Impaired synthesis of DHA in patients with X-linked retinitis pigmentosa

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Abstract Many patients with X-linked retinitis pigmentosa (XLRP) have lower than normal blood levels of the long-chain polyunsaturated ω3 fatty acid docosahexaenoic acid (DHA; 22:6ω3). This clinical trial was designed to test whether down-regulation of DHA biosynthesis might be responsible for these reduced DHA levels. DHA biosynthesis was assessed in five severely affected patients with XLRP and in five age-matched controls by quantifying conversion of [U-13C]ω3-linolenic acid (ω-LNA) to [13C]DHA. Following oral administration of [U-13C]ω-LNA, blood samples were collected at designated intervals for 21 days and isotopic enrichment of all ω3 fatty acids was determined by gas chromatography/mass spectroscopy. Activity of each metabolic step in the conversion of ω-LNA to DHA was determined by comparison of the ratios of the integrated concentration of 13C-product to 13C-precursor in plasma total lipid fractions. The ratio of [13C]DHA to [13C]18:3ω3 (the entire pathway) and that of [13C]20:5ω3 to [13C]20:4ω3 (Δ5-desaturase) were significantly lower in patients versus controls (P = 0.03 and 0.05, respectively). The estimated biosynthetic rates of [13C]20:5ω3, [13C]20:4ω3, [13C]24:5ω3, [13C]24:6ω3, and [13C]22:6ω3 were significantly lower in XLRP patients (42%, 43%, 31%, 18%, and 32% of control values, respectively; P < 0.04), supporting down-regulation of Δ5-desaturase in XLRP. The disappearance of 13C-labeled fatty acids from plasma was not greater in XLRP patients compared with controls, suggesting that XLRP was not associated with increased rates of fatty acid oxidation or other routes of catabolism. Thus, despite individual variation among both patients and controls, the data are consistent with a lower rate of Δ5-desaturation, suggesting that decreased biosynthesis of DHA may contribute to lower blood levels of DHA in patients with XLRP.

DHA comprises a small percentage (1–4%) of the total fatty acids of the membranes of most human tissues; however, it accounts for 30–40% of fatty acids in rod photoreceptor outer segments of the human retina (13). The high concentration of this highly unsaturated fatty acid can increase membrane fluidity and, in turn, may modify the mobility of vital proteins and the activities of retinal enzymes (14, 15). Indeed, deficiencies of ω3 fatty acids result in abnormal electroretinographic (ERG) responses in rats (16–19), guinea pigs (20), and monkeys (21–23). Less

Retinitis pigmentosa (RP) is a family of hereditary diseases characterized by photoreceptor degeneration with progressive night blindness and constriction of peripheral vision leading to functional blindness (1). Multiple inheritance patterns exist for RP, including autosomal dominant, autosomal recessive, and X-linked. Patients with the X-linked form of RP (XLRP) are among the most severely affected, with an onset of night blindness due to rod photoreceptor loss often detectable by age 5 years (2–4). Cone degeneration typically results in legal blindness by the second or third decade of life. With the exception of visual impairment, patients with RP including XLRP are commonly considered “healthy.” However, a number of investigations have shown that many patients with RP have lower plasma and red blood cell (RBC) lipid levels of the ω3 polyunsaturated fatty acid docosahexaenoic acid (DHA; 22:6ω3) than non-affected individuals (reviewed in (5)). In two studies, the majority of patients with XLRP had 30–40% lower DHA levels in RBC lipids than normally sighted controls (6, 7).

Lower plasma levels of DHA and other ω3 fatty acids also have been found in miniature poodles with progressive rod-cone degeneration (prcd), a model of inherited retinal degeneration that closely resembles human RP (8–10). Other animal models of RP including prcd dogs (11) and transgenic rats and mice with rhodopsin mutations (12) also have reduced DHA levels in lipid membranes of rod outer segments.

Abbreviations: AUC, area under the curve; DHA, docosahexaenoic acid; ERG, electroretinographic; GC, gas chromatography; LNA, linolenic acid; MS, mass spectrometry; prcd, progressive rod-cone degeneration; RBC, red blood cell; RP, retinitis pigmentosa; XLRP, X-linked retinitis pigmentosa.

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mature ERG responses and/or lower visual acuity, as well as lower neurodevelopmental scores also have been associated with reduced blood lipid levels of DHA in preterm and term infants fed conventional formula versus breast milk or DHA-supplemented formula (24–29). Significant correlations between RBC levels of DHA and ERG responses also have been reported such that patients with XLRP and low blood DHA levels have correspondingly poor ERG function (7). Thus, DHA may participate in optimizing the lipid microenvironment in photoreceptor membranes, thereby influencing enzyme and protein interactions.

DHA cannot be synthesized de novo by the human species, and therefore must be formed from shorter chain ω3 precursors [e.g., α-linolenic acid (α-LNA), an essential fatty acid] or ingested intact (30). The currently accepted pathway for ω3 fatty acid biosynthesis occurs through a series of desaturations (insertion of additional double bonds) and elongations (addition of two-carbon units). Thus, α-LNA (18:3ω3) is sequentially converted to 18:4ω3, 20:4ω3, 20:5ω3, 22:5ω3, 24:5ω3, 24:6ω3, and 22:6ω3 (DHA). The respective enzymatic steps involved in α-LNA conversion to DHA are desaturation (Δ^6-desaturase), elongation, desaturation (Δ^5-desaturase), elongation, desaturation (Δ^5-desaturase), and finally β-oxidation. The desaturation and elongation steps occur in smooth endoplasmic reticulum (31), whereas the final β-oxidation step occurs in peroxisomes (32). Liver is considered the primary site of DHA synthesis, but other tissues including retina, retinal pigment epithelium, and testes (33, 34) also convert α-LNA to DHA via the 24-carbon intermediates.

There are three possible explanations for the low blood lipid DHA levels in patients with XLRP: 1) reduced synthesis of DHA; 2) increased catabolism of DHA; and 3) impaired transport, uptake, and/or trafficking of DHA, reducing the availability of DHA to target tissues. Tracing stable isotopically labeled α-LNA [i.e., [U-13C]α-LNA] through the ω3 fatty acid intermediates to DHA in vivo permits critical examination of the first hypothesis and provides some insight into the possibility of increased catabolism of DHA associated with XLRP. In this clinical trial, 13C-enrichment of the ω3 fatty acid intermediates in the plasma total lipid fraction of five patients with XLRP at various times following administration of [U-13C]α-LNA was compared with enrichment of the same fatty acids at the same times in plasma lipids of five age-matched controls. The lower accumulation of 13C-label in DHA in patients with XLRP indicates that their metabolic conversion of α-LNA to DHA is reduced. The lack of difference between groups both in expiration of 13CO₂ and disappearance of 13C-labeled fatty acids from total plasma lipids suggests that oxidation and other routes of catabolism of ω3 fatty acids do not differ between XLRP patients and controls.

MATERIALS AND METHODS

Subjects

All patients with XLRP had been diagnosed by retinal specialists and were in the advanced stages of disease. The trial included only males, as they are most severely affected by this disease. Each had an early onset of night blindness followed by significant loss of peripheral vision. All had nondetectable rod ERG responses, dark-adaptation values elevated by greater than three log units, and characteristically poor cone ERG function. Family histories of patients were consistent with the XLRP inheritance pattern; that is, presence of at least two affected male relatives, absence of male-to-male transmission, and expression of characteristics of carrier heterozygotes in either the patient’s mother or daughter. Four patients with XLRP were Caucasian and one was Hispanic; ages ranged from 28 to 52 years (mean ± SD = 41 ± 8). One patient smoked about eight packages of cigarettes per week; no other patient smoked. None consumed more than one alcoholic drink per week. Body mass index (BMI; weight/height^2) ranged from 26 to 36 (mean ± SD = 32 ± 5). All patients underwent a comprehensive physical examination including medical history, review of systems, review of diet and health habits, electrocardiograph, chest X-ray, blood chemistries, and baseline carbon dioxide production.

Normal-sighted male volunteers of roughly the same age as the patients (±2 years) were recruited as controls (range = 29–50 years; mean ± SD = 39 ± 7). Controls also underwent physical and ophthalmic examinations. All were Caucasian, nonsmokers, and consumed less than one alcoholic drink per week. BMI ranged from 26–32 (mean ± SD = 28 ± 2; significantly less than patients, P = 0.04).

Participants were informed of the objectives and protocol of the study and written informed consent of each was obtained. The study was approved by the Institutional Review Boards of Presbyterian Hospital of Dallas, TX and Baylor College of Medicine and Affiliated Institutions, Houston, TX.

Experimental design

All subjects were provided a low fat, low ω3 fatty acid diet for 1 week prior to and 2 weeks following tracer administration. Meals (breakfast, lunch, dinner, and snacks) were prepared to our specifications and delivered to each subject’s home or work. The purpose of this “stabilization” diet was to reduce variability among participants due to differences in the individuals’ typical diets and to stabilize fatty acid intake during the study, thus preventing “spikes” in blood levels of ω3 fatty acids.

After admission to Presbyterian Hospital, baseline blood and breath samples were obtained and an in-dwelling catheter was placed in the subject’s arm for blood sampling during a 50 h hospital stay. [U-13C]α-LNA (0.5 g) obtained from Martek Biosciences Corporation, Columbia, MD, was sonicated into 3 oz of a low fat chocolate drink (Yoo-hoo, Carlstadt, NJ) and ingested immediately by all subjects. Subsequently, blood samples [collected in 5-ml Vacutainer® (Becton Dickinson, Franklin Lakes, NJ) tubes containing EDTA as anticoagulant] were obtained at 1, 1.5, 2, 3, 4, 6, 8, 12, 18, and 24 h. Breath samples were obtained every half hour for the first 12 h, and then every hour for the next 12 h. These were collected in gas sampling bags and transferred to sterile 10-ml evacuated glass tubes for analysis of 13CO₂. The subjects were discharged but returned for blood samples at 2, 3, 4, 5, 7, 10, 14, and 21 days.

Plasma was separated by centrifugation (3,000 g x 10 min) and frozen at ~80°C until analysis. Total plasma fatty acids were extracted according to the method of Bligh and Dyer (35) following addition of heptadecanoic acid (17:0) in phospholipid and triglyceride forms (Sigma-Aldrich Chemical Co., St. Louis, MO) as an internal standard for determination of ω3 fatty acid concentrations. Fatty acids in the total lipid extracts were converted to methyl esters and perfluorobenzyl derivatives using the methods of Morrison and Smith (36) and Hachey et al. (37), respectively. Methyl and perfluorobenzyl esters were separated

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by gas chromatography (GC) on 30-m DB-225 (J & W Scientific, Folsom, CA) and 60-m SP-2380 (Supelco, Bellefonte, PA) capillary columns, respectively, using a Hewlett-Packard 5890 gas chromatograph.

Detection of GC-separated pentafluorobenzyl esters was accomplished by mass spectrometry (MS) using a Hewlett-Packard 5890A quadrupole mass spectrometer. Signals of the tracer ([M]) and tracer ([M + 18]) isotopomers of the α3 fatty acids were determined by selective ion monitoring for their corresponding molecular masses under negative chemical ionization (37). Peaks were identified by comparison with α3 fatty acid reference standards (Sigma-Aldrich Chemical Co.) or with authentic preparations of 24:5α3 and 24:6α3 (provided by Dr. H. Sprecher, Columbus, OH). Areas of the trace and tracer MS signal peaks were converted to percentage enrichment of the tracer in its fatty acid pool (% enrichment = [tracer/(tracer + tracer)] × 100%).

Detection of GC-separated methyl esters was accomplished by flame ionization. Identities were confirmed against GC of α3 fatty acid standards. Although 20:4α3 was detectable in the plasma lipid extracts by GC/MS, it could not be quantified directly by GC; thus, its concentration was estimated by comparison of its MS signal area with that of 20:4α6 (arachidonic acid). All subjects had plasma lipid concentrations of α3 fatty acids determined at 0, 8, 24, 168, and 504 h. Because the concentrations differed minimally, individual averaged values were used in all subsequent calculations.

Breath samples were analyzed for the 13C/12C ratio of CO2 by isotope ratio MS using a Europa Scientific 20-20 Stable Isotope Analyzer mass spectrometer (Franklin, OH). Atom percentage enrichments for 13CO2 in breath samples were converted to 13CO2 expiration rates (nmol/min/kg) and cumulative percentage expiration (% of [U-13C]α-LNA dose) using CO2 production rates (ml/kg/min) measured at baseline.

Data analysis

Data are expressed as nmol [13C]fatty acid/ml plasma (i.e., the product of the 13C-enrichment of each fatty acid and the plasma concentration of the fatty acid). Total accumulations of 13C-fatty acids are reported as a function of time after isotope administration. Area under the curve (AUC; nmol [13C]fatty acid/ml plasma/504 h) was calculated by trapezoidal integration using IGOR software (Wavemetrics Inc., Lake Oswego, OR). An estimate of the initial biosynthetic rate (nmol [13C]fatty acid/ml plasma/h) of each intermediate fatty acid was established by linear regression of the leading edge of the 13C-accumulation curve. Rates of fatty acid disappearance were estimated as the rate of decline in [13C]fatty acid concentrations from the time of peak enrichment to 504 h. Product/precursor ratios of individual metabolic steps in conversion of α-LNA to DHA were established from AUC data. α-LNA oxidation was estimated by respiratory expiration of 13CO2 and expressed as percentage of [U-13C]α-LNA administered. Statistically significant differences (P < 0.05) in mean values of each variable between patients and controls were detected by Student’s t-test.

RESULTS

Mean baseline blood chemistry values of the two groups of subjects are summarized in Table 1. As expected, patients with XLRP had significantly lower RBC lipid DHA content than controls (Table 1). Neither plasma lipoprotein profiles nor other blood chemistries differed significantly between groups; however, alanine aminotransferase activity was somewhat higher in XLRP patients versus controls (P = 0.06). Baseline carbon dioxide production of the control group was 300 ± 50 ml/min; that of the XLRP group was 340 ± 60 ml/min (P = 0.28).

Figure 1 shows the concentrations of [13C]α-LNA and [13C]DHA in plasma lipids of individual control subjects and XLRP patients, as well as the mean concentrations of both groups. Although these values were variable in both groups, the difference in maximal mean peak concentration of [13C]α-LNA in plasma lipids between controls and patients was not statistically significant (see Fig. 1A and B; 42 ± 24 nmol/ml and 51 ± 18 nmol/ml, respectively; P > 0.5). The time to reach the maximal peak concentration also did not differ between groups (4 vs. 3.5 h). In contrast, there were marked differences between controls and XLRP patients in both mean plasma lipid [13C]DHA concentration and the time to reach peak concentration (Fig. 1C and D). The mean concentration of [13C]DHA in plasma lipids of controls peaked at 0.11 nmol/ml, approximately 144 h after tracer administration, whereas the mean peak plasma lipid concentration of [13C]DHA was lower in patients with XLRP (0.06 nmol/ml; P = 0.21), and occurred 96 h later (240 h; P = 0.03) than controls.

A comparison of the integrated concentrations of 13C-labeled α3 fatty acids in plasma lipid of patients with XLRP and controls is shown in Fig. 2A. Concentrations of [13C]18:3, [13C]18:4, and [13C]20:4 were 46%, 82%, and 136% higher, respectively, in patients with XLRP than in controls, whereas the integrated concentrations of [13C]20:5, [13C]22:5, [13C]24:5, [13C]24:6, and [13C]22:6 were 3%, 2%, 2%, 38%, and 46% lower, respectively, in patients with XLRP than in controls. These data, although not statistically significant (P ≥ 0.06), are consistent with a block in conversion of 18:3α3 to 22:6α3 at the Δ5-desaturation step (i.e., 20:4α3→20:5α3).

The estimated biosynthetic rates of formation of each intermediate fatty acid were determined by the slope of the 13C-enrichment curve as a function of time up to the peak 13C concentration. Estimated biosynthetic rates of 18:4 and 20:4 were nearly identical in XLRP patients and controls (Fig. 2B), whereas in patients, the rates of 20:5, 22:5, 24:5, 24:6, and 22:6 biosynthesis were 58%, 76%, 69%,

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Table 1. Baseline laboratory measures

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=5)</th>
<th>Patients with XLRP (n=5)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td><strong>Lung function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO2 volume (ml/min)</td>
<td>300 ± 50</td>
<td>340 ± 60</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Blood chemistry</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>RBC-DHA (% of total fatty acids)</td>
<td>4.2 ± 0.5</td>
<td>2.7 ± 0.3</td>
<td>0.002</td>
</tr>
<tr>
<td>Plasma triglycerides (mg/dl)</td>
<td>171 ± 70</td>
<td>224 ± 96</td>
<td>0.35</td>
</tr>
<tr>
<td>Plasma total cholesterol (mg/dl)</td>
<td>218 ± 48</td>
<td>211 ± 22</td>
<td>0.77</td>
</tr>
<tr>
<td>Plasma LDL cholesterol (mg/dl)</td>
<td>115 ± 53</td>
<td>126 ± 32</td>
<td>0.51</td>
</tr>
<tr>
<td>Plasma HDL cholesterol (mg/dl)</td>
<td>40 ± 7</td>
<td>40 ± 10</td>
<td>1.0</td>
</tr>
<tr>
<td>Plasma AST (U/l)</td>
<td>23 ± 3</td>
<td>30 ± 8</td>
<td>0.12</td>
</tr>
<tr>
<td>Plasma ALT (U/l)</td>
<td>25 ± 7</td>
<td>42 ± 16</td>
<td>0.06</td>
</tr>
<tr>
<td>Plasma GGT (U/l)</td>
<td>33 ± 13</td>
<td>60 ± 47</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Values are given as means ± SD. RBC-DHA, docosahexaenoic acid in red blood cell lipids; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase.
82%, and 69% lower than in controls, respectively (all differences were significant, \( P < 0.05 \)). These data also are consistent with down-regulation at the \( \Delta^2 \)-desaturase step.

Further assessment of conversion activity was made by comparing product-to-precursor ratios. Thus, ratios of the integrated concentration of the \( [\text{13C}] \) product of each step in conversion of \( 18:3 \) to \( 22:6 \) to the \( [\text{13C}] \) precursor of each step were calculated (Fig. 3). The ratio of \( [\text{13C}] \) DHA (product of the pathway) to \( [\text{13C}] 18:3 \) (precursor) in patients with XLRP was roughly half that of the control group (\( P = 0.029 \)). The ratio of the integrated concentration of \( [\text{13C}] 20:5 \) to that of \( [\text{13C}] 20:4 \), the step catalyzed by \( \Delta^5 \)-desaturase, also was significantly lower in patients with XLRP versus controls (\( P = 0.05 \)). The integrated concentrations of \( [\text{13C}] \) product/\( [\text{13C}] \) precursor of other steps in conversion of \( 18:3 \) to \( 22:6 \) (e.g., desaturation, elongation, and \( \beta \)-oxidation) did not differ significantly between patients and controls.

The rate of disappearance of each intermediate \( [\text{13C}] \) fatty acid was estimated as the slope of the plasma fatty acid curve beginning at the peak of \( [\text{13C}] \) label enrichment through 504 h (Table 2). There was no evidence of a greater rate of disappearance of any \( [\text{13C}] \) fatty acids in patients with XLRP. Rather, the rate of disappearance of comparing product-to-precursor ratios. Thus, ratios of the integrated concentration of the \( [\text{13C}] \) product of each step in conversion of \( 18:3 \) to \( 22:6 \) to the \( [\text{13C}] \) precursor of each step were calculated (Fig. 3). The ratio of \( [\text{13C}] \) DHA (product of the pathway) to \( [\text{13C}] 18:3 \) (precursor) in patients with XLRP was roughly half that of the control group (\( P = 0.029 \)). The ratio of the integrated concentration of \( [\text{13C}] 20:5 \) to that of \( [\text{13C}] 20:4 \), the step catalyzed by \( \Delta^5 \)-desaturase, also was significantly lower in patients with XLRP versus controls (\( P = 0.05 \)). The integrated concentrations of \( [\text{13C}] \) product/\( [\text{13C}] \) precursor of other steps in conversion of \( 18:3 \) to \( 22:6 \) (e.g., desaturation, elongation, and \( \beta \)-oxidation) did not differ significantly between patients and controls.

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most labeled intermediates beyond the Δ5-desaturase step, including DHA, appeared to be slower in XLRP patients. Oxidation of [U-13C]α-LNA was assessed by differences in breath 13CO2 of patients with XLRP versus controls following administration of the tracer (Fig. 4). Breath 13CO2 (nmol/min/kg body weight) of both patients and controls peaked 4 h post-dose and returned to baseline within about 36 h; peak 13CO2 enrichment of the two groups did not differ significantly (P = 0.37). Cumulative breath 13CO2 excretion, expressed as percentage of the [U-13C]α-LNA dose administered, also did not differ significantly between groups (P > 0.3).

### DISCUSSION

This clinical trial was conducted to assess activity of the DHA biosynthetic pathway in patients with XLRP. The reduced accumulation of [13C]DHA and lower estimated initial rates of DHA synthesis in patients with XLRP implicate a defect in the metabolic conversion pathway of α-LNA to DHA. Furthermore, the statistically significant differences in product/precursor ratios between controls and patients are highly suggestive that down-regulation of Δ5-desaturase contributes to the lower circulating pools of DHA associated with XLRP. The reduced blood lipid DHA levels in XLRP patients do not appear to be attributable to higher rates of oxidation of ω3 fatty acids, as estimated by expiration of 13CO2 in the breath. Although by no means conclusive, the [13C]fatty acid disappearance rates reported here are not consistent with an elevated catabolism of DHA as a cause for the lower circulating levels of DHA in XLRP patients. Further evaluation of this possibility will require studies with in vivo [13C]DHA administration.

Nevertheless, a number of assumptions and confounding variables may affect the interpretation of these data. The major such assumption is that the plasma pool is homogeneous with other lipid pools and that periodic sampling of plasma reflects the metabolic activity in the whole body. Although this appears to be true generally, plasma is a dynamic medium with numerous tissues releasing and absorbing varying amounts of fatty acids; this makes kinetic analysis of metabolism tentative at best. Another potential problem is intersubject variability; however, despite this, accumulation of [13C]DHA was consistently lower in patients with XLRP than in controls.

A break in metabolic activity at the Δ5-desaturase step was evident in XLRP patients from integrated [13C]fatty acid concentrations, biosynthetic activities, and product/precursor ratios. By examination of both integrated [13C]24:5 and 24:6 concentrations and biosynthetic rates, it appears that the metabolic flux of 13C-label through these 24-carbon intermediates also was attenuated in both controls and patients with XLRP (Fig. 2). This may be associated with slower transport of these fatty acids into and out of peroxisomes prior to the final β-oxidation step to produce DHA and subsequent esterification to phospholipid (38).

A reduction in the synthesis of DHA in patients with RP is also consistent with previous studies (7). A crude comparison of product/precursor ratios of RBC concentrations of ω3 fatty acid intermediates of the individual desaturation and elongation reactions in the pathway revealed significant reductions in the final steps of DHA biosynthesis in patients with XLRP versus controls. These results are supported by a dietary supplementation study in patients with autosomal dominant RP (39). In this study, both patients and controls received a low ω3 fatty acid “stabilization” diet followed by 3 weeks of supplementation with eicosapentaenoic acid (20:5ω3). Plasma lipid eicosapentaenoic acid content increased similarly in both patients and controls. However, plasma lipid DHA content of controls increased by 26% over the 3-week period of supplementation, whereas that of the RP patients did not change, which is consistent with decreased metabolic production of DHA.

Mutations of retinal-specific genes are considered the

### TABLE 2. Estimated rates of 13C-labeled fatty acid disappearance from plasma

<table>
<thead>
<tr>
<th>ω3 Fatty Acid</th>
<th>Controls (n = 5)</th>
<th>XLRP Patients (n = 5)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:3ω3</td>
<td>3.530 ± 2.690</td>
<td>2.340 ± 1.090</td>
<td>0.38</td>
</tr>
<tr>
<td>18:4ω3</td>
<td>0.62 ± 35</td>
<td>0.47 ± 27</td>
<td>0.46</td>
</tr>
<tr>
<td>20:4ω3</td>
<td>0.20 ± 15</td>
<td>0.20 ± 14</td>
<td>0.77</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>3.3 ± 1.9</td>
<td>3.8 ± 2.8</td>
<td>0.76</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>1.0 ± 0.4</td>
<td>0.6 ± 0.6</td>
<td>0.25</td>
</tr>
<tr>
<td>24:5ω3</td>
<td>0.21 ± 0.13</td>
<td>0.11 ± 0.10</td>
<td>0.21</td>
</tr>
<tr>
<td>24:6ω3</td>
<td>0.23 ± 0.01</td>
<td>0.07 ± 0.02</td>
<td>0.003</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>0.42 ± 0.50</td>
<td>0.06 ± 0.06</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Values given as means ± SD. Disappearance rates are based on linear regression of slopes of individual [13C]fatty acid concentration curves as a function of time from peak to 504 h.

![Fig. 4. Expiration rate of 13CO2 following tracer administration in controls (solid triangles and solid lines) and in patients with XLRP (open squares and dashed lines). Inset: cumulative expiration of 13CO2, given as percentage of tracer dose.](https://www.jlr.org)

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primary cause of RP. The focus has been on mutations in genes controlling enzymes involved in the phototransduction cascade and proteins of structural significance in the photoreceptor. Despite persistent observations of low blood lipid levels of DHA associated with retinal dysfunction, it is not currently known whether alterations in ω3 fatty acid metabolism are involved in the pathophysiology of RP. Because the lipid anomaly occurs in various genetic forms of RP, it may be a secondary phenomenon (10). Anderson et al. (10) hypothesized that the genetic mutations resulting in retinal degenerations in patients with RP also produce a metabolic stress that involves adaptive structural and biochemical modifications in retinal photoreceptors so as to reduce stress-induced damage to the photoreceptors. Because DHA has numerous unsaturated bonds and may be a target of oxidative stress, reduction of tissue levels of DHA may serve to limit intracellular damage. Such a mechanism also may explain local regulation of DHA levels within the retina. However, a reduction in circulating DHA would supposedly be regulated by the liver. Bazan and Rodriguez de Turco (40) proposed recently that the retina generates some signal to the liver, recirculating DHA would supposedly be regulated by the liver. Bazan and Rodriguez de Turco (40) proposed that the retina generates some signal to the liver, recirculating DHA would supposedly be regulated by the liver, resulting in diminished blood lipid levels of DHA in patients with XLRP.

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