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

Dennis R. Hoffman,[1,*] James C. DeMar,[†] William C. Heird,[†] David G. Birch,[*] and Robert E. Anderson[§]

Retina Foundation of the Southwest,* 9900 North Central Expressway, Dallas, TX 75231; USDA/ARS Children's Nutrition Research Center,[†] Department of Pediatrics, Baylor College of Medicine, Houston, TX; and Departments of Ophthalmology and Cell Biology,[§] University of Oklahoma Health Sciences Center, Dean A. McGee Institute, Oklahoma City, OK

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-^{13}C]α-linolenic acid (α-LNA) to [^{13}C]DHA. Following oral administration of [U-^{13}C]α-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 α-LNA to DHA was determined by comparison of the ratios of the integrated concentration of ^{13}C-product to ^{13}C-precursor in plasma total lipid fractions. The ratio of [^{13}C]DHA to [^{13}C]18:3ω3 (the entire pathway) and that of [^{13}C]20:5ω3 to [^{13}C]20:4ω3 (Δ^5-desaturase) were significantly lower in patients versus controls (P = 0.03 and 0.05, respectively). The estimated biosynthetic rates of [^{13}C]20:5ω3, [^{13}C]22:5ω3, [^{13}C]24:5ω3, [^{13}C]24:6ω3, and [^{13}C]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 Δ^5-desaturase in XLRP. The disappearance of ^{13}C-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 Δ^5-desaturation, suggesting that decreased biosynthesis of DHA may contribute to lower blood levels of DHA in patients with XLRP.**—Hoffman, D. R., J. C. DeMar, W. C. Heird, D. G. Birch, and R. E. Anderson. **Impaired synthesis of DHA in patients with X-linked retinitis pigmentosa. *J. Lipid Res.* 2001.** 42: **1395–1401.**

Supplementary key words omega-3 fatty acids • retinal degeneration • delta-5 desaturase • stable isotopes • lipid metabolism • linolenic acid

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 ω-3 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.

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

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.

[1] To whom correspondence should be addressed.
e-mail: dhoffman@retinafoundation.org

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 though 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^6-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-^{13}C]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, ^{13}C-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-^{13}C]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 ^{13}C-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 $^{13}CO_2$ and disappearance of ^{13}C-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 ± 3). 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 30-h hospital stay. [U-^{13}C]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 $^{13}CO_2$. 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 $g \times 10$ 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 pentafluorobenzyl derivatives using the methods of Morrison and Smith (36) and Hachey et al. (37), respectively. Methyl and pentafluorobenzyl esters were separated 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 5989A quadrupole mass spectrometer. Signals of the tracee ([M]) and tracer ([M + 18]) isotopomers of the ω3 fatty acids were determined by selective ion monitoring for their corresponding molecular masses under negative chemical ionization (37). Peaks were identified by comparison with ω3 fatty acid reference standards (Sigma-Aldrich Chemical Co.) or with authentic preparations of 24:5ω3 and 24:6ω3 (provided by Dr. H. Sprecher, Columbus, OH). Areas of the tracee and tracer MS signal peaks were converted to percentage enrichment of the tracer in its fatty acid pool (% enrichment = [tracer/(tracee + tracer)] × 100%).

Detection of GC-separated methyl esters was accomplished by flame ionization. Identities were confirmed against GC of ω3 fatty acid standards. Although 20:4ω3 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 20:4ω6 (arachidonic acid). All subjects had plasma lipid concentrations of ω3 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 $^{13}C/^{12}C$ ratio of CO_2 by isotope ratio MS using a Europa Scientific 20-20 Stable Isotope Analyzer mass spectrometer (Franklin, OH). Atom percentage enrichments for $^{13}CO_2$ in breath samples were converted to $^{13}CO_2$ expiration rates (nmol/min/kg) and cumulative percentage expiration (% of [U-^{13}C]α-LNA dose) using CO_2 production rates (ml/kg/min) measured at baseline.

Data analysis

Data are expressed as nmol [^{13}C]fatty acid/ml plasma (i.e., the product of the ^{13}C-enrichment of each fatty acid and the plasma concentration of the fatty acid). Total accumulations of ^{13}C-fatty acids are reported as a function of time after isotope administration. Area under the curve (AUC; nmol [^{13}C]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 [^{13}C]fatty acid/ml plasma/h) of each intermediate fatty acid was established by linear regression of the leading edge of the ^{13}C-accumulation curve. Rates of fatty acid disappearance were estimated as the rate of decline in [^{13}C]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 $^{13}CO_2$ and expressed as percentage of [U-^{13}C]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 ($P = 0.28$).

TABLE 1. Baseline laboratory measures

	Controls (n = 5)	Patients with XLRP (n = 5)	*P*
Lung function			
CO_2 volume (ml/min)	300 ± 50	340 ± 60	0.28
Blood chemistry			
RBC-DHA (% of total fatty acids)	4.2 ± 0.5	2.7 ± 0.3	0.002
Plasma triglycerides (mg/dl)	171 ± 70	224 ± 96	0.35
Plasma total cholesterol (mg/dl)	218 ± 48	211 ± 22	0.77
Plasma LDL cholesterol (mg/dl)	145 ± 53	126 ± 32	0.51
Plasma HDL cholesterol (mg/dl)	40 ± 7	40 ± 10	1.0
Plasma AST (U/l)	23 ± 3	30 ± 8	0.12
Plasma ALT (U/l)	25 ± 7	42 ± 16	0.06
Plasma GGT (U/l)	33 ± 13	60 ± 47	0.27

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.

Figure 1 shows the concentrations of [^{13}C]α-LNA and [^{13}C]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 [^{13}C]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 [^{13}C]DHA concentration and the time to reach peak concentration (Fig. 1C and D). The mean concentration of [^{13}C]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 [^{13}C]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 ^{13}C-labeled ω3 fatty acids in plasma lipid of patients with XLRP and controls is shown in **Fig. 2A**. Concentrations of [^{13}C]18:3, [^{13}C]18:4, and [^{13}C]20:4 were 46%, 82%, and 136% higher, respectively, in patients with XLRP than in controls, whereas the integrated concentrations of [^{13}C]20:5, [^{13}C]22:5, [^{13}C]24:5, [^{13}C]24:6, and [^{13}C]22:6 were 3%, 2%, 2%, 38%, and 46% lower, respectively, in patients with XLRP than in controls. These data, although not statistically significant ($P \geqslant 0.06$), are consistent with a block in conversion of 18:3ω3 to 22:6ω3 at the Δ^5-desaturation step (i.e., 20:4ω3→20:5ω3).

The estimated biosynthetic rates of formation of each intermediate fatty acid were determined by the slope of the ^{13}C-enrichment curve as a function of time up to the peak ^{13}C 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%,

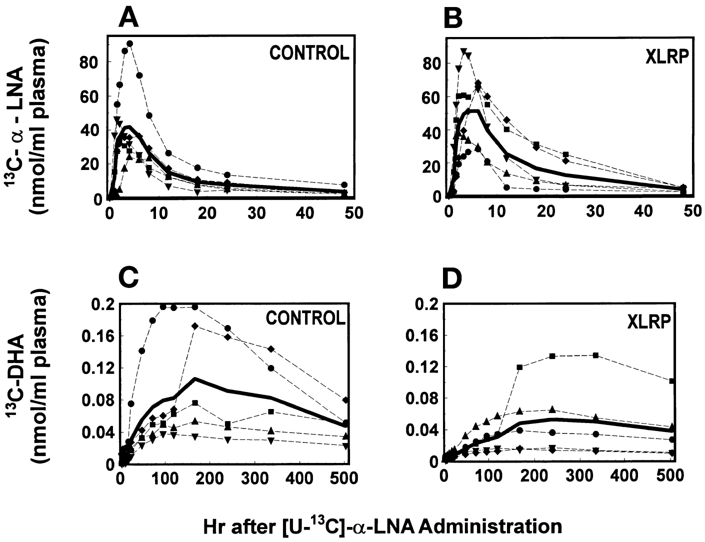

Fig. 1. Concentrations of ^{13}C-labeled α-LNA and DHA in plasma lipids of five controls and five patients with XLRP as a function of time following [U-^{13}C]α-LNA administration. Mean (solid black line) and individual data (solid symbols and dashed lines) are given for the [^{13}C]α-LNA plasma concentration in controls (A) and in XLRP patients (B). Data for [^{13}C]DHA plasma concentrations of controls are shown in C, and those of patients with XLRP are shown in D.

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 Δ^5-desaturase step.

Further assessment of conversion activity was made by comparing product-to-precursor ratios. Thus, ratios of the integrated concentration of the ^{13}C-product of each step in conversion of 18:3ω3 to 22:6ω3 to the ^{13}C-precursor of each step were calculated (**Fig. 3**). The ratio of [^{13}C]DHA (product of the pathway) to [^{13}C]18:3ω3 (precursor) in patients with XLRP was roughly half that of the control group ($P = 0.029$). The ratio of the integrated concentration of [^{13}C]20:5ω3 to that of [^{13}C]20:4ω3, the step catalyzed by Δ^5-desaturase, also was significantly lower in patients with XLRP versus controls ($P = 0.05$). The integrated concentrations of ^{13}C-product/^{13}C-precursor of other steps in conversion of 18:3ω3 to 22:6ω3 (e.g., desaturation, elongation, and β-oxidation) did not differ significantly between patients and controls.

The rate of disappearance of each intermediate [^{13}C]fatty acid was estimated as the slope of the plasma fatty acid curve beginning at the peak of ^{13}C-label enrichment through 504 h (**Table 2**). There was no evidence of a greater rate of disappearance of any [^{13}C]fatty acids in patients with XLRP. Rather, the rate of disappearance of

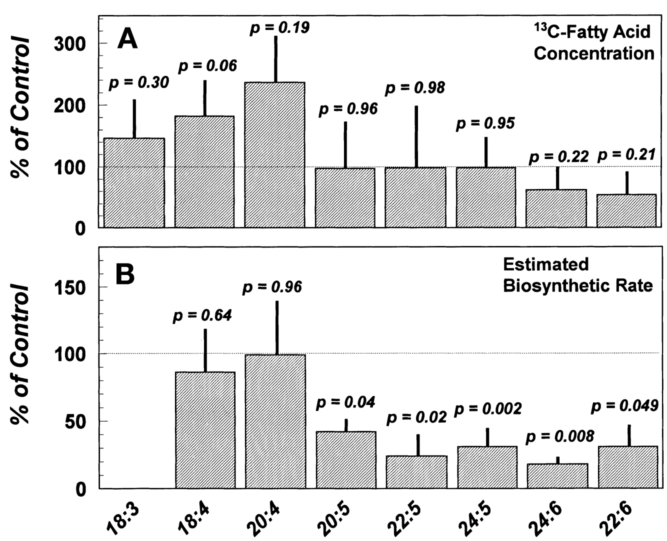

Fig. 2. A: Integrated plasma concentrations of ^{13}C-labeled ω3 fatty acids, and (B) estimated [^{13}C]fatty acid biosynthetic rates in XLRP patients compared with controls. Bars represent mean (±SD, vertical black lines) values of integrated [^{13}C]fatty acid concentrations (AUC) from XLRP patients as a percentage of control. Control AUC values (nmol/ml plasma/504 h) for [^{13}C]18:3ω3 were 720 ± 340; [^{13}C]18:4ω3, 29 ± 12; [^{13}C]20:4ω3, 82 ± 61; [^{13}C]20:5ω3, 120 ± 58; [^{13}C]22:5ω3, 62 ± 18; [^{13}C]24:5ω3, 7 ± 2; [^{13}C]24:6ω3, 10 ± 4; and [^{13}C]22:6ω3, 37 ± 20. Estimated biosynthetic rates of ^{13}C-labeled fatty acids were determined by the linear regression slope of the leading edge of [^{13}C]fatty acid accumulation curves as a function of time up to peak enrichment. Estimated biosynthetic rates for XLRP patients are given as percentage of control; control values (pmol/ml plasma/h) for [^{13}C]18:4ω3 were 258 ± 139; [^{13}C]20:4ω3, 277 ± 181; [^{13}C]20:5ω3, 72 ± 38; [^{13}C]22:5ω3, 22 ± 12; [^{13}C]24:5ω3, 5.1 ± 1.4; [^{13}C]24:6ω3, 5.6 ± 2.8; and [^{13}C]22:6ω3, 0.82 ± 0.51. Significant differences are identified by *P* values. Data for [^{13}C]18:3ω3 is omitted, as it results from accumulation rather than synthesis.

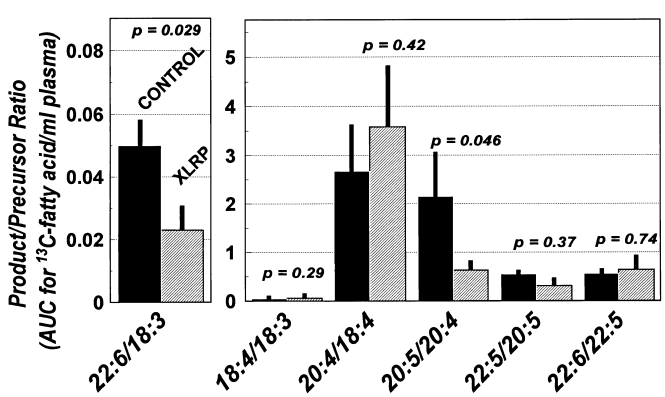

Fig. 3. Product/precursor ratios of ^{13}C-labeled ω3 fatty acids. Values are means (±SD, vertical black lines) and derived from integrated AUC values for [^{13}C]fatty acid concentrations in plasma of controls (solid bars) and patients with XLRP (hatched bars). Significant differences are identified by *P* values.

TABLE 2. Estimated rates of ^{13}C-labeled fatty acid disappearance from plasma

ω3 Fatty Acid	Controls (n = 5)	XLRP Patients (n = 5)	*P*
	pmol/ml plasma/h		
18:3ω3	3,530 ± 2,690	2,340 ± 1,090	0.38
18:4ω3	62 ± 35	47 ± 27	0.46
20:4ω3	20 ± 15	23 ± 14	0.77
20:5ω3	3.3 ± 1.9	3.8 ± 2.8	0.76
22:5ω3	1.0 ± 0.4	0.6 ± 0.6	0.25
24:5ω3	0.21 ± 0.13	0.11 ± 0.10	0.21
24:6ω3	0.23 ± 0.01	0.07 ± 0.02	0.003
22:6ω3	0.42 ± 0.50	0.06 ± 0.06	0.15

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

most labeled intermediates beyond the Δ^5-desaturase step, including DHA, appeared to be slower in XLRP patients.

Oxidation of [U-^{13}C]α-LNA was assessed by differences in breath $^{13}CO_2$ of patients with XLRP versus controls following administration of the tracer (**Fig. 4**). Breath $^{13}CO_2$ (nmol/min/kg body weight) of both patients and controls peaked 4 h post-dose and returned to baseline within about 36 h; peak $^{13}CO_2$ enrichment of the two groups did not differ significantly (P = 0.37). Cumulative breath $^{13}CO_2$ excretion, expressed as percentage of the [U-^{13}C]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 [^{13}C]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 Δ^5-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 $^{13}CO_2$ in the breath. Although by no means conclusive, the [^{13}C]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 [^{13}C]DHA administration.

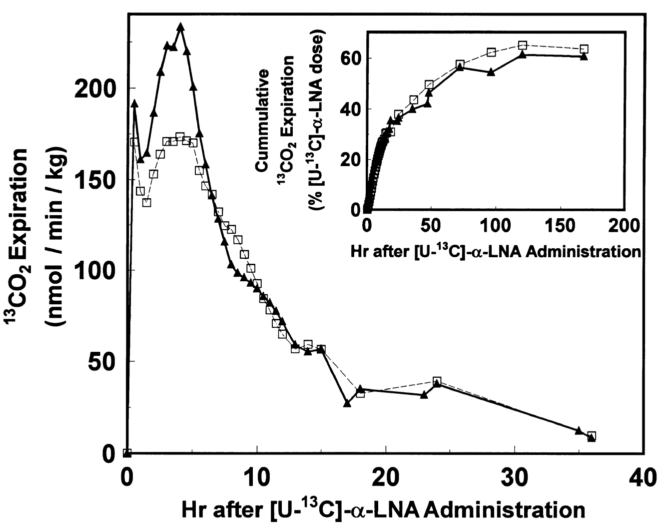

Fig. 4. Expiration rate of $^{13}CO_2$ following tracer administration in controls (solid triangles and solid lines) and in patients with XLRP (open squares and dashed lines). Inset: cumulative expiration of $^{13}CO_2$, given as percentage of tracer dose.

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 [^{13}C]DHA was consistently lower in patients with XLRP than in controls.

A break in metabolic activity at the Δ^5-desaturase step was evident in XLRP patients from integrated [^{13}C]fatty acid concentrations, biosynthetic activities, and product/precursor ratios. By examination of both integrated [^{13}C]24:5 and 24:6 concentrations and biosynthetic rates, it appears that the metabolic flux of ^{13}C-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 retina-specific genes are considered the 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 invokes 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, resulting in regulation of hepatic DHA production or circulatory transport mechanisms. The current finding of apparently lower Δ^5-desaturase activity in XLRP is consistent with this concept. Δ^5-Desaturase is known to be influenced by a number of circulatory and environmental factors including insulin, glucocorticoids, and dietary fatty acids (41–43). Thus, we conclude that Δ^5-desaturase activity is lower in patients with XLRP, and speculate that regulation of Δ^5-desaturase activity by factors originating from the retina or by stress-induced factors may account for down-regulation of hepatic production and/or release of DHA by the liver, resulting in diminished blood lipid levels of DHA in patients with XLRP.

This study was funded by the Foundation Fighting Blindness. Investigators were supported by the National Eye Institute (R.E.A., D.G.B.), National Institute for Child Health and Human Development (J.C.D.), Research to Prevent Blindness (R.E.A.) and the Food and Drug Administration (D.R.H.). This work has been funded, in part, with federal funds from the U.S. Department of Agriculture, Agricultural Research Service, under Cooperative Agreement No. 38-6250-1-003. The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does the mention of trade names, commercial products, or organizations imply endorsement by the United States Government. We appreciate the organizational and technical assistance of Dr. David Hachey, Beverly Romero, Deidre Capper, Malou Arnold, and Jennifer Sayne. We are also grateful to Dr. Tony Babb and Darren DeLorey of the Institute for Exercise and Environmental Medicine, Dallas, TX for determination of baseline CO_2 production, and for medical assistance from Dr. Rand Spencer (Texas Retina Associates, Dallas, TX) and from Drs. Robert Harris, Jeffrey Phillips, and Mark Fleschler of Texas Internal Medicine Associates, Dallas, TX. We are also grateful to Tyler's Restaurant of the Cooper Aerobics Center, Dallas, TX for preparation of meals. We thank our patients and control volunteers for participating in this clinical trial.

Manuscript received 19 January 2001 and in revised form 3 May 2001.

REFERENCES

1. Heckenlively, J. R. 1988. Retinitis Pigmentosa. Lippincott, Philadelphia.
2. Bird, A. C. 1975. X-linked retinitis pigmentosa. *Br. J. Ophthalmol.* **59:** 177–199.
3. Fishman, G. A., M. D. Farber, and D. J. Derlacki. 1988. X-linked retinitis pigmentosa. Profile of clinical findings. *Arch. Ophthalmol.* **106:** 369–375.
4. Birch, D. G., J. L. Anderson, and E. E. Birch. 1993. Early abnormalities of rod function in children with X-linked retinitis pigmentosa. *Clin. Vision Sci.* **8:** 329–335.
5. Hoffman, D. R. 2000. Fatty acids and visual dysfunction. *In* Fatty Acids in Foods and Their Health Implications. C. K. Chow, editor. Marcel Dekker, New York. 817–842.
6. Schaefer, E. J., S. J. Robins, G. M. Patton, M. A. Sandberg, C. A. Weigel-DiFranco, B. Rosner, and E. L. Berson. 1995. Red blood cell membrane phosphatidylethanolamine fatty acid content in various forms of retinitis pigmentosa. *J. Lipid Res.* **36:** 1427–1433.
7. Hoffman, D. R., and D. G. Birch. 1995. Docosahexaenoic acid in red blood cells of patients with X-linked retinitis pigmentosa. *Invest. Ophthal. Vis. Sci.* **36:** 1009–1018.
8. Aguirre, G., J. Alligood, P. O'Brien, and N. Buyukmichi. 1982. Pathogenesis of progressive rod-cone degeneration in miniature poodles. *Invest. Ophthalmol. Vis. Sci.* **23:** 610–630.
9. Anderson, R. E., M. B. Maude, R. A. Alvarez, G. M. Acland, and G. D. Aguirre. 1991. Plasma lipid abnormalities in the miniature poodle with progressive rod-cone degeneration. *Exp. Eye Res.* **52:** 349–355.
10. Anderson, R. E., M. B. Maude, R. A. Alvarez, G. Acland, and G. D. Aguirre. 1999. A hypothesis to explain the reduced blood levels of docosahexaenoic acid in inherited retinal degenerations caused by mutations in genes encoding retina-specific proteins. *Lipids.* **34:** S235–237.
11. Aguirre, G., G. Acland, M. Maude, and R. Anderson. 1997. Diets enriched in docosahexaenoic acid fail to correct progressive rod-cone degeneration (*prcd*) phenotype. *Invest. Ophthalmol. Vis. Sci.* **38:** 2387–2407.
12. McClellan, M., R. E. Anderson, M. B. Maude, H. Chen, M. I. Naash, R. A. Bush, P. A. Sieving, T. Matthes, D. Yasumura, and M. M. LaVail. 2000. Lower ROS docosahexaenoic acid phenotype in rats and mice with rhodopsin mutations. *Invest. Ophthalmol. Vis. Sci.* **41:** S199.
13. Fliesler, S. J., and R. E. Anderson. 1983. Chemistry and metabolism of lipids in the vertebrate retina. *Prog. Lipid Res.* **22:** 79–131.
14. Treen, M., R. D. Uauy, D. M. Jameson, V. Thomas, and D. R. Hoffman. 1992. Effect of docosahexaenoic acid on membrane fluidity and function in intact cultured Y-79 retinoblastoma cells. *Arch. Biochem. Biophys.* **294:** 564–570.
15. Litman, B. J., and D. C. Mitchell. 1996. A role for phospholipid polyunsaturation in modulating membrane protein function. *Lipids.* **31:** S193–197.
16. Benolken, R. M., R. E. Anderson, and T. G. Wheeler. 1973. Membrane fatty acids associated with the electrical response in visual excitation. *Science.* **182:** 1253–1254.
17. Wheeler, T. G., R. M. Benolken, and R. E. Anderson. 1975. Visual membranes: specificity of fatty acid precursors for the electrical response to illumination. *Science.* **188:** 1312–1314.
18. Watanabe, I., M. Kato, H. Aonuma, A. Hishimoto, Y. Naito, A. Moriuchi, and H. Okuyama. 1987. Effect of dietary alpha-linolenate/linoleate balance on the lipid composition and electroretinographic responses in rats. *In* Advances in the Biosciences. Vol. 62: Research in Retinitis Pigmentosa. E. Zrenner, H. Krastel, and H. H. Goebel, editors. Pergamon Journals Ltd., Oxford, UK. 563–570.
19. Bourre, J-M., M. Francois, A. Youyou, O. Dumont, M. Piciotti, G. Pascal, and G. Durand. 1989. The effects of dietary α-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. *J. Nutr.* **119:** 1880–1892.
20. Weisinger, H. S., A. J. Vingrys, and A. J. Sinclair. 1996. Effect of dietary n-3 deficiency on the electroretinogram in the guinea pig. *Ann. Nutr. Metab.* **40:** 91–98.
21. Neuringer, M., W. E. Connor, C. Van Patten, and L. Barstad. 1984. Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys. *J. Clin. Invest.* **73:** 272–276.
22. Neuringer, M., and W. E. Connor. 1986. n-3 Fatty acids in the

brain and retina: evidence for their essentiality. *Nutr. Rev.* **44:** 285–294.
23. Neuringer, M., W. E. Connor, D. S. Lin, L. Barstad, and S. Luck. 1986. Biochemical and functional effects of prenatal and postnatal ω3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proc. Natl. Acad. Sci. USA.* **83:** 4021–4025.
24. Uauy, R. D., D. G. Birch, E. E. Birch, J. E. Tyson, and D. R. Hoffman. 1990. Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-weight neonates. *Pediatr. Res.* **28:** 485–492.
25. Birch, D. G., E. E. Birch, D. R. Hoffman, and R. D. Uauy. 1992. Retinal development in very-low-birth-weight infants fed diets differing in omega-3 fatty acids. *Invest. Ophthalmol. Vis. Sci.* **33:** 2365–2376.
26. Birch, E. E., D. G. Birch, D. R. Hoffman, and R. Uauy. 1992. Dietary essential fatty acid supply and visual acuity development. *Invest. Ophthal. Vis. Sci.* **33:** 3242–3253.
27. Carlson, S. E., S. H. Werkman, and E. A. Tolley. 1996. Effect of long-chain n-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia. *Am. J. Clin. Nutr.* **63:** 687–697.
28. SanGiovanni, J. P., C. S. Berkey, J. T. Dwyer, and G. A. Colditz. 2000. Dietary essential fatty acids, long-chain polyunsaturated fatty acids, and visual resolution acuity in healthy fullterm infants: a systematic review. *Early Hum. Develop.* **57:** 165–188.
29. Birch, E. E., S. Garfield, D. R. Hoffman, and R. Uauy. 2000. A randomized controlled trial of early dietary supply of long chain polyunsaturated fatty acids and mental development in term infants. *Dev. Med. Child Neurol.* **42:** 174–181.
30. Tinoco, J. 1982. Dietary requirements and function of α-linolenic acid in animals. *Prog. Lipid Res.* **21:** 1–45.
31. Voss, A., M. Reinhart, S. Sankarappa, and H. Sprecher. 1991. The metabolism of 7, 10, 13, 16, 19-docosapentaenoic acid to 4, 7, 10, 13, 16, 19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. *J. Biol. Chem.* **266:** 19995–20000.
32. Luthria, D. L., B. S. Mohammed, and H. Sprecher. 1996. Regulation of the biosynthesis of 4,7,10,13,16,19-docosahexaenoic acid. *J. Biol. Chem.* **271:** 16020–16025.
33. Wang, N., and R. E. Anderson. 1993. Synthesis of docosahexaenoic acid by retina and retinal pigment epithelium. *Biochemistry.* **32:** 13703–13709.
34. Yin, F. Q., Q. Chen, and H. Sprecher. 1999. A comparison of the metabolism of [3–14C]-labeled 22- and 24-carbon (n-3) and (n-6) unsaturated fatty acids by rat testes and liver. *Lipids.* **34:** S153–156.
35. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
36. Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethyl acetals from lipids with boron tri-fluoride-methanol. *J. Lipid Res.* **5:** 600–608.
37. Hachey, D. L., B. W. Patterson, P. J. Reeds, and L. J. Elsas. 1991. Isotopic determination of organic keto acid pentafluorobenzyl esters in biological fluids by negative chemical ionization gas chromatography/mass spectrometry. *Anal. Chem.* **63:** 919–923.
38. Sprecher, H., and Q. Chen 1999. Polyunsaturated fatty acid biosynthesis: a microsomal-peroxisomal process. *Prostaglandins Leukot. Essent. Fatty Acid*s. **60:** 317–321.
39. Hoffman, D. R., R. Uauy, and D. G. Birch. 1995. Metabolism of omega-3 fatty acids in patients with autosomal dominant retinitis pigmentosa. *Exp. Eye Res.* **60:** 279–289.
40. Bazan, N. G., and E. B. Rodriguez de Turco. 1996. Alterations in plasma lipoproteins and DHA transport in progressive rod-cone degeneration (*prcd). In* Retinal Degeneration and Regeneration. S. Kato, N. N. Osborne, and M. Tamai, editors. Kugler Publications, Amsterdam. 89–97.
41. Mimouni, V., and J. P. Poisson. 1992. Altered desaturase activities and fatty acid composition in liver microsomes of spontaneously diabetic Wistar BB rat. *Biochim. Biophys. Acta.* **1123:** 296–302.
42. De Alaniz, M. J., and C. A. Marra. 1992. Glucocorticoid and mineralcorticoid hormones depress liver delta 5 desaturase activity through different mechanisms. *Lipids.* **27:** 599–604.
43. Keelan, M., M. T. Clandinin, and A. B. Thomson. 1997. Dietary lipids influence the activity of delta 5-desaturase and phospholipid fatty acids in rat enterocyte microsomal membranes. *Can. J. Physiol. Pharmacol.* **75:** 1009–1014.