Docosahexaenoic Acid in Red Blood Cells of Patients With X-Linked Retinitis Pigmentosa

Dennis R. Hoffman*† and David G. Birch*‡

Purpose. Abnormalities in the distribution of long-chain polyunsaturated fatty acids (LCPUFA) have been documented in plasma of patients with X-linked retinitis pigmentosa (XLRP). In this study, fatty acid profiles of red blood cells (RBC) were used as an index for LCPUFA metabolism in patients with XLRP because RBC lipids reflect membrane-associated fatty acids. Correlations between LCPUFA content and electroretinographic (ERG) function were assessed.

Methods. Mean ages for the XLRP group (n = 18) and control group (n = 28) were 22 ± 18 years and 24 ± 16 years, respectively. Electroretinographic assessment included the International Society for the Clinical Electrophysiology of Vision standard protocol. Methyl esters of RBC fatty acids were analyzed by capillary column gas chromatography.

Results. The content of the ω3 fatty acid, docosahexaenoic acid (DHA), was 40% lower in the group with XLRP (23.1 ± 5.9 μg/ml RBC [mean ± 1 SD]) than in normal subjects (38.6 ± 9.4 μg/ml RBC, t = 6.24, P < 0.0001). Total ω3 LCPUFA content in patients with XLRP was reduced by 30% from normal levels compared to a 10% reduction in ω6 LCPUFA levels. Elongation reactions for ω3, ω6, saturated fatty acids, and monounsaturated fatty acids were markedly lower for patients with XLRP than for normal subjects. Multiple regression analysis revealed that RBC–DHA was a significant determinant for amplitude and implicit time of cone ERG responses.

Conclusions. The overwhelming majority of patients with XLRP have lower levels of DHA in RBCs compared to normally sighted control subjects. An analysis of fatty acid profiles suggests a metabolic defect in fatty acid chain elongation mechanisms. The significant association between DHA content and cone ERG response parameters is consistent with an effect of lipid abnormalities on membrane environment and physiology in retinal photoreceptors.

Retinitis pigmentosa (RP) is a group of hereditary retinal degenerations characterized by progressive night blindness and loss of peripheral visual field resulting from photoreceptor degeneration. Multiple inheritance patterns exist for RP, including autosomal dominant (ADRP), autosomal recessive, and X-linked (XLRP). Based on the prevalence of visual loss in preadolescence to early adolescence, XLRP is considered the most severe genetic type of RP. In a recent study of the natural history of rod photoreceptor functional loss in patients with RP, patients with XLRP showed a significantly faster rate of progression in rod electroretinographic (ERG) threshold than patients with other modes of inheritance. Recently, we also reported the first longitudinal measures of rod function in children with XLRP. At 5 years of age, average rod ERG thresholds were elevated above normal by approximately 2 log units, and the rate of rod threshold elevation averaged 0.29 log units/year through age 9. These results suggest that rod abnormalities are present at or soon after birth in children with XLRP.

In ADRP, specific mutations in the genes for proteins and enzymes vital to visual transduction presently account for RP in approximately one third of the pa-
Approximately 80 different loci in the gene for rhodospin have been identified as mutation sites; mutations also have been described in the peripherin gene. In some families, a combination of the unlinked alleles for two rod outer segment membrane proteins, peripherin and ROM1, appears to act in a recessive manner, resulting in digenic inheritance of the retinal degeneration. Gene mutations for the rod β-subunit of cGMP phosphodiesterase have been associated with photoreceptor degeneration in autosomal recessive RP. In XLRP, two chromosomal defects have been localized near Xp21 and Xp11.3, although candidate genes have not been identified.

In addition to the identification of gene mutations in specific photoreceptor proteins as a causative factor in RP, a persistent observation in patients is a reduced content in systemic levels of ω3 long-chain polyunsaturated fatty acids (LCPUFA). The evidence for lipid abnormalities associated with retinal degenerations has been accumulating for the past decade. A number of investigators have reported abnormal levels of ω3 fatty acids in plasma lipids of patients with Usher's syndrome and ADRP. Most investigators have reported anomalies in the levels of the end-product of ω3 LCPUFA metabolism, docosahexaenoic acid (DHA). In our studies of patients with ADRP, red blood cell (RBC) levels of ω3 LCPUFAs were reduced significantly compared to that of normal subjects and probed for potential associations with visual function. A preliminary report of this work has been presented. The purpose of the present study was to analyze ω3 fatty acids in RBCs of patients with XLRP and to evaluate possible abnormalities in fatty acid metabolism. The ω3 fatty acid content in the patients was compared to that of normal subjects and probed for potential associations with visual function. A preliminary report of this work has been presented.

METHODS

Subjects

The subject cohort included 18 patients with confirmed X-linked inheritance and 28 normally sighted control subjects. XLRP inheritance was defined as the presence of at least two affected male relatives, an absence of male-to-male transmission, and either the patient's mother or daughter expressing characteristics consistent with the XLRP carrier state. Carriers were characterized by a slightly reduced ERG amplitude and a slight delay in implicit time.

The patients with XLRP ranged in age from 5 to 67 years, with a mean age (±1 SD) of 22 ± 18 years. Patients were from 13 different families; two relatives from five of these families participated in the study. Normally sighted control subjects ranged in age from 4 to 61 years, with a mean age (±1 SD) of 24 ± 16 years and only provided blood samples. All subjects were recruited into the study in accordance with the tenets of the Declaration of Helsinki and after the subject and parent or legal guardian, if appropriate, had been informed of the purpose of blood analysis and visual function tests. Consent forms were approved by the Institutional Review Board on Human Subjects of the University of Texas Southwestern Medi-
Fatty Acid Analysis

Red blood cells were separated immediately by centrifugation (3000g for 10 minutes), and lipids were extracted using methanol/chloroform (2:1) containing 0.02% butylated hydroxytoluene as an antioxidant. Lipid samples were stored briefly (<3 days) under N₂ and at −20°C. Red blood cell lipids were saponified, and fatty acids were methylated under N₂ with 14% boron trifluoride–methanol heated at 100°C for 20 minutes. Thirty fatty acid methyl esters in hexane were fractionated and quantified using capillary column gas chromatography and flame ionization detection on a Hewlett-Packard 5890 gas chromatograph equipped with a 0.25-mm inner diameter, 30-m capillary column containing Omegawax stationary phase (Supelco, Bellefonte, PA). Chromatographic parameters included helium as a carrier gas flowing at 1.0 ml/minute, a split ratio of 10-to-1, injector temperature of 240°C, detector temperature of 250°C, and an oven temperature program of 195°C for 2 minutes, decrease at 0.5°C/minute to 180°C, 180°C for 1 minute, increase at 5°C/minute to 210°C and 210°C for 25 minutes. At this point, biologic contaminants were removed from the column by increasing the oven temperature at 20°C/minute to 245°C and running at 245°C for 40 minutes. Peak identification was confirmed by comparison of retention times to a standard mixture prepared from GLC68A (NuChek Prep, Elysian, MN) that was enriched with 11 individual fatty acid methyl ester standards obtained from Sigma Chemical (St. Louis, MO) or Cayman Chemical (Ann Arbor, MI). Peak identity of 22:5ω6 was tentatively established by comparison to a preparation from ovine retina enriched in this fatty acid. The standard mixture was run daily along with samples as a standard laboratory practice.

The relative fatty acid content of each sample was expressed as percent of total fatty acid (weight percent) equal to or greater than 14 carbons. Fatty acid mass was determined by comparison of individual fatty acid peak areas with that of an internal standard (10 µg of 23:0 fatty acid) added to the methanol/chloroform before extraction. A customized software program (EMASTER, T. Burns, Kalamazoo, MI) was used to aid in peak identification, quantitation, and allocation of data to Excel spreadsheets.

Visual Function

The eye with the best visual acuity, as measured with a Bailey-Lovie eye chart, was selected for further testing of visual function. Kinetic visual fields were measured with the test light (Goldmann IV4e or equivalent) moving from nonseeing to seeing areas. After pupil dilation and 45 minutes of dark adaptation, final dark-adapted visual thresholds (average of 10 determinations) were obtained with an 11° test stimulus located 7° below fixation on a Goldmann–Weekers dark adaptometer. Full-field ERGs were obtained with a Burian–Allen bipolar contact-lens electrode. Signals were amplified (×10,000) and averaged (n = 20 to 50) by computer after eliminating responses containing artifacts over twice the signal amplitude. Testing followed the protocol recommended by the International Standards Committee of the International Society for the Clinical Electrophysiology of Vision (IS-CEV)⁶⁵. Rod responses were elicited from the dark-adapted eye with short-wavelength (~0.1 log scot td-sec) flashes. Mixed cone–rod responses were elicited by maximal intensity (2.0 log phot td-sec) white flashes. Cone responses were elicited by 30-Hz flicker (1.3 log phot td-sec) and by single flashes of maximal intensity in the presence of a background (3.2 log phot td). Dark-adapted rod responses, maximal responses, and light-adapted cone responses were considered to be nondetectable if the peak-to-peak amplitude measured less than 2.0 µV. If the amplitude of responses to 30-Hz flicker was less than 2.0 µV, narrowband amplification (×20; Q = 16) at the stimulus frequency was used to enhance responses. With this tuned amplification, responses greater than 0.05 µV could be reliably distinguished from noise. Normal values and lower limits of normal for each response have been published.³⁷
### TABLE 1. Distribution of Fatty Acids in Red Blood Cell Lipids of Patients With X-Linked Retinitis Pigmentosa and Normal Controls

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<tr>
<th>Mass (µg/ml Packed Red Blood Cells)</th>
<th>Percent of Total Fatty Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>XLRP</strong></td>
<td><strong>Controls</strong></td>
</tr>
<tr>
<td><strong>n = 18</strong></td>
<td><strong>n = 28</strong></td>
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</table>

| UI                                | 164 ± 42.7                  | 164 ± 42.8        | 0.0001 |
| 16:0                              | 189.4 ± 38.7                | 163.9 ± 44.3      | 0.050  |
| 18:0                              | 128.4 ± 21.4                | 157.3 ± 49.4      | 0.020  |
| Total Saturates*                  | 357.5 ± 60.7                | 360.4 ± 131.5     | 0.927  |
| 18:1                              | 175.4 ± 27.5                | 162.4 ± 15.9      | 0.048  |
| 22:1                              | 3.9 ± 2.4                   | 11.4 ± 7.1        | < 0.0001 |
| Total                              |                             |                  |        |
| Monounsaturates                    | 203.7 ± 30.5                | 198.0 ± 39.9      | 0.613  |
| 20:3ω9                            | 1.01 ± 0.81                 | 0.56 ± 0.45       | 0.025  |
| 18:2ω6                            | 119.9 ± 17.5                | 112.9 ± 23.3      | 0.235  |
| 18:3ω6                            | 0.99 ± 0.42                 | 1.54 ± 1.15       | 0.059  |
| 20:2ω6                            | 2.44 ± 0.64                 | 3.32 ± 1.22       | 0.007  |
| 20:3ω6                            | 15.7 ± 5.1                  | 30 ± 13.9         | 0.051  |
| 20:4ω6                            | 145.2 ± 26.5                | 164.1 ± 30.1      | 0.035  |
| 22:4ω6                            | 41.4 ± 7.7                  | 46.0 ± 13.2       | 0.188  |
| 22:5ω6                            | 8.36 ± 1.96                 | 10.1 ± 2.58       | 0.018  |
| SUMω6 LCPUFA                      | 214.1 ± 37.6                | 242.8 ± 47.1      | 0.035  |
| 18:2ω6                            | 14.4 ± 0.48                 | 1.09 ± 0.27       | 0.007  |
| 18:3ω6                            | 3.38 ± 0.96                 | 3.95 ± 2.99       | 0.439  |
| 20:5ω3                            | 17.5 ± 3.1                  | 21.8 ± 5.4        | 0.004  |
| 22:6ω3                            | 23.1 ± 5.9                  | 79.6 ± 9.4        | < 0.0001 |
| SUMω3 LCPUFA                      | 45.1 ± 7.5                  | 65.9 ± 15.9       | 0.009  |
| UI                                | 1545 ± 296                  | 1755 ± 303        | 0.017  |

**Ratios**

| 20:4ω6/18:2ω6                      | 1.21 ± 0.28                 | 1.47 ± 0.36       | 0.013  |
| 22:6ω3/18:3ω3                      | 22.1 ± 6.7                  | 28.4 ± 6.5        | 0.003  |
| 22:6ω3/22:5ω6                      | 2.81 ± 0.68                 | 4.04 ± 1.44       | 0.001  |
| 22:6ω3/20:4ω6                      | 0.16 ± 0.04                 | 0.24 ± 0.06       | < 0.0001 |
| 22:5ω3/22:6ω3                      | 1.35 ± 0.40                 | 1.82 ± 0.53       | 0.003  |
| 22:6ω3/22:4ω6                      | 0.20 ± 0.04                 | 0.22 ± 0.10       | 0.425  |
| ω3 LCPUFA/ω6 LCPUFA                | 0.21 ± 0.03                 | 0.28 ± 0.07       | 0.002  |
| ω6 DI                              | 16.3 ± 2.2                  | 15.4 ± 2.0        | 0.159  |
| ω3 DI                              | 2.2 ± 1.3                   | 2.0 ± 0.8         | 0.521  |
| ω6 EI                              | 18.3 ± 6.1                  | 23.7 ± 9.9        | 0.042  |
| ω3 EI                              | 7.8 ± 1.4                   | 9.9 ± 2.7         | 0.004  |
| Sat EI                             | 138 ± 80                    | 245 ± 144         | 0.006  |
| Mono El                            | 64 ± 14                     | 96 ± 27           | < 0.0001 |

Values are mean ± 1 SD.

* Indicates that the group of patients with XLRP is significantly different from the controls.

† Includes 14.0, 15.0, 16.0, 17.0, 18.0, 20.0, 22.0, and 24.0.

LCPUFA (long-chain polyunsaturated fatty acids) are greater than 18 carbons.

UI (unsaturation index) is calculated by the sum of double bonds to total fatty acid × % of each fatty acid.


ω3 DI = [(18:2ω6/18:3ω3) + (22:5ω3/22:6ω3)].


Sat El (saturated fatty acid elongation index) = [(15:0/14:0) + (18:0/16:0) + (20:0/18:0) + (22:0/20:0) + (24:0/22:0)]


lower in these patients. Fatty acid mass values showed similar trends. The levels of other saturates were equivalent between the two groups. Total monounsaturates were not different between control and XLRP groups; however, oleic acid (18:1) was elevated by 10% in the group with XLRP, and erucic acid (22:1) was significantly reduced in RBCs of the patients. No other mono-unsaturates differed in content between the groups. The level of eicosatetraenoic acid (20:3ω9) was increased twofold in RBCs of patients with XLRP compared to controls.
Distribution of docosahexaenoic acid (DHA) in red blood cells of patients with XLRP. Mean normal value ± SD is 38.6 ± 9.4 µg/ml packed red blood cells (n = 28). Eighteen members of 13 families were tested; related family members are indicated by similar letters on vertical bars. XLRP = X-linked retinitis pigmentosa.

Figure 1 shows the DHA concentration in RBCs from individual patients with XLRP. Individuals from the same family are indicated by the same letter on the bar. The average reduction in DHA concentration compared to control subjects was 40%. None of the patients had a DHA concentration that reached the mean of the control subjects, and only four had levels within 1 SD of the mean.

By examining differences in the levels of the individual fatty acids of each pathway for control subjects and patients with XLRP, precursor-to-product shifts can be used to localize specific impairments in fatty acid metabolism. In Figure 2, the concentrations of each fatty acid in the ω6 pathway are presented for control subjects and patients with XLRP. The mean concentrations of 20- and 22-carbon ω6 polyunsaturates in the patients with XLRP were reduced compared to control subjects; however, only 20:2ω6 was reduced significantly based on our strict criterion of P < 0.01. Although there was a trend toward reduced levels of ω6 LCPUFAs in patients, an inhibitory step in ω6 metabolism could not be identified. In contrast, there was a break in ω3 fatty acid metabolism (Fig. 3) at eicosapentaenoic acid (20:5ω3) such that the patients with XLRP had higher concentrations of the 18-carbon precursors (18:3ω3 and 18:4ω3), no difference in 20:5ω3, and 20% to 40% lower levels of the ω3 long-chain derivatives, 22:5ω3 and 22:6ω3. The ω6 and ω3 fatty acid profiles based on relative percent content were comparable to those based on mass analysis.

Further insight into the efficiency of the metabolic pathways can be derived from fatty acid product-
whereas the ω6 ratio was comparable between the 0.003), with XLRP compared to control subjects (P =

 saturated fatty acid El and monounsaturated fatly acid

and control groups (Table 1). In contrast, the elongation (P <

fatty acids 22:6ω3/20:4ω6

0.0001) and 22:6ω3/

fatty acid, α-linolenic acid (18:3ω3) to DHA, the major

conversion of the ω6 essential fatty acid, linoleic acid, to arachidonic acid was impaired as re-
flected by an 18% reduction in the ratio of 20:4ω6/

18:2ω6 in XLRP. The conversion of the ω3 essential fatty acid, α-linolenic acid (18:3ω3) to DHA, the major

ω3 fatty acid, (22:6ω3/18:3ω3) was similarly reduced

by 22% (Table 1, Fig. 3). Activity of the final steps
of the two pathways is often considered a ω3 fatty
acid sufficiency index. 

The unsaturation index (UI) is the sum of all double bonds in fatty acids of the RBCs. There was a trend (P = 0.017) toward a lower UI based on mass in the patients with XLRP and a significant reduction based on relative percentage of unsaturation (Table 1). Because the unsaturation of fatty acids is considered a major determinant of overall membrane fluidity,80 this decreased UI may reflect a reduced membrane fluidity in RBCs of patients with XLRP.

Clinical findings for each patient are shown in Table 2. In response to standard ISCEV protocol stim-
ulii, cone responses to 30-Hz flicker were significantly reduced in amplitude and delayed in b-wave implicit time. Only four patients had detectable (>2.0 μV) rod responses. Because the majority were nondetect-
able, rod responses were not considered in subsequent statistical analysis. All patients showed significant elevations in final visual threshold after 45 minutes of dark adaptation. Visual fields showed varying degrees of constriction in general agreement with ERG amplitu-
des. Because of intrafamilial similarities, subsequent statistical analysis of visual function was conducted with a subgroup of patients with XLRP comprised of one member per family. Excluded family members (ID# 874, 2762, 2998, 4362, 4365) were randomly se-
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To examine the relationship between retinal func-
tion and the level of DHA in RBCs of the patients, we conducted a stepwise multiple regression analysis (Table 3). With the exception of the single-flash cone amplitude, the partial r² values for DHA in equations for all response parameters were significant. Because of the association between retinal function and age in XLRP,2 this variable was also included in the regression model. The only signif-
icant factor in the equations for 30 Hz (r² = 0.38; P = 0.033) and single-flash cone implicit times (r² = 0.78; P = 0.0017) was RBC–DHA. In combination, RBC–DHA and age could account for 72% of the variance in ampli-
tude to the maximal stimulus (P = 0.002) and 80% of the variance in cone amplitude to 30-Hz flicker (P = 0.0003). Docosahexaenoic acid was not a significant factor in the regression equations for dark-adapted threshold and visual acuity. Limited data were available for Gold-
mann fields.

Regression plots for cone responses to RBC–DHA mass are given in Figure 4. Because rod responses in most

FIGURE 3. Profiles of ω3 fatty acids (FA) in patients with XLRP (n = 18) and normally sighted control subjects (n = 28). Mean mass (μg/ml packed red blood cells) of individual ω6 fatty acids is given. Significant differences between patients with XLRP and control subjects were determined by t-tests and indicated by P-values.

to-precursor ratios. Based on mass values for the ω6 pathway (Table 1, Fig. 2), the 18-carbon precursor linoleic acid (18:2ω6) was marginally elevated, whereas the major ω6 fatty acid, arachidonic acid (20:4ω6) was significantly reduced in the group with XLRP. Thus, conversion of the ω6 essential fatty acid, linoleic acid, to arachidonic acid was impaired as ref-
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ω3 fatty acid, (22:6ω3/18:3ω3) was similarly reduced

by 22% (Table 1, Fig. 3). Activity of the final steps
of ω3 and ω6 metabolism can be estimated by end-
product-to-precursor ratios. The greater impact of XLRP on ω3 metabolism is evident by comparison of
the ω3 and ω6 ratios of 22:6ω3/22:5ω3 and 22:5ω6/22:4ω6. The ω3 ratio was reduced by 26% in patients with XLRP compared to control subjects (P = 0.003), whereas the ω6 ratio was comparable between the two groups (Table 1). The ratio of total ω3 LCPUFA-to-
ω6 LCPUFA also was significantly reduced in XLRP
(P = 0.002), as were the ω3/ω6 ratios of the major
fatty acids 22:6ω3/20:4ω6 (P < 0.0001) and 22:6ω3/
22:5ω6 (P = 0.002). The latter ratio of end-products
of the two pathways is often considered an ω3 fatty
acid sufficiency index.

Metabolic activities of the two pathways can be com-
pared by examining the overall desaturation and elonga-
tion reactions. The product-to-precursor ratios of all desat-
uration steps in either the ω3 or ω6 pathways (i.e., desat-
uration index, DI) was not different between the XLRP
and control groups (Table 1). In contrast, the elongation indices (EI) for both ω3 and ω6 pathways, as well as the saturated fatty acid EI and monounsaturated fatty acid EL, were markedly lower in the group with XLRP. Because metabolism of 22:5ω3 to 22:6ω3, according to the pathway described by Voss et al,17 involves elongation and desaturation reactions, these reactions have been in-
cluded in calculations of ω3 EI and ω3 DI. Similarly, the 22:4ω6/22:5ω6 ratio has been included in the indices for the ω6 pathway.

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TABLE 2. Clinical Findings: X-Linked Retinitis Pigmentosa

<table>
<thead>
<tr>
<th>Identification Number</th>
<th>Acuity (Snellen units)</th>
<th>Rod Amplitude* (µV)</th>
<th>Maximal Amplitude* (µV)</th>
<th>30-Hz Implicit Time (msec)</th>
<th>Cone Amplitude (µV)</th>
<th>Cone Implicit Time (msec)</th>
<th>Dark-adapted Threshold #</th>
<th>Goldmann Field**</th>
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<td>58.6</td>
<td>2.7</td>
<td>46.1</td>
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</tr>
<tr>
<td>4575††</td>
<td>20/25</td>
<td>ND</td>
<td>0.38</td>
<td>35.9</td>
<td>ND</td>
<td>ND</td>
<td>—</td>
<td>3.92</td>
</tr>
<tr>
<td>2926††</td>
<td>20/400</td>
<td>ND</td>
<td>13.5</td>
<td>4.62</td>
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<td>9.2</td>
<td>38.3</td>
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<tr>
<td>2738††</td>
<td>20/50</td>
<td>ND</td>
<td>7.4</td>
<td>0.30</td>
<td>44.5</td>
<td>5.9</td>
<td>47.7</td>
<td>4.70</td>
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<tr>
<td>251††</td>
<td>45/20</td>
<td>ND</td>
<td>6.3</td>
<td>0.24</td>
<td>43.5</td>
<td>ND</td>
<td>—</td>
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<tr>
<td>874</td>
<td>20/120</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>—</td>
<td>ND</td>
<td>5.22</td>
</tr>
<tr>
<td>4542††</td>
<td>20/100</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>6.20</td>
</tr>
</tbody>
</table>

Normal (mean ± 1 SD) — 20/20 141 ± 38 355 ± 92 65 ± 19 28.8 ± 1.5 102 ± 29 28.2 ± 1.4 1.65 ± 0.24 >120°

* Dark-adapted rod amplitude, −0.1 log scot td-sec short-wavelength flash.
† Maximal amplitude, 2.0 log phot td-sec flash.
‡ Cone amplitude to 30-Hz flicker, 1.5 log phot td-sec.
§ 30-Hz cone b-wave implicit time.
¶ Light-adapted cone amplitude, 2.0 log phot td-sec flash, 3.2 log phot td background.
‖ Ligh-adapted cone implicit time.
¶° Dark-adapted threshold, log microapostilbs after 45-minute dark adaptation (11° white test).
** Goldmann IVe or equivalent (+ indicates temporal island).
†† Patient included in single-family-member subgroup.
ND = not detectable.
NA = not available.

patients with XLRP were nondetectable, the maximal amplitudes primarily reflect cone components and the regression line paralleled that of 30-Hz cone amplitudes. Cone b-wave implicit times to the 30-Hz stimuli were negatively associated with DHA levels.

DISCUSSION

The most notable deviation from normal levels of RBC fatty acids found in patients with XLRP was a 40% reduction in the ω3 end-product, DHA. Because RBC fatty acid profiles have been demonstrated to correlate with retinal and brain levels of fatty acids in animal and human studies,18-30 the low systemic concentration of DHA might reflect low retinal DHA content in these patients. A role for DHA in optimizing retinal function is consistent with a significant association between DHA levels and cone ERG function.

Consistently lower activity was evident in fatty acid elongation as the elongation indices (EI) for ω6 fatty

TABLE 3. Multiple Regression Analysis of Photoreceptor Function, DHA, and Age

<table>
<thead>
<tr>
<th>DHA</th>
<th>DHA + Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Partial r²</td>
</tr>
<tr>
<td>Maximal amplitude*</td>
<td>0.55</td>
</tr>
<tr>
<td>30-Hz amplitude*</td>
<td>0.45</td>
</tr>
<tr>
<td>30-Hz implicit time</td>
<td>0.38</td>
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<tr>
<td>Cone amplitude*</td>
<td>0.25</td>
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<tr>
<td>Cone implicit time</td>
<td>0.73</td>
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</tbody>
</table>

Stepwise analysis was conducted with RBC-DHA mass (µg/ml packed red blood cells) and functional values from the individual family member subgroup (n = 13). 2 µV values were assigned for nondetectable maximal (n = 2) and light-adapted cone amplitudes (n = 4); 0.05 µV values were assigned for nondetectable 30-Hz amplitude response (n = 1).

* Log unit values were used for analysis.
Aldiough a defect in the elongation reactions might with an impairment in the final steps of DHA biosynthesis, patients with XLRP. Numerous studies have associated the fluidity of the membrane. Suboptimal membrane fluidity has been demonstrated to alter biochemical and biophysical properties unique to the highly unsaturated hydrocarbon chain of DHA.

These results extend our previous findings in RBCs of patients with RP. In a study of ADRP, a subset of patients was found to have lower than normal levels of RBC–DHA. This group also had reductions in chain elongation, ω3 fatty acid desaturation, and unsaturation indices compared to normally sighted control subjects. The ADRP population was heterogeneous with respect to lipid abnormalities because many patients had normal DHA levels. In contrast, all patients with XLRP had subnormal levels of RBC–DHA. The patients with XLRP also had reduced elongation indices, whereas desaturation indices were comparable to control subjects. A finding common to patients with ADRP and patients with XLRP was a reduction in the fatty acid unsaturation index, which may reflect abnormalities in membrane fluidity in both groups. In all patients with ADRP, significant correlations were found between retinal function, defined as the ratio of rod-to-cone ERG amplitudes, and RBC–DHA levels, total ω3 PUFA, and overall fatty acid unsaturation. Only four patients with XLRP in the present study retained detectable rod responses to the ISCEV standard stimuli. Therefore, all responses were mediated by cones in the majority of patients. Stepwise multiple regression analysis indicated that RBC–DHA was a significant determinant of cone ERG response parameters.

The presence of low blood levels of DHA in patients with RP suggests involvement of systemic lipid regulatory mechanisms. Points of regulation may include hepatic fatty acid biosynthesis, packaging of fats for transport, and activities of specific DHA-binding proteins in the circulation and retinal tissues. Dietary supplementation of DHA would bypass several of the biosynthetic and transport steps and may restore blood levels of DHA to normal regardless of the specific mechanism impaired in the disease. It now becomes important to consider the potential for early nutritional intervention to delay the degenerative rod loss in patients with XLRP.

**Key Words**

retinitis pigmentosa, docosahexaenoic acid, red blood cells, electroretinography, ω3 fatty acids

**Acknowledgments**

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