Plasma Docosahexaenoic Acid Levels in Various Genetic Forms of Retinitis Pigmentosa

Junxian Gong,* Bernard Rosner,† Deirdre G. Rees,* Eliot L. Berson,† Carol A. Weigel-DiFranco,† and Ernst J. Schaefer*

In 188 patients from separate families with various forms of retinitis pigmentosa (RP) and 91 normal subjects, plasma fatty acids were measured as a percentage of total plasma fatty acids, and their concentrations were determined using capillary-column gas-liquid chromatography. After controlling for the effects of age and gender, those with RP had significantly lower (P < 0.01) mean plasma percentages and concentrations of the omega-3 fatty acids: 18:3ω-3 (alpha-linolenic acid), 22:3ω-3 (13,16,19 docosatriaenoic acid), and 22:6ω-3 (docosahexaenoic acid, DHA) compared with the group of normal subjects. The mean percentages were reduced 15%, 14%, and 10%, respectively, below the mean percentages in normal subjects. Analysis by genetic type revealed that the X-linked and isolate forms of RP had significantly lower (P < 0.01) mean percentage values for DHA (18% and 17%, respectively). Dominant and recessive forms of RP had DHA levels close to normal. Mean absolute plasma DHA concentrations in X-linked RP were not significantly different from the concentrations in the control subjects, although these levels were significantly lower in patients with isolate RP. These data identify the possibility that some forms of RP may have alterations in plasma omega-3 fatty acid metabolism resulting in decreased plasma DHA content. These observations await additional confirmation using an analysis of the fatty acid content of specific erythrocyte phospholipid classes. Invest Ophthalmol Vis Sci 33:2596-2602, 1992

Several investigators have observed abnormalities of plasma docosahexaenoic acid (DHA) in various common forms of retinitis pigmentosa (RP). In one study,1 the DHA (22:6ω-3) content of plasma phospholipids was 36% lower (P < 0.05) in affected patients in three kindreds with Usher's syndrome, an inherited disorder involving congenital deafness and retinitis pigmentosa, compared with that in control subjects. Others2 found decreased plasma DHA content in autosomal dominant RP and isolate (simplex) RP. In other studies,3-5 affected patients in four of five X-linked kindreds had a reduced plasma content of DHA compared with their unaffected relatives.1 These reductions were significantly different (P < 0.05) from control levels in two of the four families.1-4 Hyperlipidemia also was seen in some of these kindreds.1-5 It was suggested that RP is associated with abnormal plasma lipoproteins6 and an increased frequency of the rare apoE isoform of apolipoprotein E2.7 However, apoE is not essential for normal fatty acid metabolism. In its absence, plasma fatty acid levels are normal, and no RP is found.8,9 In another study, the DHA (22:6ω-3) content of plasma phospholipids was elevated in 26 affected relatives in a large family with RP from the same geographic area.10 Fatty acid abnormalities recently were discovered in a strain of miniature poodles with progressive rod-cone degeneration; in affected dogs, the plasma DHA levels were significantly reduced (P < 0.005).11,12 These studies suggested a genetic defect in the delta-4 desaturase step that converts 22:5ω-3 to 22:6ω-3 and 22:4ω-6 to 22:5ω-6 in poodles.

Rod outer segment membranes contain exceptionally large amounts of polyunsaturated fatty acids, especially DHA, which comprises almost one half of the esterified fatty acid in outer segment phospholipids.13,14 Outer segment membranes are also unusual because of their high phospholipid content (80-90 mol% of total lipid) and low cholesterol content (8-9 mol% of total lipid). Moreover, the fatty acid content of the phospholipids is exceptional because of their high degree of polyunsaturation. These factors...
combine to produce membranes of high fluidity. The proportion of DHA in phosphatidylethanolamine and phosphatidylserine in the membranes of outer segments of photoreceptor cells is typically 45–60% higher than that in any other tissue. It comprises 75–100% of the fatty acids in the 2 position of these phospholipids, the position usually occupied by polyunsaturated fatty acids. In contrast, the plasma fatty acid content of DHA is only 2–3% of the total plasma fatty acids. In addition, 5–25% of retinal phospholipid and phosphatidylserine in the membranes of outer segments of photoreceptor cells contain DHA in the 1 position, which is unusual because this position usually is occupied by a saturated fatty acid. The significance of the special lipid composition of the rod photoreceptor outer segment is not understood, but it is thought to be necessary for the normal functioning of rhodopsin in the initial stage of phototransduction. Activation by a photon of light causes a rapid change in the conformation of rhodopsin, which undergoes lateral and rotational movement in the rod outer segment membranes. The high degree of fluidity and flexibility of disc membranes appears to be essential for this dynamic behavior of rhodopsin.

Although DHA turnover in the retina must be rapid, prolonged dietary deficiency of this fatty acid and its omega-3 precursors is necessary to decrease its retinal levels. It appears to be retained by the retina even when animals are fed diets that are deficient in omega-3 fatty acids. The mechanism of conservation is not clear. It may be recycled in the photoreceptors before the tips are shed and phagocytized, DHA may be recycled directly to the pigment epithelium by the phagosomes, or it may be released by the phagosomes into the bloodstream and recycled through the liver and plasma.

Current evidence indicates that alpha linolenic acid (18:3ω3), its major dietary precursor, can be converted to DHA in the liver by elongation and desaturation. Little is known about the biologic pathways in DHA (22:6ω3) synthesis and transport. In normal dogs, their retinas have a limited capacity for elongation and desaturation of 18:3ω3 and 22:6ω3. Through the bloodstream, DHA is distributed from the liver to other tissues. Major food sources are fish and some plant oils. The vertebrate retina may depend on blood lipids and/or other carriers to supply essential fatty acids for normal outer-segment phospholipid synthesis. Specific carriers may be involved in transporting DHA (1) from the liver to the eye and (2) across the pigment epithelium and interphotoreceptor matrix to supply the photoreceptor cells.

If DHA is important for normal retinal function in terms of maintaining outer segment membrane fluidity, then deficiencies of this fatty acid or abnormalities in its metabolism could lead to alterations in membrane function (and possibly to RP). This possibility and the reported abnormalities found in plasma DHA in some patients with RP prompted us to investigate the plasma levels of additional patients with different genetic types of this condition.

### Materials and Methods

#### Sample Selection

We evaluated the plasma fatty acid levels in 188 patients (age range, 18–49 yr), one per family, with common forms of RP. The diagnosis in each patient was based on findings on ocular examinations, including electroretinography done at the Berman-Gund Laboratory. These patients were classified genetically after their family histories were reviewed as autosomal dominant, autosomal recessive, X-linked, or isolate, using previously described criteria. Plasma fatty acid levels also were evaluated in 91 normal control subjects (age range, 18–49 yr), none of whom had any family history of RP. In patients and control subjects, blood (20 ml) was drawn after they had fasted overnight (12–14-hr). The sample was placed in 0.1% ethylenediaminetetraacetic acid-containing tubes, and the plasma was separated from the erythrocytes by centrifugation at 2500 rpm and 4°C. The plasma was stored immediately at −70°C under nitrogen gas before analysis.

#### Lipid and Fatty Acid Analysis

All analyses were done in a masked fashion. Only numbered samples were provided to the Lipid Metabolism Laboratory at Tufts University. A modification of Lepage’s method was used for lipid extraction and transesterification of the fatty acids. Plasma (100 μl) and 40 μg of an internal standard (margaric acid, 17:0) were mixed in 13 100-mm glass tubes. We added 2 ml of a 4:1 (v/v) methanol–benzene solution and 200 μl of acetyl chloride with slow stirring. Nitrogen gas was added to each tube before they were sealed with Teflon tape, tightly closed with Teflon-lined caps (Fisher Scientific, Springfield, NJ), and then methanolized at 100°C for 1 hr in a Reacti-Therm (Pierce Chemical Co., Rockford, IL) heating-stirring dry block. Then the tubes were cooled in water, and 1 ml of isooctane was added to dilute the solution. We added 5 ml of 6% potassium carbonate to stop the reaction and neutralize the mixture. The tubes were centrifuged at 1500 × g for 15 min. Subsequently, the clear upper phase that contained the fatty acid methyl esters (FAME) was transferred to a 1-ml autosampler vial. After being overlaid with anhydrous sodium sulfate (1 mm layer), the tubes were filled with nitrogen, and the vials were sealed with crimp caps. They were ready for injection into the measuring device. An au-
troming for age and sex using the Statistical Analysis System General Linear Model procedure.

**Statistical Methods**

Analysis of covariance was used to compare (1) plasma fatty acid mole percentages in all patients with RP patients versus those in the group of normal subjects and (2) each group of patients by genetic type versus the group of normal subjects, after controlling for age and gender. We followed the SAS General Linear Model procedure (Statistical Analysis System Institute, Cary, NC). Fatty acid levels did not follow a normal distribution. To normalize the distribution, the values were transformed using the natural logarithm log10 (x + 1) transformation in the analyses of covariance.28,29

In the first analysis, a class variable with two categories was used to distinguish affected patients from normal subjects. In the second analysis, differences among each of the four genetic types versus those in normal subjects were assessed using a class variable with five categories. First, an F test was done to assess overall group differences after controlling for age and gender. Differences in the mean fatty acid percentages between pairs of groups were analyzed using the least-significant difference approach. Contingency-table methods were used to compare the percentage of patients with RP of specific genetic types who were below the tenth percentile in their amount of plasma DHA. Fatty acid concentration data were reported in moles percent (ie, milligrams per deciliter).

**Results**

The mean levels of fatty acids as a percentage (normalized mass percent) of total fatty acids in patients with RP and in normal control subjects are shown in Table 1. These fatty acid values reflect approximately 90% of all free and bound fatty acids in the plasma, including those bound to albumin and those in triglycerides, phospholipids, and cholesterol esters. Patients with RP had significantly lower (P < 0.01) percentages of alpha linolenic acid (18:3ω3); 13,16,19 docosatrienoic.

**Table 1. Plasma fatty acid normalized mass percentage in retinitis pigmentosa patients and normal controls**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Affected (n = 188)</th>
<th>Normal (n = 91)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 (palmitic)</td>
<td>21.81 ± 0.15</td>
<td>21.48 ± 0.22</td>
<td>NS</td>
</tr>
<tr>
<td>18:0 (stearic)</td>
<td>10.19 ± 0.23</td>
<td>9.93 ± 0.27</td>
<td>NS</td>
</tr>
<tr>
<td>18:1ω9 (oleic)</td>
<td>18.17 ± 0.22</td>
<td>17.24 ± 0.26</td>
<td>NS</td>
</tr>
<tr>
<td>18:2ω6 (linoleic)</td>
<td>27.53 ± 0.35</td>
<td>28.15 ± 0.44</td>
<td>NS</td>
</tr>
<tr>
<td>18:3ω3 (alpha linolenic—GLA)</td>
<td>0.93 ± 0.05</td>
<td>1.27 ± 0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18:3ω6 (gamma linolenic—DGLA)</td>
<td>0.30 ± 0.01</td>
<td>0.32 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>20:4ω6 (arachidonic)</td>
<td>1.48 ± 0.03</td>
<td>1.41 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>20:5ω3 (5,8,11,14,17 eicosapentaenoic—EPA)</td>
<td>6.85 ± 0.10</td>
<td>6.35 ± 0.13</td>
<td>NS</td>
</tr>
<tr>
<td>22:3ω3 (13,16,19 docosatrienoic)</td>
<td>0.44 ± 0.03</td>
<td>0.49 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>22:4ω6 (7,10,13,16,19 docosapentaenoic)</td>
<td>0.32 ± 0.02</td>
<td>0.36 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>22:6ω3 (4,7,10,11,16,19 docosahexaenoic—DHA)</td>
<td>1.10 ± 0.05</td>
<td>1.34 ± 0.06</td>
<td>0.005</td>
</tr>
<tr>
<td>20:3ω3/22:6ω3†</td>
<td>0.46 ± 0.03</td>
<td>0.40 ± 0.03</td>
<td>NS</td>
</tr>
</tbody>
</table>

*P values for differences between affected and normal subjects after controlling for age and sex using the Statistical Analysis System General Linear Model procedure.

† Molar ratio that allows for quantification of the relative amounts of polyunsaturated fats in the plasma. (n = 185 for 20:5ω3/22:6ω3 for affected patients.)

NS, not significant.
saturated (22:5ω3) and DHA (22:6ω3). These omega-3 fatty acids were, respectively, 15%, 14%, and 10% lower than the mean normal values after controlling for age and gender. All patients and control subjects had plasma cholesterol values over 100 mg/dl, excluding the diagnoses of abetalipoproteinemia and homozygous hypobetalipoproteinemia.²⁰,³¹ Phytic acid was not seen in any sample, excluding the diagnosis of Refsum's disease.

The mean levels of plasma fatty acids as percentages of total fatty acids by genetic type of RP (after controlling for age and gender) are shown in Table 2. For 52 patients with autosomal dominant and 48 patients with autosomal recessive RP, no significant differences from normal were found with respect to mean DHA percentages. The patients with dominant RP had significantly lower mean percentages of alpha linolenic acid (18:3ω3; P < 0.05) and 13,16,19 docosatrienoic acid (22:3ω3; P < 0.01). These fatty acids were decreased 13% and 15%, respectively, below the mean normal levels. The patients with recessive RP had a significantly lower mean percentage of plasma 13,16,19 docosatrienoic acid (22:3ω3; P < 0.01). Their mean percentage of plasma arachidonic acid (20:4ω6) was also slightly higher than normal (P < 0.05).

By contrast, 45 patients with X-linked RP (Table 2) had mean percentages of 13,16,19 docosatrienoic acid (22:3ω3) and DHA (22:6ω3) that were significantly lower than normal (P < 0.01 and P < 0.05, respectively). These omega-3 fatty acids were 17% and 18% below the mean normal percentages, respectively. These patients also had a 12% higher mean percentage of oleic acid (18:1ω9; P < 0.01) than the control group (Table 2). Furthermore, the ratio of 5,8,11,14 eicosapentaenoic acid (EPA) to DHA (20:5ω3/22:6ω3) was 0.59 in the X-linked patients versus 0.40 in the control subjects (P < 0.05). In this group of patients with X-linked RP, the percentage of those with DHA values below the tenth percentile of normal was 13.3% (P not significant).

Forty-three patients with isolate RP (Table 2) also had significantly lower (P < 0.01) mean percentages of DHA (22:6ω3). Their mean percentage was 17% below the mean normal level. These patients also had significantly lower (P < 0.01) mean percentages of linolenic acid (18:3ω3), EPA (20:5ω3), and 13,16,19 docosatrienoic acid (22:3ω3). Their mean percentages were reduced 27%, 10%, and 19%, respectively, below the mean normal levels. In this group of patients with isolate RP, the percentage of those with DHA values below the tenth percentile of normal was 32.6% (P < 0.05).

Data on each plasma fatty acid's absolute concentration, corrected for lack of complete recovery with an internal standard, in patients with RP and control subjects are shown in Table 3. In this analysis, patients with RP had significantly lower (P < 0.013) concentrations of plasma alpha linolenic acid, docosatrienoic acid, and docosahexaenoic acid than control subjects. Data on each plasma fatty acid's absolute concentrations in patients with specific genetic subtypes of RP and control subjects are listed in Table 4. In the autosomal dominant type of RP, alpha linolenic acid concentrations were significantly lower than normal (P < 0.05). In recessive RP, no differ-

### Table 2. Plasma fatty acid normalized mass percentage by genetic type of retinitis pigmentosa

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Dominant (n = 52)</th>
<th>Recessive (n = 48)</th>
<th>X-linked (n = 45)</th>
<th>Isolate (n = 43)</th>
<th>Normal (n = 91)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x ± SE</td>
<td>x ± SE</td>
<td>x ± SE</td>
<td>x ± SE</td>
<td>x ± SE</td>
</tr>
<tr>
<td>16:0 (palmitic)</td>
<td>21.45 ± 0.33</td>
<td>21.79 ± 0.28</td>
<td>22.08 ± 0.27</td>
<td>21.96 ± 0.32</td>
<td>21.48 ± 0.22</td>
</tr>
<tr>
<td>18:0 (stearic)</td>
<td>9.79 ± 0.38</td>
<td>9.59 ± 0.37</td>
<td>9.12 ± 0.41</td>
<td>12.46 ± 0.58</td>
<td>9.93 ± 0.27</td>
</tr>
<tr>
<td>18:1ω9 (oleic)</td>
<td>17.84 ± 0.46</td>
<td>17.47 ± 0.36</td>
<td>19.85 ± 0.42</td>
<td>17.59 ± 0.41</td>
<td>17.24 ± 0.26</td>
</tr>
<tr>
<td>18:2ω6 (linoleic)</td>
<td>27.67 ± 0.72</td>
<td>28.66 ± 0.70</td>
<td>26.42 ± 0.64</td>
<td>27.27 ± 0.74</td>
<td>28.15 ± 0.44</td>
</tr>
<tr>
<td>18:3ω3 (alpha linolenic)</td>
<td>0.94 ± 0.08</td>
<td>1.00 ± 0.09</td>
<td>1.14 ± 0.10</td>
<td>0.60 ± 0.07</td>
<td>1.27 ± 0.08</td>
</tr>
<tr>
<td>18:3ω6 (gamma linolenic—GLA)</td>
<td>0.25 ± 0.03</td>
<td>0.29 ± 0.03</td>
<td>0.31 ± 0.02</td>
<td>0.30 ± 0.02</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>20:3ω6 (dihomogamma linolenic—DGLA)</td>
<td>1.46 ± 0.06</td>
<td>1.47 ± 0.06</td>
<td>1.48 ± 0.05</td>
<td>1.52 ± 0.06</td>
<td>1.41 ± 0.04</td>
</tr>
<tr>
<td>20:4ω6 (arachidonic acid)</td>
<td>6.77 ± 0.20</td>
<td>6.78 ± 0.17</td>
<td>6.02 ± 0.19</td>
<td>6.71 ± 0.18</td>
<td>6.35 ± 0.13</td>
</tr>
<tr>
<td>20:5ω3 (5,8,11,14,17 eicosapentaenoic—EPA)</td>
<td>0.43 ± 0.07</td>
<td>0.47 ± 0.03</td>
<td>0.52 ± 0.08</td>
<td>0.35 ± 0.04</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td>22:3ω3 (13,16,19 docosatrienoic)</td>
<td>0.31 ± 0.03</td>
<td>0.28 ± 0.04</td>
<td>0.25 ± 0.04</td>
<td>0.14 ± 0.02</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>22:5ω3 (7,10,13,16 docosapentaenoic)</td>
<td>0.30 ± 0.02</td>
<td>0.35 ± 0.03</td>
<td>0.34 ± 0.02</td>
<td>0.30 ± 0.05</td>
<td>0.36 ± 0.03</td>
</tr>
<tr>
<td>22:6ω3 (4,7,10,11,16,19 docosahexaenoic—DHA)</td>
<td>1.27 ± 0.12</td>
<td>1.13 ± 0.06</td>
<td>0.98 ± 0.09</td>
<td>1.01 ± 0.10</td>
<td>1.34 ± 0.06</td>
</tr>
<tr>
<td>20:5ω3/22:6ω3 δ</td>
<td>0.35 ± 0.03</td>
<td>0.44 ± 0.03</td>
<td>0.59 ± 0.06</td>
<td>0.49 ± 0.12</td>
<td>0.40 ± 0.03</td>
</tr>
</tbody>
</table>

* F statistic for the Type III sum of squares comparing overall differences among genetic types after controlling for age and sex using the SAS GLM procedure.
† P < 0.01 for different from normal.
‡ P < 0.05 for different from normal.
§ Molar ratio that allows for quantification of the relative amounts of polyunsaturated fats in the plasma. (n = 51 for dominants and n = 41 for isolates for 20:5ω3/22:6ω3). NS, not significant.
Table 3. Plasma fatty acid concentrations in retinitis pigmentosa and normal controls

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Affected (n = 188)</th>
<th>Normal (n = 91)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 (palmitic)</td>
<td>81.87 ± 2.04</td>
<td>76.38 ± 2.91</td>
<td>NS</td>
</tr>
<tr>
<td>18:0 (stearic)</td>
<td>37.66 ± 1.04</td>
<td>34.97 ± 1.37</td>
<td>NS</td>
</tr>
<tr>
<td>18:1ω9 (oleic)</td>
<td>68.91 ± 2.03</td>
<td>62.16 ± 2.95</td>
<td>NS</td>
</tr>
<tr>
<td>18:2ω6 (linoleic)</td>
<td>100.26 ± 1.66</td>
<td>97.20 ± 2.24</td>
<td>NS</td>
</tr>
<tr>
<td>18:3ω3 (alpha linolenic)</td>
<td>3.52 ± 0.20</td>
<td>4.50 ± 0.33</td>
<td>0.010</td>
</tr>
<tr>
<td>18:3ω6 (gamma linolenic—GLA)</td>
<td>1.13 ± 0.05</td>
<td>1.11 ± 0.09</td>
<td>NS</td>
</tr>
<tr>
<td>20:3ω6 (arachidonic)</td>
<td>23.98 ± 0.44</td>
<td>21.69 ± 0.50</td>
<td>0.032</td>
</tr>
<tr>
<td>20:4ω6 (5,8,11,14,17 eicosapentaenoic—EPA)</td>
<td>1.65 ± 0.12</td>
<td>1.71 ± 0.13</td>
<td>NS</td>
</tr>
<tr>
<td>22:3ω3 (13,16,19 docosatrienoic)</td>
<td>0.80 ± 0.06</td>
<td>1.56 ± 0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>22:3ω6 (7,10,13,16,19 docosapentaenoic)</td>
<td>1.19 ± 0.06</td>
<td>1.21 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>22:5ω3 (4,7,10,11,16,19 docosahexaenoic—DHA)</td>
<td>4.01 ± 0.17</td>
<td>4.55 ± 0.22</td>
<td>0.013</td>
</tr>
<tr>
<td>20:5ω3/22:6ω3</td>
<td>0.46 ± 0.03</td>
<td>0.40 ± 0.03</td>
<td>NS</td>
</tr>
</tbody>
</table>

* P values for differences between affected and normal subjects after controlling for age and sex using the SAS GLM procedure.
† Molar ratio that allows for quantification of the relative amounts of polyunsaturated fats in the plasma. (n = 185 for 20:5ω3/22:6ω3 for affected patients.)
NS, not significant.

Discussion

Our study showed that mean plasma DHA levels (as percentages of total plasma fatty acids in plasma) were reduced below the mean normal levels in patients with X-linked and isolate forms of RP. Although the reductions were statistically significant, the actual levels were reduced only 17–18% below normal. Because DHA normally comprises approximately 3% of the total plasma fatty acids, it is unknown whether these observed differences in the X-linked and isolate forms of RP have any clinical significance. Most patients with isolate RP are thought to have significantly lower levels of DHA compared to normal controls.

Table 4. Plasma fatty acid concentration by genetic type of retinitis pigmentosa

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Dominant (n = 52)</th>
<th>Reccessive (n = 48)</th>
<th>X-linked (n = 45)</th>
<th>Isolate (n = 43)</th>
<th>Normal (n = 91)</th>
<th>F*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 (palmitic)</td>
<td>79.56 ± 3.27</td>
<td>82.11 ± 3.33</td>
<td>90.31 ± 6.15†</td>
<td>75.57 ± 2.64†</td>
<td>76.38 ± 2.91</td>
<td>2.37</td>
<td>0.053</td>
</tr>
<tr>
<td>18:0 (stearic)</td>
<td>35.91 ± 1.77</td>
<td>35.86 ± 1.85</td>
<td>36.08 ± 1.96</td>
<td>43.44 ± 2.62†</td>
<td>34.97 ± 1.37</td>
<td>2.25</td>
<td>NS</td>
</tr>
<tr>
<td>18:1ω9 (oleic)</td>
<td>67.33 ± 3.69</td>
<td>66.15 ± 2.94</td>
<td>82.07 ± 5.96†</td>
<td>60.11 ± 2.01†</td>
<td>62.16 ± 2.95</td>
<td>4.33</td>
<td>0.002</td>
</tr>
<tr>
<td>18:2ω6 (linoleic)</td>
<td>99.44 ± 2.71</td>
<td>105.22 ± 3.26</td>
<td>103.53 ± 4.28</td>
<td>92.30 ± 2.71†</td>
<td>97.20 ± 2.24</td>
<td>3.44</td>
<td>0.009</td>
</tr>
<tr>
<td>18:3ω3 (alpha linolenic)</td>
<td>3.47 ± 0.36‡</td>
<td>3.81 ± 0.35</td>
<td>4.71 ± 0.53</td>
<td>2.02 ± 0.21†</td>
<td>4.50 ± 0.33</td>
<td>7.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18:3ω6 (gamma linolenic—GLA)</td>
<td>1.08 ± 0.11</td>
<td>1.14 ± 0.12</td>
<td>1.25 ± 0.10</td>
<td>1.04 ± 0.08</td>
<td>1.11 ± 0.09</td>
<td>0.76</td>
<td>NS</td>
</tr>
<tr>
<td>20:3ω6 (dihomo gamma linolenic—DGLA)</td>
<td>5.38 ± 0.29</td>
<td>5.38 ± 0.27</td>
<td>5.83 ± 0.29†</td>
<td>5.19 ± 0.23</td>
<td>4.95 ± 0.19</td>
<td>1.80</td>
<td>NS</td>
</tr>
<tr>
<td>20:4ω6 (arachidonic acid)</td>
<td>24.53 ± 0.94</td>
<td>25.32 ± 0.98‡</td>
<td>23.19 ± 0.84</td>
<td>22.63 ± 0.60</td>
<td>21.69 ± 0.50</td>
<td>2.02</td>
<td>NS</td>
</tr>
<tr>
<td>20:5ω3 (5,8,11,14,17 eicosapentaenoic—EPA)</td>
<td>1.57 ± 0.26</td>
<td>1.70 ± 0.13</td>
<td>2.11 ± 0.34</td>
<td>1.17 ± 0.14†</td>
<td>1.71 ± 0.13</td>
<td>5.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>22:3ω3 (13,16,19 docosatrienoic)</td>
<td>0.74 ± 0.11†</td>
<td>1.08 ± 0.15</td>
<td>0.88 ± 0.13†</td>
<td>0.48 ± 0.08†</td>
<td>1.56 ± 0.16</td>
<td>7.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>22:3ω6 (7,10,13,16,19 docosapentaenoic)</td>
<td>1.12 ± 0.08</td>
<td>1.27 ± 0.11</td>
<td>1.36 ± 0.11</td>
<td>1.02 ± 0.16†</td>
<td>1.21 ± 0.08</td>
<td>2.43</td>
<td>0.048</td>
</tr>
<tr>
<td>22:6ω3 (4,7,10,11,16,19 docosahexaenoic—DHA)</td>
<td>4.48 ± 0.39</td>
<td>4.19 ± 0.23</td>
<td>3.82 ± 0.37</td>
<td>3.43 ± 0.35†</td>
<td>4.55 ± 0.22</td>
<td>4.07</td>
<td>0.002</td>
</tr>
<tr>
<td>20:5ω3/22:6ω3</td>
<td>0.35 ± 0.03</td>
<td>0.44 ± 0.03</td>
<td>0.39 ± 0.06†</td>
<td>0.48 ± 0.11</td>
<td>0.40 ± 0.03</td>
<td>2.96</td>
<td>0.020</td>
</tr>
</tbody>
</table>

* F statistic for the Type III sum of squares comparing overall differences among genetic types after controlling for age and sex using the SAS GLM procedure.
† P < 0.01 for different from normal.
‡ P < 0.05 for different from normal.
inherit their condition by an autosomal recessive mode, and it is not clear why the patients with autosomal recessive RP do not show this deficiency. The finding of normal levels of DHA in our group of patients with dominant RP contrasts with previous reports of reduced levels in one group and elevated levels in another group, both with dominant RP. There is no evidence in humans that photoreceptor outer segment lipids are altered significantly in the those with modest changes in plasma fatty acid levels.

Studies in rhesus monkeys have documented that a low dietary intake of omega-3 fatty acids during prenatal and postnatal development leads to reductions of 80–90% in the DHA content of plasma and tissues and compensatory increases in long-chain omega-6 fatty acids. Such increases were not observed in the plasma in our patients with RP. The visual acuity of omega-3 fatty acid-deficient infant monkeys was subnormal. These data suggest that DHA depletion leads to defects in the structure and functioning of central retinal cones. In the common forms of human RP, rod function is affected early, and central cone function is relatively preserved. Similarly, the minia-
ture poodles with rod–cone degeneration and decreases in plasma DHA levels had central degeneration at an early stage; this differed from the findings in common forms of human RP.

In our studies of patients with X-linked RP, there was no deficiency in any of the detectable precursors of DHA, namely 18:3ω3, 20:5ω3, or 22:5ω3, suggesting that some patients with this form of RP may have a block in the pathways for converting 22:5ω3 to 22:6ω3. These pathways recently were studied extensively. The possibility of a block was supported by a significantly higher ratio of 20:5ω3 to 22:6ω3 in this group than in normal subjects. By contrast, in isolate RP, all omega-3 fatty acids were decreased, including the precursor 18:3ω3. This pattern raised the possibility of either a dietary deficiency of 18:3ω3 or possibly enhanced utilization of all of the omega-3 fatty acids by the body in isolate RP.

It is unlikely that any of the genetic types of RP studied had a significant abnormality in fat absorption. One of the most sensitive indicators of fat malabsorption and dietary essential fatty acid deficiency is a decreased plasma percentage of linoleic acid (18:2ω6). In all types of RP, normal percentages of this major essential fatty acid (approximately 27% of total fatty acids) were found. Similarly, it is unlikely that these patients with RP consumed a substantially different diet with regard to saturated fatty acid intake than do normal subjects. The major saturated fatty acid in human plasma, palmitic acid (16:0), which comprises approximately 22–23% of plasma fatty acids, was virtually identical in all types of RP compared with that in the group of normal subjects.

It is known that the major essential fatty acids (linoleic and alpha linolenic acid) are converted to derivative fatty acids. An interesting feature of our data was that, in all categories of patients with RP, there was a consistent decrease in the percent of plasma fatty acids as 22:3ω3, a minor fatty acid (Table 2). This fatty acid is produced as a result of a minor fatty acid elongation pathway, whereby alpha linolenic acid (18:3ω3) is elongated directly to 11,14,17 eicosatrienoic acid (20:3ω3) and then to 13,16,19 docosatrienoic acid (22:3ω3) instead of being converted to EPA and DHA (the major pathway). Therefore, in some patients with RP, there may be excess conversion of 18:3ω3 to these major derivatives (EPA and DHA) instead of the minor derivative, 22:3ω3, perhaps signaling a channeling of precursors to maximize the EPA and DHA available to target tissues. With regard to the major elongation and desaturation product of 18:3ω3, namely 20:5ω3, only in isolate types of RP was there a significant decrease. In all other forms of RP, the percentage of plasma fatty acids as EPA was similar to normal. This was also the case for the next elongation product of 20:5ω3, which is 22:5ω3. This fatty acid was decreased on a percentage basis only in isolate RP but not in the other forms of RP.

Our results differ from an earlier study as they relate to dominant RP, even though both studies were done on outpatient populations with the common forms of RP and no known clinical differences. Moreover, we found no definitive evidence that these patients had a clinically significant deficiency of fatty acids. Patients with essential fatty acid deficiency secondary to intestinal disease with no known retinal degeneration have had percentages of plasma linoleic acid that were approximately one third of those observed in normal subjects. These differences were much greater than those we observed comparing patients with RP and normal subjects.

In addition, we examined plasma fatty acids and not fatty acids in specific lipid classes. Lecithin (or phosphatidylcholine) is the major plasma phospholipid; phosphatidylethanolamine and phosphatidylserine are the major cell membrane phospholipids. Erythrocyte membrane phospholipid fatty acids are similar in composition to those of brain and retina, and they change in parallel, at least in rats, after dietary manipulation. Studies are ongoing to determine if the small plasma fatty acid percentage changes we observed in X-linked RP can be correlated with alterations in fatty acids in erythrocyte membrane phospholipids in these same patients.

Key words: retinitis pigmentosa, docosahexaenoic acid (22:6ω3; 4,7,10,13,16,19-docosahexaenoic acid), fatty acids, retinal degenerations, plasma
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References


