebo. Blood samples were collected every 6 months. Biological safety analysis included fatty acids, vitamin A and E concentrations, antioxidant capacity, platelet aggregation, alanine aminotransferase activity, and lipoprotein cholesterol and triglyceride profiles.

Results: Mean plasma docosahexaenoic acid levels were elevated 2.5-fold by supplementation compared with baseline. Patients receiving placebo capsules exhibited no change (\(P=.35\)) in plasma docosahexaenoic acid content. All adverse events reported were minor and equivalently distributed between groups. Plasma vitamin A concentrations remained unchanged during the trial. Mean plasma vitamin E concentrations were correlated with age (\(P=.005\)), such that as patients with XLRP matured, plasma vitamin E concentrations increased to approach normal values. There was a trend (\(P=.10\)) toward lower mean vitamin E concentrations in the docosahexaenoic acid–supplemented group after 4 years. Docosahexaenoic acid supplementation did not compromise plasma antioxidant capacity, platelet aggregation, liver function enzyme activity, or plasma lipoprotein lipid content in patients with XLRP.

Conclusion: Long-term docosahexaenoic acid supplementation to patients with XLRP was associated with no identifiable safety risks in this 4-year clinical trial.

Arch Ophthalmol. 2003;121:1269-1278

Retinitis Pigmentosa (RP) is a genetic eye disease characterized by progressive retinal degeneration. Clinical manifestations include disturbances of retinal pigment, vessel attenuation, and thinning of the retinal pigment epithelium. Degeneration typically occurs first in the peripheral retina, affecting the rod photoreceptors and resulting in night blindness. The disease progresses inward toward the macula, leading to progressive restriction of the peripheral visual field (ie, tunnel vision). As cone function within the macula becomes progressively impaired, legal blindness and loss of ambulatory vision often result. Retinitis pigmentosa is genetically heterogeneous and can be inherited in an autosomal dominant, autosomal recessive, X-linked recessive, or sporadic manner. To date, no cure for RP has been identified and clinical interventions to retard disease progression are limited.

Numerous studies have reported reduced blood levels of the \(\omega3\) fatty acid, docosahexaenoic acid, in patients with RP.1,2 This reduction is particularly striking in patients with XLRP (XLRP).3,5 After adjusting for the effects of age, concentrations of docosahexaenoic acid in red blood cell (RBC) lipids of patients with XLRP were positively correlated with retinal function measured by electrophysiography (ERG), such that higher blood docosahexaenoic acid concentrations were
associated with higher (ie, better) rod and cone ERG amplitudes. Heterozygous carriers of XLRP have demonstrable reductions in blood docosahexaenoic acid levels and cone ERG function similar to, but less dramatic than, that described in the hemizygous male.

Docosahexaenoic acid (22:6ω3), with 6 double bonds, is the most unsaturated membrane fatty acid present in biological systems, and comprises 30% to 40% of the total fatty acids in phospholipids of the cerebral cortex and retina. Although no functional role for docosahexaenoic acid has been identified in the visual cascade, research implies that this ω3 fatty acid is biologically significant to neural tissue. Within the retina, docosahexaenoic acid is concentrated in highly specialized membranes that make up photoreceptor outer segments, and is found in phospholipids that are tightly associated with the visual chromophore rhodopsin. The dependency on docosahexaenoic acid–containing phospholipid bilayers has recently been described for the kinetic conversion of metarhodopsin I to metarhodopsin II, the formation of activated rhodopsin–transducin complex, and activation of phosphodiesterase.

A reduced level of docosahexaenoic acid in blood lipids provides a rationale for a clinical intervention using docosahexaenoic acid supplementation in patients with RP. The X-linked form of the disease is considered one of the most severe forms of RP based on early onset of visual loss and functional blindness at a young age. As a group, patients with XLRP have the lowest blood docosahexaenoic acid levels and the earliest onset of disease severity, making this an ideal population for an early treatment intervention. A randomized, placebo-controlled, clinical trial was undertaken to assess the potential benefit of nutritional docosahexaenoic acid supplementation. The primary goals of this trial were 2-fold: (1) to determine if daily oral supplementation with docosahexaenoic acid would elevate blood docosahexaenoic acid levels and (2) to determine if variations in the RBC docosahexaenoic acid levels of patients are related to the rate of disease progression. The primary outcomes have been reported separately. Briefly, RBC docosahexaenoic acid levels were significantly elevated in supplemented patients; however, the rate of disease progression, as measured by cone ERG, was not significantly different between the 2 groups using an intent-to-treat analysis. Nevertheless, cone and rod photoreceptor loss correlated with RBC docosahexaenoic acid levels such that patients with higher docosahexaenoic acid levels had reduced ERG loss.

As a long-chain polyunsaturated fatty acid (LCPUFA), docosahexaenoic acid is considered a potential target molecule for free radical oxidative damage. Increased LCPUFA intake may lead to an elevated state of oxidative stress, and subsequently result in membrane damage. Additional findings, such as decreased platelet aggregation, increased bleeding time, and alterations in lipoprotein cholesterol level, have also been reported in previous supplementation studies with LCPUFAs. Therefore, a parallel goal of this treatment trial was to assess the biological safety of long-term docosahexaenoic acid supplementation in patients with XLRP. We report the outcome of biochemical assays that were selected to monitor the bioavailability and safety of long-term LCPUFA supplementation.

**METHODS**

**SUBJECTS**

Patients diagnosed as having XLRP were recruited from the Southwest Eye Registry (Retina Foundation of the Southwest) for participation in this clinical trial. Recruitment was extended nationally through the referral of clinical centers supported by the Foundation Fighting Blindness, Owings Mills, Md. Major eligibility criteria for study participation included a diagnosis of RP by an ophthalmologist specializing in retinal disease, a family history consistent with X-linked inheritance, detectable cone ERG responses to a 31-Hz flicker (ie, >0.34 µV), and willingness to return annually for a visual function assessment.

Fifty-two patients were screened for inclusion in the trial; 4 patients did not meet the enrollment criteria and 4 declined to participate. All participants were male, because the disease is more severe in hemizygous males than heterozygous carriers. The study was explained in detail, and informed consent was obtained from participants and/or parents of minors. This research protocol observed the tenets of the Declaration of Helsinki, and was approved by the Institutional Review Board of The University of Texas Southwestern Medical Center at Dallas. In addition, the use of docosahexaenoic acid in this trial that included minors was conducted under an investigational new drug approval (No. 45942) issued by the US Food and Drug Administration, Rockville, Md.

**STUDY DESIGN**

Forty-four patients (mean±SD age, 16±9 years; range, 4-38 years) (see Table 1 for baseline demographic characteristics) were randomized to parallel arms of the study, and received capsules containing either docosahexaenoic acid–enriched oil or a placebo oil (corn/soy oil) for the duration of the 4-year trial. Assignments were made following a 10 per block randomization schedule. Close relatives were randomized together to eliminate a potential for mixing of capsules; there were 5 sibling pairs in each cohort.

Both oil formulations were gelatin encapsulated to provide a total fat content of 500 mg per capsule. Each capsule also contained vitamin E, 12.5 IU, and ascorbyl palmitate, 12.5 mg, as antioxidants to reduce oxidative degradation of LCPUFAs. Placebo and docosahexaenoic acid–enriched capsules were identical in appearance, taste, and smell. Patients were instructed to take two 500-mg capsules per day with no recommended changes in their normal dietary practices. Oral supplementation with 2 enriched capsules provided 400 mg of docosahexaenoic acid daily. Thus, patients receiving the enriched capsules averaged about 10 mg of docosahexaenoic acid per kilogram of body weight per day. Seven of the youngest patients were provided half-sized capsules to ease swallowing; taking 4 of these capsules provided the full dosage. As the study progressed, all patients were switched to full-sized capsules. All supplements were labeled either A or B by the manufacturer (Martek Biosciences Corporation, Columbia, Md) to mask study participants and research staff. The manufacturer retained the code and was willing to divulge group assignment to the Data and Safety Monitoring Committee, the Institutional Review Board, or a patient’s physician in case of a medical emergency. This option was not used during the trial.

The docosahexaenoic acid–enriched oil used for the capsules was derived from a single-cell algal source and provided as a highly purified triacylglycerol (DHASCO oil; Martek Bio-
The docosahexaenoic acid content of these capsules is standardized at 40% of the total fatty acids (Table 2); the remaining 60% of the fatty acids are all naturally occurring and presumably do not compromise the specificity of the study. Extensive toxicology studies were conducted in animal models to establish safety for human consumption. This docosahexaenoic acid–enriched oil has been used in Phase 1 clinical trials with healthy volunteers and has been well tolerated.

Table 1. Baseline Demographic Characteristics of the Cohorts

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo Group (n = 23)</th>
<th>Docosahexaenoic Acid–Supplemented Group (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y</td>
<td>18.0 ± 10.1</td>
<td>14.3 ± 9.0</td>
</tr>
<tr>
<td>White-African American–</td>
<td>19.0 ± 2.0</td>
<td>19.1 ± 13.0</td>
</tr>
<tr>
<td>Latin American–other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, mean ± SD, kg</td>
<td>62 ± 29</td>
<td>58 ± 30</td>
</tr>
<tr>
<td>Body mass index†</td>
<td>21.8 ± 6.5</td>
<td>21.3 ± 6.6</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>20-30</td>
<td>9</td>
</tr>
<tr>
<td>&gt;30</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Plasma docosahexaenoic, % of total fatty acids</td>
<td>1.48 ± 0.33</td>
<td>1.46 ± 0.37</td>
</tr>
<tr>
<td>Range</td>
<td>0.94-2.18</td>
<td>0.98-2.19</td>
</tr>
<tr>
<td>Daily vitamin supplement use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivitamin</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*Data are given as number of patients unless otherwise indicated. All patients were male. Four patients in each group smoked 1 pack of cigarettes per day or more. No statistically significant group differences were found for age (t = 1.06, P = .29), body mass index (t = 0.25, P = .80), or plasma docosahexaenoic acid level (t = 0.19, P = .85). The normative values of the Retina Foundation of the Southwest, Dallas, Tex, are as follows: n = 20; mean ± SD age, 17.2 ± 6.6 years (range, 10-30 years); white–African American–Latin American–other ratio, 18:2:0:0; smoking, 0 patients; mean ± 95% confidence interval for body weight, 66.2 ± 11.6 kg; mean ± 95% confidence interval for body mass index, 21.9 ± 2.3 (range, 15.6-38.0); mean ± 95% confidence interval for plasma docosahexaenoic acid percentage of total fatty acids, 1.90 ± 0.33; and 2 control subjects supplemented with a daily multivitamin.

†Calculated as weight in kilograms divided by the square of height in meters.

Table 2. Fatty Acid Composition of Intervention Capsules

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Common Name</th>
<th>Docosahexaenoic Acid–Enriched Capsules (DHASCO Oil)</th>
<th>Placebo Capsules (Corn/Soy Oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:0</td>
<td>Capric acid</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>12:0</td>
<td>Lauric acid</td>
<td>5.0</td>
<td>0</td>
</tr>
<tr>
<td>14:0</td>
<td>Myristic acid</td>
<td>21.6</td>
<td>0</td>
</tr>
<tr>
<td>16:0</td>
<td>Palmitic acid</td>
<td>19.8</td>
<td>11.0</td>
</tr>
<tr>
<td>16:1</td>
<td>Palmitoleic acid</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>18:0</td>
<td>Stearic acid</td>
<td>0.4</td>
<td>3.1</td>
</tr>
<tr>
<td>18:1</td>
<td>Oleic acid</td>
<td>10.1</td>
<td>25.0</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>Linoleic acid</td>
<td>0.6</td>
<td>56.2</td>
</tr>
<tr>
<td>18:3ω3</td>
<td>α-Linolenic acid</td>
<td>0</td>
<td>4.0</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>Docosahexaenoic acid</td>
<td>40.1</td>
<td>0</td>
</tr>
<tr>
<td>24:1</td>
<td>Nervonic acid</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>Not applicable</td>
<td>0.4</td>
<td>0.7</td>
</tr>
</tbody>
</table>

*Data are given as percentage of total fatty acids. Each placebo and docosahexaenoic acid–enriched capsule (500 mg) contained 12.5 mg (12.5 IU) of vitamin E and 12.5 mg of ascorbyl palmitate. Fatty acid analysis was conducted at the Retina Foundation of the Southwest, Dallas, Tex.

To address the concerns of increased bleeding time and decreased platelet activation, which had been associated with ω-3 fatty acids in some but not all studies that used fish oil as an LCPUFA source. At 4 years, plasma alanine aminotransferase (ALT) activity was measured as an index of liver function, because it was recently reported that the hepatic activity of Δ⁵ desaturase was reduced in patients with XLRP. An overview of the study design is given in Table 3. Of the 44 patients enrolled in the study, 41, 43, 44, and 41 completed testing at the 1-, 2-, 3-, and 4-year annual visits, respectively.

Near the conclusion of the study, a group of age- and sex-matched control subjects with healthy vision (mean ± SD age, 17 ± 7 years; range, 10-30 years; n = 20) provided normative data for each of the biochemical assays. Volunteers with healthy vision were subjected to a brief visual function examination (visual acuity and visual field perimeter measurements), and a 1-time blood sample was drawn as described for study participants.

**BLOOD COLLECTION**

Blood samples were drawn from an antecubital vein of subjects who had fasted for at least 8 hours. Typically, 10 to 20 mL of blood was collected in tubes containing 15% potassium EDTA as an anticoagulant. Beginning at the 3-year point, 2 additional samples (5 mL each) were collected for biological safety monitoring. EDTA was added to preserve plasma lipids and lipoproteins.

**FATTY ACID ANALYSIS**

Details of the lipid analysis have been reported previously. Briefly, plasma and RBCs were separated immediately by centrifugation (3000g for 10 minutes at 4°C), and lipids were extracted using a 2:1 ratio of methanol and chloroform containing 0.02% butylated hydroxytoluene as an antioxidant. Total lipids from plasma were transmethylated (using a combination of 14% boron trifluoride and methanol) and subsequently quantified using capillary column gas chromatography and flame ionization detection. Peak identification was confirmed by comparing retention times with those of a standard mixture of fatty acid methyl esters. Plasma fatty acid quantitation was expressed as relative weight percentage (percent of total fatty acid).

**Downloaded From:** by a Non-Human Traffic (NHT) User on 12/02/2018
**ANTIOXIDANT ANALYSIS**

**Measurement of Vitamins A and E**

The rhodopsin chromophore precursor, vitamin A (retinol), and the antioxidant, vitamin E (α-tocopherol), were measured on a high-pressure liquid chromatograph (Beckman Instruments, Fullerton, Calif). The fat-soluble vitamins were solvent extracted from EDTA-anticoagulated plasma and fractionated on a C18 reversed-phase analytical column (Alltech Associates, Deerfield, Ill) by methods previously described by Bui.26 Analytes were resolved with an isocratic flow of 1 mL/min of mobile phase (acetonitrile–tetrahydrofuran–methanol–1% ammonium acetate, 68:22:7:3 vol/vol/vol/vol) and detected at 325 nm for vitamin A and 290 nm for vitamin E. Quantitation was achieved by comparison with calibration curves derived from standards for vitamins A and E (Sigma-Aldrich Corp, St Louis, Mo), and expressed as microgram per deciliter and milligrams per deciliter, respectively.

**Total Antioxidant Capacity**

The total antioxidant capacity of plasma was measured using an enhanced chemiluminescence modification of the total peroxyl radical trapping parameter.27 The antioxidant capacity of an aliquot (20 µL) of citrate anticoagulated plasma diluted 1:10 with isotonic sodium chloride solution was established by its ability to quench a horseradish peroxidase–catalyzed reaction generated by a chemiluminescence kit (catalog number RPN H9251–tocopherol), were measured on a high-pressure liquid chromatograph (Beckman Instruments, Fullerton, Calif). The fat-soluble vitamins were solvent extracted from EDTA-anticoagulated plasma and fractionated on a C18 reversed-phase analytical column (Alltech Associates, Deerfield, Ill) by methods previously described by Bui.26 Analytes were resolved with an isocratic flow of 1 mL/min of mobile phase (acetonitrile–tetrahydrofuran–methanol–1% ammonium acetate, 68:22:7:3 vol/vol/vol/vol) and detected at 325 nm for vitamin A and 290 nm for vitamin E. Quantitation was achieved by comparison with calibration curves derived from standards for vitamins A and E (Sigma-Aldrich Corp, St Louis, Mo), and expressed as microgram per deciliter and milligrams per deciliter, respectively.

**WHOLE BLOOD PLATELET AGGREGATION**

Platelet aggregation was measured using an aggregometer (Chrono-Log Corp, Haverton, Pa). Citrate anticoagulated blood (200 µL) was diluted 1:4 with Tyrode buffered saline solution (sodium chloride, 120 mmol/L; potassium chloride, 5 mmol/L; sodium bicarbonate, 10 mmol/L; calcium chloride, 2 mmol/L; magnesium chloride, 2 mmol/L; sodium phosphate, 4 mmol/L; glucose, 10 mmol/L; and heparin, 2 U/mL), and aggregation was induced with 10 µg of Type I collagen (No. 389; Chrono-Log Corp). Results are expressed as impedance (ohms) to aggregation of the sample vs a Tyrode blank control.

**ALANINE AMINOTRANSFERASE**

Liver function was indexed by assaying ALT enzyme activity with an automated analyzer (Cholestech L-D-X; Cholestech Corp, Hayward, Calif). This analyzer measures ALT level by an enzymatic method and solid-phase technology using a cassette-based assay (No. 11-772; Cholestech Corp). The addition of heparin-anticoagulated whole blood (50 µL) to the cassette initiates the first of 3 enzyme-catalyzed reactions and terminates with the formation of a blue color at a rate proportional to the ALT activity of the sample. The resulting color is measured by reflectance photometry, with enzyme activity reported as units per liter at 37°C.

**LIPOPROTEIN LIPID PROFILES**

The analyzer (Cholestech L-D-X) was also used to determine plasma lipoprotein lipid profiles. EDTA-anticoagulated plasma (50 µL) was added to a lipid profile cassette (No. 10-989; Cholestech Corp) that allowed the simultaneous measurement of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and TGs via a series of multistep enzymatic reactions. Values for low-density lipoprotein cholesterol (LDL-C) were calculated by the analyzer using the measured values. Concentrations were recorded as milligrams per deciliter.

**ADVERSE EVENTS**

Adverse events were self-reported on a per-incident basis to the study coordinator (K.G.L.). Postevent follow-up was conducted by telephone interview, and outcome was recorded accordingly. Patients were encouraged to inform their primary care physician of their participation in the supplementation trial. A letter was prepared and given to each patient or parent on enrollment to provide to their physician in the event that specific questions pertaining to the trial or supplements were raised.

**STATISTICAL ANALYSIS**

All patient data were used as dictated by an intent-to-treat protocol. Data from previous measurements were used to replace missing values. A 2-tailed t test was used to compare fatty acid and biosafety outcome variables between the treatment groups at each time point in the trial. In addition, repeated-measures analyses of variance were used for longitudinal analysis of vitamins A and E. Statistical significance was considered at P<.05 for antioxidant, platelet aggregation, ALT, and lipoprotein lipid measures. However, for most fatty acid values, a more stringent assessment of variance were used for longitudinal analysis of vitamins A and E. Statistical significance was considered at P<.003, Bonferroni adjustment) was used. Values for patients with XLR are given as mean±SD, and those for age- and sex-matched controls as mean±95% confidence interval (CI).

---

Table 3. Study Design

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Annual Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Visual function</td>
<td>21/23</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>21/23</td>
</tr>
<tr>
<td>Vitamins</td>
<td>21/23</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>0/0</td>
</tr>
<tr>
<td>Aggregation</td>
<td>0/0</td>
</tr>
<tr>
<td>Lipoproteins</td>
<td>21/23</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>0/0</td>
</tr>
</tbody>
</table>

*Data are given as the number of patients tested at each annual visit for the placebo/docosahexaenoic acid–supplemented groups. An intent-to-treat protocol was used for data analysis at each time point.
RESULTS

FATTY ACIDS

There was no significant difference at baseline between mean plasma docosahexaenoic acid levels of patients randomized to either the placebo or the docosahexaenoic acid–supplemented group (1.48% vs 1.46%) (Table 1); values for the entire cohort ranged from 0.94% to 2.19% of total fatty acids. A group of age- and sex-matched controls with healthy vision had a mean ± SD plasma docosahexaenoic acid level of 1.90% ± 0.33% (range, 1.06%-3.68%). Thus, the study patients exhibited plasma docosahexaenoic acid levels that are approximately 75% of normal before randomized capsule assignment.

The docosahexaenoic acid–supplemented group exhibited an increased plasma docosahexaenoic acid content within the first 6 months of supplementation (Figure 1). During the 4-year trial, the plasma docosahexaenoic acid level of the docosahexaenoic acid–supplemented group (mean ± SD for years 0.5-4, 3.91% ± 1.24%; range, 2.12%-6.46%) was elevated an average of 2.5-fold compared with baseline levels. Significant group differences ($P<.003$) were maintained throughout the trial. In the placebo group, plasma docosahexaenoic acid content at each annual visit remained unchanged from the baseline value ($P>.20$).

A corresponding reduction in arachidonic acid (ARA) (20:4ω6) also was observed in the docosahexaenoic acid–supplemented cohort after the first 6 months of supplementation. The plasma ARA levels remained significantly reduced ($P<.001$), compared with the placebo group, for the duration of the trial (0.5 to 4 years) (Figure 1 and Table 4). ω6 Docosapentaenoic acid (22:5ω6), the end product of the ω6 biosynthetic pathway, was reduced by 50% in the docosahexaenoic acid–supplemented group (0.20% vs 0.41% of total fatty acids). These alterations were also reflected in the ratios of ARA/docosahexaenoic acid, ω6 docosapentaenoic acid/docosahexaenoic acid, and ω6 PUFA/ω3 PUFA, which were reduced by 62%, 75%, and 41%, respectively, in patients receiving docosahexaenoic acid (Table 4). The docosahexaenoic acid–supplemented group exhibited an overall elevation in total ω3 LCPUFAs and a decrease in total ω6 LCPUFAs; however, the total sum of saturated and monounsaturated fatty acids was not different between the 2 groups ($P>.60$). Although the placebo capsule provided the essential fatty acids, α-linolenic and linoleic acids (18:3ω3 and 18:2ω6, respectively), no significant group differences were observed for these fatty acids ($P>.55$). The plasma level of the ω3 intermediate, eicosapentaenoic acid (EPA) (20:5ω3), was not different between the 2 groups; however, ω3 docosapentaenoic acid (22:5ω3) was reduced in the docosahexaenoic acid–supplemented group.

Table 4. Fatty Acid Profiles in Total Plasma Lipids of Trial Subjects With XLRP∗

<table>
<thead>
<tr>
<th>Fatty Acid Variable</th>
<th>Placebo Group (n = 21)</th>
<th>Docosahexaenoic Acid–Supplemented Group (n = 23)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of total fatty acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ω3</td>
<td>0.44 ± 0.08</td>
<td>0.46 ± 0.13</td>
<td>.55</td>
</tr>
<tr>
<td>ω3 DPA</td>
<td>0.40 ± 0.11</td>
<td>0.40 ± 0.08</td>
<td>1.0</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>0.73 ± 0.11</td>
<td>0.46 ± 0.09†</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ω6</td>
<td>1.57 ± 0.32</td>
<td>3.91 ± 1.24†</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LA</td>
<td>26.60 ± 2.67</td>
<td>26.60 ± 1.67</td>
<td>1.0</td>
</tr>
<tr>
<td>ARA</td>
<td>9.35 ± 0.75</td>
<td>7.51 ± 1.10†</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ω6 DPA</td>
<td>0.41 ± 0.12</td>
<td>0.20 ± 0.06†</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturates‡</td>
<td>35.00 ± 1.21</td>
<td>35.20 ± 1.28†</td>
<td>.60</td>
</tr>
<tr>
<td>Monounsaturates§</td>
<td>22.20 ± 1.18</td>
<td>22.00 ± 1.92†</td>
<td>.68</td>
</tr>
<tr>
<td>LCPUFAs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ω3</td>
<td>2.66 ± 0.43</td>
<td>4.75 ± 1.16†</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ω6</td>
<td>12.90 ± 0.94</td>
<td>10.50 ± 1.19†</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Ratios</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ω6 PUFA/ω3 PUFA</td>
<td>12.80 ± 2.20</td>
<td>7.56 ± 1.63†</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ω6 DPA/docosahexaenoic acid</td>
<td>0.27 ± 0.06</td>
<td>0.07 ± 0.04†</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ARA/docosahexaenoic acid</td>
<td>6.20 ± 0.96</td>
<td>2.36 ± 1.14†</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: ARA, arachidonic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; LCPUFA, long-chain polyunsaturated fatty acid; LNA, linolenic acid; XLRP, X-linked retinitis pigmentosa.

*Data are given as mean ± SD for years 0.5 to 4.
†The difference between the placebo group and the docosahexaenoic acid–supplemented group is significant.
‡Includes 14:0, 16:0, 17:0, 18:0, 20:0, 22:0, and 24:0.
§Includes 16:1, 18:1, 20:1, 22:1, and 24:1.
vitamin A concentration of the study cohort was not significantly different between the 2 groups after 4 years of supplementation (mean ± SD, 16.8 ± 5.7 vs 14.9 ± 7.1 µmol/L; P = .35). The placebo and docosahexaenoic acid–supplemented groups demonstrated less collagen-induced platelet aggregation than controls (mean ± 95% CI, 22 ± 2 U/L; range, 13-29 U/L) (Figure 3B). Although reduced platelet aggregation was a concern of long-term supplementation with LCPUFAs, reduced aggregation was noted in both study groups and, therefore, was not associated with docosahexaenoic acid supplementation per se. As an alternative, vitamin E in placebo and docosahexaenoic acid–enriched capsules (12.5 IU of vitamin E per capsule) may accumulate in vivo to approach a level capable of inhibiting platelet aggregation.28 However, no correlation was found between plasma vitamin E content and whole blood platelet aggregation at the 4-year point for the combined cohorts with XLRP (P > .90).

**Platelet Aggregation**

A subset of patients from the placebo group (n = 16) and the docosahexaenoic acid–supplemented group (n = 14) were randomly selected for assessment of ALT activity after 4 years of supplementation. The mean ± SD ALT activity for the docosahexaenoic acid–supplemented group was 18.1 ± 7.1 U/L, and was not significantly different from the placebo group (24.8 ± 13.8 U/L) (P = .15) (Figure 3C). When compared with normative data (mean ± 95% CI, 20.6 ± 3.1 U/L; range, 12-40 U/L), the placebo group exhibited ALT activity slightly greater than the upper 95% CI, whereas the docosahexaenoic acid–supplemented group exhibited activity near the lower 95% CI.

**Lipoprotein Lipid Profiles**

No significant differences were found at year 4 between measures of plasma lipoprotein lipid profiles in the placebo and docosahexaenoic acid–supplemented groups (P > .70) (Figure 4A-D) for TC (mean ± SD, 170 ± 34 vs 160 ± 40 mg/dL [4.40 ± 0.88 vs 4.14 ± 1.03 mmol/L]), TGs...
recorded for 6 subjects from the placebo group (age range, 13-32 years) and 4 subjects from the docosahexaenoic acid–supplemented group (age range, 9-19 years). Those symptoms thought to be potentially associated with capsule/LCPUFA consumption were as follows: bruising, prolonged bleeding, and gastrointestinal symptoms.

Adverse events reported by patients in the placebo group included bruising (n=2), sinusitis (n=1), prolonged epistaxis (n=1), eructation (burp back) (n=1), nausea (n=1), and flatulence (n=1). Postevent follow-up disclosed that the 2 cases of bruising were due to physical contact sports. The prolonged epistaxis was preceded by a case of sinusitis in 1 individual. A bleeding time test conducted by the patient’s family physician subsequently determined bleeding time to be within normal limits. The case of nausea was short-term (<1 week), and eructation and flatulence were related to capsule consumption. One additional case each of apnea, fainting, and a “weird” feeling among patients in the placebo group were judged “not study related” by the parent or family physician.

The docosahexaenoic acid–supplemented group reported 1 case of eructation that was related to capsule consumption. Additional reported events included acne (n=2), headache (n=2), ear infection (n=1), fatigue (n=1), and weight gain (n=1). These events were judged by the parent or family physician to be consistent with normal events of adolescence.

Supplementation with a low dose of docosahexaenoic acid, 400 mg/d, for a 4-year duration was not associated with any identifiable safety risk. Plasma docosahexaenoic acid levels were successfully elevated by 2.5-fold in the docosahexaenoic acid–supplemented group, and remained at baseline levels for the placebo group. Both groups reported minor adverse events judged by the parent or physician not to be study related as well as minor gastrointestinal symptoms (eructation and flatulence) associated with capsule consumption. No group differences were observed for plasma antioxidant capacity, vitamin A content, and vitamin E content.

ADVERSE EVENTS

Patients were requested to report all adverse events throughout the trial; subsequently, several individuals (n=5) reported more than 1 event. Adverse events were recorded for 6 subjects from the placebo group (age range, 13-32 years) and 4 subjects from the docosahexaenoic acid–supplemented group (age range, 9-19 years). Those symptoms thought to be potentially associated with capsule/LCPUFA consumption were as follows: bruising, prolonged bleeding, and gastrointestinal symptoms.

Adverse events reported by patients in the placebo group included bruising (n=2), sinusitis (n=1), prolonged epistaxis (n=1), eructation (burp back) (n=1), nausea (n=1), and flatulence (n=1). Postevent follow-up disclosed that the 2 cases of bruising were due to physical contact sports. The prolonged epistaxis was preceded by a case of sinusitis in 1 individual. A bleeding time test conducted by the patient’s family physician subsequently determined bleeding time to be within normal limits. The case of nausea was short-term (<1 week), and eructation and flatulence were related to capsule consumption. One additional case each of apnea, fainting, and a “weird” feeling among patients in the placebo group were judged “not study related” by the parent or family physician.

The docosahexaenoic acid–supplemented group reported 1 case of eructation that was related to capsule consumption. Additional reported events included acne (n=2), headache (n=2), ear infection (n=1), fatigue (n=1), and weight gain (n=1). These events were judged by the parent or family physician to be consistent with normal events of adolescence.
concentration, whole blood platelet aggregation, liver function enzyme activity, and plasma lipoprotein lipid profiles, although both study groups had lower platelet aggregation and vitamin E values than the laboratory reference group. Plasma vitamin E analysis exhibited a trend toward lower mean concentrations of this antioxidant vitamin in the docosahexaenoic acid–supplemented group at the 3- and 4-year intervals. Although these reductions never reached statistical significance, future supplementation trials, especially those of long duration and/or elevated dosages of LCPUFAs, should consider continued surveillance of vitamin E concentrations.

The docosahexaenoic acid–supplemented cohort maintained mean plasma docosahexaenoic acid levels of 3.91% of total fatty acids during the 4 years of supplementation. This amount of plasma docosahexaenoic acid is well below physiological plasma levels of populations consuming large amounts of fish, particularly cold-water fish (salmon, tuna, mackerel, and sardines). For example, Alaskan Eskimos who consume food enriched in ω3 PUFAs had plasma docosahexaenoic acid levels of 5.22%. Clinical trials of healthy adults supplemented with relatively high dosages of docosahexaenoic acid (DHASCO oil) have not been associated with adverse safety concerns. Innis and Hansen used a dosage of 7.5 g/d of DHASCO (2.9 g of docosahexaenoic acid) for 14 days to increase docosahexaenoic acid levels greater than 2.5-fold in plasma phospholipids (from 2.1% to 6.1%). Biosafety assays, including liver enzymes, lipoprotein cholesterol, and bleeding time tests, did not identify adverse effects associated with this dosage. A study of blood clotting variables and platelet aggregation in men supplemented with 15 g/d of DHASCO (6 g/d of docosahexaenoic acid; 76 mg/kg of body weight of docosahexaenoic acid per day) for 90 days found no identifiable changes in these properties.

The total amount of LCPUFA and the ratios of the major ω6 and ω3 LCPUFAs in the blood are key indexes in assessing fatty acid status. Patients with XLRP receiving docosahexaenoic acid exhibited a corresponding increase in total ω3 series LCPUFAs and a reduction in the ω6 series LCPUFAs. Mean plasma levels of ARA, a major ω6 pathway intermediate, were reduced by 20% in supplemented patients compared with the placebo group. Although ARA is an essential substrate for prostanoid production and integral in the regulation of immune responses and inflammation, reductions in this pathway are generally associated with benefits to biological systems. Whereas ω6-derived eicosanoids are involved in promoting inflammatory and allergic responses, ω3-derived eicosanoids are antagonistic because of less potent metabolic properties. Therefore, increasing the amount of available ω3 fatty acids not only results in the competitive inhibition of ω6 fatty acid metabolism but also reduces the ω6 substrate available for prostanoid production. Metabolic properties associated with fatty acids and eicosanoids from both pathways are essential for maintenance of health; therefore, adopting a balanced intake of ω3 and ω6 fatty acids seems desirable. However, because Western diets typically have an abundance of ω6 series fats, increased dietary intake of ω3 series fats (eg, cold-water fish) or nutritional supplementation with ω3 series fatty acids (eg, α-linolenic acid, EPA, and docosahexaenoic acid) may be needed to attain a balance of these fats.

The mean total ω6 PUFA/ω3 PUFA blood lipid balance achieved in the docosahexaenoic acid–supplemented group in this trial was 8.1, compared with 13:1 in the placebo group and 12:1 in the age- and sex-matched controls. The consumption of PUFAs has been persistently indicated as beneficial to reducing the risk of CHD. Although a specific ratio of ω6/ω3 has not been established to obtain this benefit, a low ratio is desirable. The Japan Society for Lipid Nutrition recommended a dietary ω6/ω3 ratio of 2:1 or less for prevention of chronic disease in an elderly population (ie, those with CHD and cerebrovascular disease). Furthermore, maintenance of a balanced ω6/ω3 intake of 2:1 or 1:1 is considered a target goal in the infant nutrition field. The dietary ratio of ω6/ω3 consumed in the United States is 10.6:1; while this reflects a reduction from previous estimates (12.4:1), the ratio is still much higher than desirable. Alaskan Eskimos, whose diet is rich in fish and marine mammals, have a plasma ω6 PUFA/ω3 PUFA ratio

Figure 4. Mean±SD year 4 values for plasma lipoprotein total cholesterol (TC) (A), triglycerides (TGs) (B), high-density lipoprotein cholesterol (HDL-C) (C), and low-density lipoprotein cholesterol (LDL-C) (D) in the placebo group (n=21) and the docosahexaenoic acid–supplemented group (n=23). Brackets represent the mean±95% confidence interval for the age- and sex-matched control subjects (n=20) (TC, 163±15 mg/dL; TGs, 95±25 mg/dL; HDL-C, 51±5 mg/dL; and LDL-C, 94±14 mg/dL). There were no significant differences between the placebo and the docosahexaenoic acid–supplemented groups (P=.70). To convert HDL-C, LDL-C, and TG to micromoles per liter, multiply by 0.02586; and to convert TGs to micromoles per liter, multiply by 0.01129.
Dietary supplementation study of young rats fed high sanoids associated with inflammatory responses. In a tive role in the retina by suppressing ARA-derived eico-
rat, and proposed that this release may serve a protec-
strated that light elicits the in vitro release of docosa-
hexaenoic acid in their rod outer segments and were
more susceptible to light-induced retinal damage com-
pared with animals raised on ω3 fatty acid–deficient chow. Subsequently, Anderson et al20 hypothesized that ani-
imals and humans undergo an adaptive response to meta-
bolic oxidative stress to protect the retina from further damage. Such actions include up-regulating antioxidant defense mechanisms and metabolically minimizing the tissue’s susceptibility to lipid peroxidation by redu-
cing retinal docosahexaenoic acid levels.45

It remains to be determined whether docosahexae-
noic acid supplementation in humans leads to lipid per-
oxidation and subsequent membrane damage or if docosahexaenoic acid confers a protective effect.46 Docosahexaenoic acid has been shown to specifically delay the onset and slow progression of apoptotic photoreceptor cell death in vitro.47 Reinboth et al48 demonstrated that light elicits the in vitro release of docosa-
hexaenoic acid from photoreceptor phospholipids in rats, and proposed that this release may serve a protec-
tive role in the retina by suppressing ARA-derived eico-
sanoids associated with inflammatory responses. In a dietary supplementation study20 of young rats fed high levels of docosahexaenoic acid (ethyl ester; 9.69% of total calories), there was no increased tendency for in vivo hydroperoxide formation in the rod outer segment mem-
branes of supplemented animals. These and other investiga-
tions20,30 suggest that susceptibility to light damage of retinal tissues is multifactorial, and may be related more to antioxidant potential than LCPUFA status.

The efficacy of this long-term supplementation trial with docosahexaenoic acid, 400 mg/d, to retard disease progression was inconclusive as measured by cone ERG functional loss.14 Nevertheless, patients who attained the highest docosahexaenoic acid dosage (10-23 mg/kg of body weight per day) demonstrated reduced disease progression. The dose used in this trial was comparable to that received by breastfed infants (12-48 mg of docosahexaenoic acid per kilogram of body weight per day; calculated from milk compositional data23). Infants receiving 23 mg of docosahexaenoic acid per kilogram of body weight per day from docosahexaenoic acid and ARA–enriched formula had no adverse events and had a vi-
sual function equivalent to that of breastfed infants.32-34

Dietary recommendations for docosahexaenoic acid (specifically EPA plus docosahexaenoic acid) intake for healthy adults have been set at a minimum of 650 mg/d by the International Society for the Study of Fatty Acids and Lip-
ids.35 A maximum tolerated dose of EPA plus docosa-
hexaenoic acid of 300 mg/kg of body weight per day has been determined for humans.36 Recently, combined doses of 875 mg/d of EPA plus docosahexaenoic acid were beneficial in reducing myocardial infarctions, stroke, and mor-
tality in adult patients with cardiovascular disease,37 and dosages of 40 mg of EPA plus docosahexaenoic acid per kilogram of body weight per day have been reported to result in improvements in patients with rheumatoid arthritis.56

The results of this randomized, placebo-controlled, clinical trial demonstrate that long-term supplementation with docosahexaenoic acid, 400 mg/d, increased blood docosahexaenoic acid levels by 2.5-fold in patients with XLRP and was not associated with any identifiable safety risks. Monitoring biosafety, particularly plasma vitamin E levels, at increased docosahexaenoic acid dosages and/or for a longer duration would be prudent.

Submitted for publication July 2, 2002; final revision re-
ceived April 3, 2003; accepted April 17, 2003.

This study was supported by grant FD-R-001232 from the Orphan Products Development Program of the US Food and Drug Administration; grant EY05235 from the Na-
tional Institutes of Health, Bethesda, Md; and the Founda-
tion Fighting Blindness. The study capsules were donated by Martek Biosciences Corporation.

We thank the patients and families; the retinal special-
ists affiliated with the Texas Retina Associates, Dallas (Rand Spencer, MD, and Gary Fish, MD) and the Foundation Fighting Blindness (Gerald Fishman, MD, Chicago, Ill; John Heckenlively, MD, Los Angeles, Calif; Samuel Jacob-
son, MD, PhD, Philadelphia, Pa; Richard Lewis, MD, Houston, Tex; Paul Sieving, MD, PhD, Ann Arbor, Mich; and Richard Weleber, MD, Portland, Ore) for referring pa-
tients for participation in this study; Argye Hills, PhD, for statistical advice during the trial; Katherine Franke, MS, Maia Lapus, BS, and Scott Urban, BS, for their technical assistance in conducting the biochemistry safety assays; and members of the Data and Safety Monitoring Committee (Gerald Fishman, MD [chair]; Norman Salem, Jr, PhD; Joh-
anna Seddon, MD; and Barbara Philippon, RN, MS) for their time and effort invested throughout the conduct of this study.

Corresponding author and reprints: Dianna H. Wheaton, MS, retina Foundation of the Southwest, 9900 N Central Expressway, Dallas, TX 75231 (e-mail: dwheaton @retinafoundation.org).

REFERENCES

3. Hoffman DR, Birch DG. Docosahexaenoic acid in red blood cells of patients with