# Identification of a fatty acid $\Delta^6$-desaturase deficiency in human skin fibroblasts

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**Abstract Polyunsaturated fatty acid (PUFA) utilization was investigated in skin fibroblasts cultured from a female patient with an inherited abnormality in lipid metabolism. These deficient human skin fibroblasts (DF) converted 85–95% less [1-$^{14}$C]linoleic acid (18:2n-6) to arachidonic acid (20:4n-6), 95% less [3-$^{14}$C]tetracosatetraenoic acid (24:4n-6) to docosapentaenoic acid (22:5n-6), and 95% less [1-$^{14}$C]-linolenic acid (18:3n-3) and [3-$^{14}$C]tetracosapentaenoic acid (24:5n-3) to docosahexaenoic acid (22:6n-3) than did normal human skin fibroblasts (NF). The only product formed by the DF cultures from [1-$^{14}$C]tetradecadienoic acid (14:2n-6) was 18:2n-6. However, they produced 50–90% as much 20:4n-6 as the NF cultures from [1-$^{14}$C]hexadecatrienoic acid (16:3n-6), [1-$^{14}$C]γ-linolenic acid (18:3n-6), and [1-$^{14}$C]dihomo-γ-linolenic acid (20:3n-6), PUFA substrates that contain $\Delta^6$ double bonds. DF also contained 80% more 18:2n-6 and 25% less 20:4n-6. These results suggested that DF are deficient in $\Delta^6$ desaturation. This was confirmed by Northern blots demonstrating an 81–94% decrease in $\Delta^6$-desaturase mRNA content in the DF cultures, whereas the $\Delta^5$-desaturase mRNA content was reduced by only 14%. This is the first inherited abnormality in human PUFA metabolism shown to be associated with a $\Delta^6$-desaturase deficiency. Furthermore, the finding that the 18- and 24-carbon substrates are equally affected suggests that a single enzyme carries out both $\Delta^6$ desaturation reactions in human PUFA metabolism.**—Williard, D. E., J. O. Nwankwo, T. L. Kaduce, S. D. Harmon, M. Irons, H. W. Moser, G. V. Raymond, and A. A. Spector. **Identification of a fatty acid $\Delta^6$-desaturase deficiency in human skin fibroblasts. *J. Lipid Res.* 2001.** 42: **501–508.**

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The mammalian polyunsaturated fatty acid (PUFA) metabolic pathway consists of a series of elongation and desaturation reactions that convert the 18-carbon essential fatty acids to 24-carbon intermediates, followed by a peroxisomal retroconversion reaction that forms the final 22-carbon products (1–5). Two fatty acid desaturase enzymes operate in this pathway, the $\Delta^6$- and $\Delta^5$-desaturases. The human, mouse, and rat $\Delta^6$-desaturase (6, 7), and the human $\Delta^5$-desaturase (8), have been cloned. $\Delta^5$-Desaturase acts once in the pathway, inserting a double bond into the 20-carbon intermediates formed during n-3 and n-6 PUFA metabolism. $\Delta^6$-Desaturase acts twice, once on the 18-carbon PUFA substrates and again after they are converted to 24-carbon derivatives. The first $\Delta^6$-desaturase reaction is the rate-limiting step in the conversion of linoleic acid (18:2n-6) and α-linolenic acids (18:3n-3) to the longer, more highly unsaturated members of the n-6 and n-3 PUFA families.

Both of these fatty acids can go through the entire PUFA metabolic pathway to form the final 22-carbon products (2–4). However, under most conditions, this occurs only with n-3 PUFA. The main product formed from 18:2n-6 ordinarily is arachidonic acid (20:4n-6), the principal substrate for eicosanoid synthesis. This conversion requires only the initial segment of the PUFA metabolic pathway, $\Delta^6$ desaturation of 18:2n-6 followed by chain elongation to a 20-carbon intermediate and then $\Delta^5$ desaturation (1). Thus, $\Delta^6$-desaturase usually acts only once in n-6 PUFA metabolism. On the other hand, 18:3n-3 ordinarily goes through the complete pathway because the main n-3 PUFA produced by mammalian tissues is docosahexaenoic acid (22:6n-3, DHA), the product formed by

Abbreviations: 14:2n-6, tetradecadienoic acid; 16:0, palmitic acid; 16:3n-6, hexadecatrienoic acid; 18:2n-6, linoleic acid; 18:3n-3, α-linolenic acid; 18:3n-6, γ-linolenic acid; 18:4n-3, stearidonic acid; 20:3n-6, dihomo-γ-linolenic acid; 20:4n-6, arachidonic acid; 22:4n-6, docosatetraenoic acid; 22:5n-6 or n-3, docosapentaenoic acid; 22:6n-3 or DHA, docosahexaenoic acid; 24:4n-6, tetracosatetraenoic acid; 24:5n-3 or n-6, tetracosapentaenoic acid; 24:6n-3, tetracosahexaenoic acid; DF, deficient human skin fibroblasts; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLC, gas-liquid chromatography; HPLC, high performance liquid chromatography; NF, normal human skin fibroblasts; PUFA, polyunsaturated fatty acid.

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peroxisomal β-oxidation of the 24-carbon n-3 intermediate (5). Therefore, n-3 PUFA metabolism generally involves both $\Delta^6$ desaturation steps (2, 3). Studies with rat liver microsomes indicate that a single $\Delta^6$-desaturase acts on both 18- and 24-carbon PUFA substrates (9), whereas similar studies with human malignant cell lines suggest that separate $\Delta^6$-desaturases act on these substrates (10). This possibility was heightened by the cloning of a third fatty acid desaturase gene, designated FADS3, from the region of human chromosome 11q12-q13.1 that contains the $\Delta^5$- and $\Delta^6$-desaturase genes (11). Thus, whether one or two $\Delta^6$-desaturases function in PUFA metabolism remains open to question, especially in human tissues.

Deficiencies in $\Delta^6$ desaturation have been observed in metabolic studies done with radiolabeled PUFA in several rodent cell lines (12–15), primary cultures of rat cerebellar neurons (16), and the human K562 leukemia and MCF-7 breast carcinoma cell lines (17, 18). The metabolic data suggest that the MCF-7 cells also are deficient in $\Delta^5$ desaturation (18). In addition, clinical studies suggest that a $\Delta^6$-desaturase deficiency may be present in patients with the Sjögren-Larsson syndrome, a genetic disease that can produce paraplegia and mental retardation (19), and a $\Delta^5$-desaturase deficiency was detected in two brothers with multineuronal degeneration, mental retardation, and adrenal failure (20). The evidence that desaturase deficiencies occur in these diseases is based entirely on plasma fatty acid compositional analysis by gas-liquid chromatography (GLC). No confirmatory metabolic or molecular studies were presented, and the patients with the $\Delta^5$-desaturase deficiency were subsequently demonstrated to have neonatal adrenoleukodystrophy, a peroxisomal biogenesis defect.[2]

In the present study we have investigated PUFA utilization in skin fibroblasts cultured from a female patient with clinical evidence of an inherited abnormality in fatty acid metabolism. The patient had a history of serious medical problems since shortly after birth, and GLC analysis of her plasma fatty acid composition indicated a low level of 20:4n-6 and DHA. Some symptomatic improvement occurred when her diet was supplemented with black currant seed oil and fish oil and, subsequently, when this was replaced by a mixture of 20:4n-6 and DHA. Our results indicate that the conversion of radiolabeled PUFA precursors to 20:4n-6 and DHA was markedly reduced in the patient's fibroblasts. A substantial decrease in $\Delta^6$-desaturase mRNA expression also was observed in the fibroblasts, providing evidence that the metabolic abnormality is due to an inherited deficiency in $\Delta^6$-desaturase.

## MATERIALS AND METHODS

### Clinical history

The patient is at present 9 years of age. She was the product of a normal pregnancy, labor, and delivery and weighed 3.95 kg at birth. The patient developed corneal ulceration at a few weeks of age. She also developed hematemesis and feeding intolerance. Her clinical course was characterized by persistence of the corneal ulcerations, feeding intolerance, growth failure, and skin abnormalities. The problems with her skin included cheilosis, a hyperkeratotic rash over the arms and legs, dystrophic nails, and perineal dermatitis. Secondary to her corneal ulcerations, she had marked photophobia. When seen at 4 years of age, she was a small child with marked photophobia and sparse, brittle hair with a maximum length of only a few centimeters. She had cheilosis, but dentition was normal. Hyperkeratotic lesions were present on her arms and legs, and several of her nails were dystrophic. The neurological examination was normal.

Because of abnormalities noted in her plasma fatty acid composition by GLC analysis, the patient was started on black currant seed oil, fish oil capsules, and a vitamin A supplement. Her fatty acid therapy was switched subsequently to a mixture of 20:4n-6 and DHA (Martek Biosciences, Columbia, MD). She has tolerated these dietary supplements and replacements well. Growth accelerated with this dietary supplementation, and she is now normal (10th–20th percentile) for her age. She has had some amelioration of her skin manifestations but continues to have abnormalities of her hair and nails.

### Cell culture

Eagle's minimal essential medium (MEM), L-glutamine, penicillin, streptomycin, and trypsin were purchased from Life Technologies (Grand Island, NY), nonessential amino acids and basal medium Eagle vitamins from Sigma (St. Louis, MO), and fetal bovine serum (FBS) from HyClone (Logan, UT). A skin biopsy was obtained at the Kennedy Krieger Institute (Johns Hopkins University, Baltimore, MD), and a cultured fibroblast cell line (deficient human skin fibroblasts, DF) was established. The protocol received institutional approval, and the patient's parents gave informed consent. Four normal human fibroblast (NF) lines were tested for comparison; three were maintained by the University of Iowa Cardiovascular Center Tissue Culture Laboratory (designated NF1, NF2, and NF3), and one, designated NF4, was provided by the Kennedy Krieger Institute. All the fibroblasts were grown and maintained in 75-$cm^2$ vented flasks at 37°C in Eagle's MEM containing 10% FBS (21, 22). When confluent, the cells were suspended by incubation with trypsin-ethylenediaminetetraacetic acid solution and seeded in 10-$cm^2$ wells for each experiment (21).

### Incubation and analysis

After reaching 85–90% confluence, the fibroblasts were incubated for 24 h in 10-$cm^2$ wells with Eagle's MEM containing 2% FBS. This medium was removed, and the incubations were continued for 72 h in Eagle's MEM containing 2% FBS and 5 μM fatty acid (22). The gas phase contained 5% $CO_2$. Control experiments with each radiolabeled fatty acid substrate indicated that there was no fatty acid breakdown during the 72-h incubation period in a cell-free medium. [1-$^{14}$C]18:2n-6, [1-$^{14}$C]18:3n-3, [1-$^{14}$C]γ-linolenic acid (18:3n-6), and [1-$^{14}$C]dihomo-γ-linolenic acid (20:3n-6) were purchased from American Radiolabeled Chemicals (St. Louis, MO), and the corresponding unlabeled fatty acids were obtained from Cayman Chemical (Ann Arbor, MI). H. Sprecher (Department of Medical Biochemistry, Ohio State University, Columbus, OH) generously provided [1-$^{14}$C]tetradecadienoic acid (14:2n-6), [1-$^{14}$C]hexadecatrienoic acid (16:3n-6), [3-$^{14}$C]docosapentaenoic acid (22:5n-6), [3-$^{14}$C]tetracosatetraenoic acid (24:4n-6), [3-$^{14}$C]tetracosapentaenoic acid (24:5n-3), and [3-$^{14}$C]tetracosahexaenoic acid (24:6n-3), together with the corresponding unlabeled fatty acids.

[2] A. Moser (Kennedy Krieger Institute, Johns Hopkins University, Baltimore, MD), personal communication.

At the end of the incubation the medium was removed and centrifuged at 1,000 *g* to sediment any cell debris. The supernatant solution was removed, acidified to pH 4 with formic acid, and extracted three times with 4 volumes of ethyl acetate. An aliquot of the combined ethyl acetate extracts was dried under $N_2$, dissolved in scintillator solution, and assayed for radioactivity in a liquid scintillation spectrometer (21). Quenching was monitored with the external standard.

As soon as the medium was removed, the cells were washed with warm Dulbecco's phosphate-buffered saline solution containing 0.1 μM albumin, followed immediately by ice-cold Dulbecco's solution alone. The washed cells were scraped with a rubber policeman into 2 ml of ice-cold methanol containing 1% acetic acid, 4 ml of chloroform and 1.5 ml of acidified NaCl were added, and the chloroform phase was isolated (21). After the solvent was evaporated under a stream of $N_2$, the lipid residue was suspended in 0.5 ml of a 2:1 mixture of chloroform and methanol, and the radioactivity contained in an aliquot of this mixture was measured by liquid scintillation spectrometry (21).

### Chromatography

The radioactive fatty acids contained in the medium and cell lipid extracts were separated by high performance liquid chromatography (HPLC) (22, 23). Aliquots of the medium and cell lipid extract containing 20,000–50,000 dpm were dried under $N_2$, transesterified with 12% $BF_3$ in methanol, and separated on an Alltech (Deerfield, IL) 3-μm Adsorbosphere 4.6 × 150 mm reversed-phase $C_{18}$ column (23). A Gilson (Middleton, WI) dual-pump gradient HPLC system equipped with an automatic sample injector was utilized for these separations (22). The solvent system consisted of acetonitrile and water adjusted to pH 3.4 with phosphoric acid. For most separations, the gradient began at 76% acetonitrile and was increased stepwise over 10 min to 86% acetonitrile, maintained at 86% acetonitrile for 25 min, and then increased over 2 min to 100% acetonitrile, where it was maintained for an additional 23 min. To separate 18:3n-3 from 22:6n-3, the initial 76% acetonitrile concentration was maintained for 45 min, increased over 1 min to 100% acetonitrile, and then maintained for 14 min (5). The effluent was mixed with liquid scintillation solution, and the radioactivity was measured by passing the mixture through an on-line flow scintillation detector. Radiolabeled fatty acid methyl ester standards were included with each set of chromatograms.

The fatty acid composition of the fibroblast lipids was determined by GLC (24). Margaric acid was added to each sample as an internal standard. After extraction with a chloroform-methanol 2:1 (v/v) mixture, the cell lipids were transesterified with 12% $BF_3$ in methanol at 95°C for 45 min. The methyl esters were extracted into *n*-heptane and separated with a Hewlett-Packard (Palo Alto, CA) 5890 chromatograph with a 1.9 m × 2 mm glass column containing 10% SP2330 on 100-120 mesh Chromsorb WAW (Supelco, Bellefonte, PA). The carrier gas was $N_2$ at a flow rate of 25 ml/min. Fatty acids were detected by flame ionization at 250°C, and they were identified by comparison of retention times with fatty acid methyl ester standards.

### $\Delta^6$-Desaturase and $\Delta^5$-desaturase mRNA analysis

Total RNA was extracted from two confluent 75-$cm^2$ cultures of the DF cell line and each NF cell line, and 10 μg was separated by electrophoresis in 1.2% agarose gels containing 2.2 M formaldehyde. Separate experiments with different cultures of the cells were done to measure $\Delta^6$- and $\Delta^5$-desaturase expression. The DF cells were compared with three separate NF cell lines in the $\Delta^6$-desaturase experiment, and another passage of DF cells was compared with two of the NF cell lines in the $\Delta^5$-desaturase experiment. After transfer to nylon membranes (GeneScreen; DuPont NEN, Boston, MA) in an NaCl-sodium citrate solution (10×), the samples were cross-linked with ultraviolet light, using a Stratalinker 1800 (Stratagene, La Jolla, CA) in the Autocrosslink mode. cDNA probes for $\Delta^6$- and $\Delta^5$-desaturase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts were, respectively, 204-, 202-, and 876-base pair (bp) sequences synthesized by reverse transcriptase-polymerase chain reaction using previously published primers (6, 8, 25). Sequences were further cloned with a Qiagen (Valencia, CA) TA cloning kit. Cloned sequences were confirmed by analysis in the University of Iowa (Iowa City, IA) DNA Core. Before their use as probes in Northern blot analysis, the plasmids were digested with *Eco*RI and the cDNA fragments were purified from 1.5% agarose gels with a Qiagen gel extraction kit. The cDNA probes were $^{32}P$ labeled with a Prime-it II random priming kit (Stratagene) according to the manufacturer's instructions. Blots were prehybridized, hybridized, and washed according to standard techniques. After autoradiography with the desaturase probes, each blot was stripped according to the manufacturer instructions and probed again with the $^{32}P$-labeled GAPDH sequence to enable relative quantification of the $\Delta^6$- and $\Delta^5$-desaturase transcripts.

## RESULTS

### 18:2n-6 utilization

The utilization of [1-$^{14}$C]18:2n-6 was compared in NF and DF cultures. These cultured fibroblasts accumulated roughly equivalent amounts of radiolabeled 18:2n-6 during a 72-h incubation with 5 μM [1-$^{14}$C]18:2n-6. However, a substantial decrease in the conversion of the 18:2n-6 to radiolabeled fatty acid products occurred in the DF cultures as determined by reversed-phase HPLC analysis of the hydrolyzed cell lipid extract (**Fig. 1**). The three NF cell lines that were tested converted 8–10% of the incorporated radioactivity to 20:3n-6, 12–19% to 20:4n-6, and 2–3% to 22:4n-6 (Fig. 1A–1C). By contrast, the DF cells converted only 2% of the incorporated radioactivity to 20:3n-6, 3% to 20:4n-6, and 1% to 22:4n-6 (Fig. 1D). Values as low as 1% for the conversion of [1-$^{14}$C]18:2n-6 to 20:4n-6 were obtained in additional experiments with the DF cultures.

The culture medium was isolated at the end of the incubation, and the lipids were extracted and analyzed for radiolabeled fatty acids by HPLC. Much of the radioactivity in the medium remained as 18:2n-6, but small amounts of the radiolabeled fatty acid products also were detected. However, in every case, the qualitative difference between the NF and DF cultures was the same as noted in the cells. An analysis of the medium was included in experiments with all of the other fatty acids tested, and as observed with [1-$^{14}$C]18:2n-6, the results obtained from the medium reflected what was observed in the cells.

### Utilization of other n-6 PUFA

A deficiency in $\Delta^6$ desaturation could account for the inability of the DF cultures to effectively convert 18:2n-6 to 20:4n-6. To investigate this possibility further, the utilization of [1-$^{14}$C]14:2n-6 and [1-$^{14}$C]16:3n-6 was compared in DF and NF cultures. This pair of fatty acids was selected because [1-$^{14}$C]14:2n-6 requires $\Delta^6$ desaturation for con-

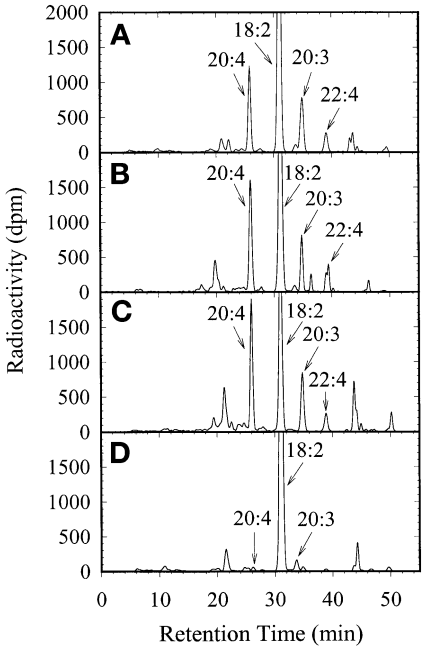


**Fig. 1.** Radiolabeled fatty acids contained in the cell lipids of fibroblast cultures incubated with [1-$^{14}$C]18:2n-6. The time of incubation was 72 h, and the [1-$^{14}$C]18:2n-6 concentration was 5 μM. After the cell lipids were extracted and transmethylated, the radiolabeled material was analyzed by high performance liquid chromatography (HPLC) with an on-line flow liquid scintillation detector. The compounds were identified by comparison of retention times with those of radiolabeled fatty acid methyl ester standards included with each set of analyses. The HPLC tracings shown are from the normal human skin fibroblast cell lines NF1 (A), NF2 (B), NF3 (C), and the deficient human skin fibroblasts (DF) cell line cultured from the patient (D). A single chromatogram is shown for each of the cell lines, but almost identical chromatograms were obtained from at least two additional cultures in every case.

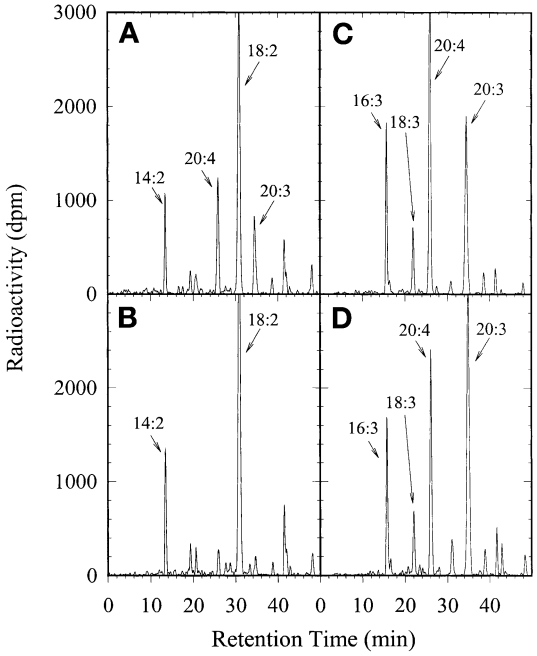


**Fig. 2.** Radiolabeled fatty acids contained in the cell lipids of fibroblast cultures incubated with 14- and 16-carbon n-6 PUFA substrates. The experimental design, analysis, number of replicates, and reproducibility were the same as described in Fig. 1. The n-6 PUFA tested were as follows: (A and B) [1-$^{14}$C]14:2n-6; (C and D) [1-$^{14}$C]16:3n-6. These radiolabeled PUFA were incubated with normal human skin fibroblast cultures from the NF1 cell line (A and C) or the DF cell line obtained from the patient (B and D).

version to 20:4n-6, whereas [1-$^{14}$C]16:3n-6 contains a $\Delta^6$ double bond and, therefore, does not require $\Delta^6$ desaturation. **Figure 2** illustrates the results. The main fatty acid formed from [1-$^{14}$C]14:2n-6 in the NF cultures was 18:2n-6, but substantial amounts of radiolabeled 20:3n-6 and 20:4n-6 also were produced during a 72-h incubation (Fig. 2A). The DF cultures also converted [1-$^{14}$C]14:2n-6 to 18:2n-6, a sequence that requires two chain elongations, but only trace amounts of radiolabeled 20:3n-6 and 20:4n-6 were formed (Fig. 2B). By contrast, the NF (Fig. 2C) and DF cultures (Fig. 2D) converted a large fraction of the [1-$^{14}$C]16:3n-6 uptake to 20:3n-6 and 20:4n-6. These results are consistent with the presence of a $\Delta^6$ desaturation deficiency in the DF cultures. They also demonstrate that chain elongation is not reduced in the DF cultures. However, more radiolabeled 20:4n-6 than 20:3n-6 was formed from [1-$^{14}$C]16:3n-6 by the NF than the DF cultures, suggesting that the DF cultures also might have some reduction in $\Delta^5$ desaturation.

Studies were done with additional n-6 PUFA to evaluate further the possibility of an associated decrease in $\Delta^5$ desaturation. The results are shown in **Fig. 3**. When the NF cultures were incubated with [1-$^{14}$C]18:3n-6 for 72 h, 26% of the radioactivity taken up by the cells was converted to 20:3n-6 and 57% was converted to 20:4n-6 (Fig. 3A), amounting to 83% of the total radioactivity retained in the cells. The sum of the 20:3n-6 and 20:4n-6 in the cells also accounted for 83% of the uptake when the DF cultures were incubated with [1-$^{14}$C]18:3n-6 (Fig. 3B), again indicating that chain elongation is not reduced. However, as noted with [1-$^{14}$C]16:3n-6 (Fig. 2C and D), the distribution of radioactivity between 20:3n-6 and 20:4n-6 in the DF cell lipids was reversed as compared with the NF cells. Twice as much was present as 20:3n-6 and half as much as 20:4n-6 in the DF cultures.

To test more directly the possibility of a partial reduction in $\Delta^5$ desaturation, studies were done with [1-$^{14}$C]-20:3n-6, the substrate for $\Delta^5$-desaturase. Both cultures converted almost the same amount of [1-$^{14}$C]20:3n-6 to 20:4n-6, 40% of the uptake in the NF cultures (Fig. 3C) and 35% in the DF cultures (Fig. 3D). These results indicate that factors other than $\Delta^5$ desaturation must be responsible for the substantial decrease in 20:4n-6 production observed when the DF cultures were incubated with [1-$^{14}$C]16:3n-6 or [1-$^{14}$C]18:3n-6.

Studies also were done to determine whether a decrease in $\Delta^6$ desaturation could be detected in the DF cultures with the 24-carbon n-6 PUFA substrate. As shown in Fig. 3E, the NF cultures converted 3% of the [3-$^{14}$C]24:4n-6 uptake to radiolabeled 24:5n-6 and 17% to 22:5n-6 in a 72-h incubation. These conversions require $\Delta^6$ desaturation. Although the DF cells retained 55% more of the incorporated radioactivity as 24:4n-6, they converted only 1% of

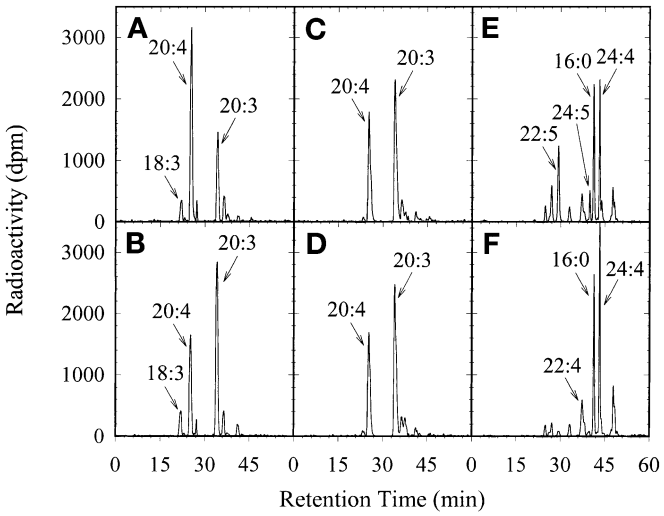


**Fig. 3.** Radiolabeled fatty acids contained in the cell lipids of fibroblast cultures incubated with 18-, 20-, and 24-carbon n-6 PUFA substrates. The experimental design, analysis, number of replicates, and reproducibility were the same as described in Fig. 1. The n-6 PUFA tested were as follows: (A and B) [1-$^{14}$C]18:3n-6; (C and D) [1-$^{14}$C]20:3n-6; (E and F) [3-$^{14}$C]24:4n-6. These radiolabeled PUFA were incubated with normal human skin fibroblast cultures from the NF1 cell line (A, C, and E) or the DF cell line obtained from the patient (B, D, and F).

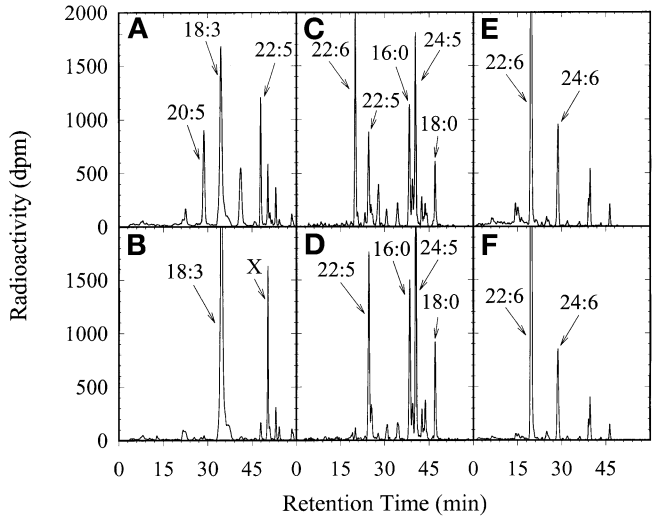


**Fig. 4.** Radiolabeled fatty acids contained in the cell lipids of fibroblast cultures incubated with n-3 PUFA substrates. The experimental design, analysis, number of replicates, and reproducibility were the same as described in Fig. 1. The n-3 PUFA tested were as follows: (A and B) [1-$^{14}$C]18:3n-3; (C and D) [3-$^{14}$C]24:5n-3 (E and F) [3-$^{14}$C]24:6n-3. These radiolabeled PUFA were incubated with normal human skin fibroblast cultures from the NF2 cell line (A, C, and E) or the DF cell line obtained from the patient (B, D, and F).

the uptake to 22:5n-6 and produced no radiolabeled 24:5n-6 (Fig. 3F). However, the DF and NF cells converted similar amounts of [3-$^{14}$C]24:4n-6 to radiolabeled docosatetraenoic acid (22:4n-6) and palmitic acid (16:0), indicating that β-oxidation was not defective in the DF cultures.

### Utilization of n-3 PUFA

As is shown in **Fig. 4**, the $\Delta^6$ desaturation of n-3 PUFA also was reduced in the DF cultures. While the NF cultures converted 19% of the [1-$^{14}$C]18:3n-3 uptake to 20:5n-3 and 17% to 22:5n-3 in a 72-h incubation (Fig. 4A), the DF cultures did not produce these products. Instead, they formed a single major radiolabeled product designated X, with a retention time of 50 min (Fig. 4B). This product did not comigrate with any of our labeled standards, but on the basis of previous studies (16), compound X probably is 20:3n-3, the elongation product of 18:3n-3.

The utilization of the 24-carbon n-3 PUFA intermediates also was investigated. The main radiolabeled product formed by the NF cultures from [3-$^{14}$C]24:5n-3 was 22:6n-3 (Fig. 4C), whereas the DF cultures produced only trace amounts of 22:6n-3 (Fig. 4D). This conversion requires an initial $\Delta^6$ desaturation step. However, both NF and DF cultures produced large amounts of radiolabeled 22:5n-3 and 16:0 from the [3-$^{14}$C]24:5n-3, reactions that require β-oxidation but no desaturation. Likewise, there was no difference in the amount of [3-$^{14}$C]24:6n-3 converted to 22:6n-3 by the NF (Fig. 4E) and DF cultures (Fig. 4F), a reaction that requires only β-oxidation. These findings indicate that the lesser conversion of 24:5n-3 to 22:6n-3 by the DF cultures is due to a decrease in $\Delta^6$ desaturation, not to a decrease in retroconversion.

### Fatty acid composition

The fatty acid composition of the DF and NF cell lipids grown in medium containing 2% FBS was compared by GLC (**Table 1**). There was little difference in their content

TABLE 1. Fatty acid composition of total cell lipids extracted from fibroblast cultures[a]

| Fatty Acid[b] | Amount[c] | |
|---|---|---|
| | NF2 Cell Line | DF Cell Line |
| | *% of Total* | |
| 14:0 | 1.2 ± 0.3 | 1.2 ± 0.3 |
| 16:0 | 19.8 ± 1.1 | 21.5 ± 0.2 |
| 16:1 | 3.7 ± 0.4 | 3.3 ± 0.5 |
| 18:0 | 22.4 ± 1.0 | 23.7 ± 1.3 |
| 18:1 | 22.4 ± 0.5 | 21.9 ± 0.9 |
| 18:2 | 2.6 ± 0.1 | 4.7 ± 0.2[d] |
| 18:3 | 0.6 ± 0.2 | 0.6 ± 0.2 |
| 20:3 | 1.9 ± 0.1 | 2.0 ± 0.1 |
| 20:4 | 11.6 ± 0.4 | 8.8 ± 0.3[e] |
| 20:5 | 0.3 ± 0.1 | 0.2 ± 0.1 |
| 22:4 | 3.9 ± 0.2 | 4.2 ± 0.1 |
| 22:5 | 1.4 ± 0.1 | 1.7 ± 0.1 |
| 22:6 | 4.2 ± 0.7 | 3.4 ± 0.3 |
| Others[f] | 4.0 | 2.7 |

[a] The lipids extracted from confluent NF2 and DF cultures in 10-cm$^2$ wells were transmethylated and analyzed by GLC. The fatty acids were identified by comparison of retention times with those of fatty acid methyl ester standards.

[b] The fatty acids are abbreviated as number of carbons:number of double bonds.

[c] Mean ± SEM of values obtained from five separate cultures.

[d] $P < 0.001$.

[e] $P < 0.005$.

[f] Others consists of 14:1, 20:1, 22:1, 24:0, and 24:1.

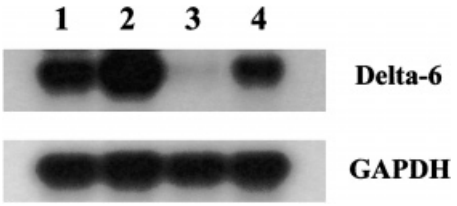


**Fig. 5.** $\Delta^6$-Desaturase mRNA expression in fibroblast cell lines. Total RNA extracted from two 75-cm$^2$ cultures of each cell line was combined and assayed by Northern blotting. GAPDH mRNA was utilized as a control for RNA loading. The cultures tested are as follows: lane 1, NF1; lane 2, NF3; lane 3, DF; lane 4, NF4. These results are from a single experiment in which total RNA isolated from two 75-cm$^2$ cultures of each cell line was combined. Similar results were obtained in two additional experiments in which the DF cell line obtained from the patient was compared with the NF2 cell line.

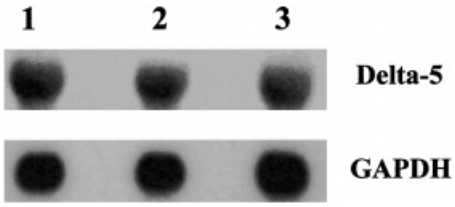


**Fig. 6.** $\Delta^5$-Desaturase mRNA expression in fibroblast cell lines. The procedure was the same as described in Fig. 5, except that only one experiment was done. The fibroblast cultures tested are as follows: lane 1, NF3; lane 2, DF; lane 3, NF1.

of total saturated, monounsaturated, or PUFA. However, the DF lipids contained 80% more 18:2n-6 ($P < 0.001$) and 25% less 20:4n-6 ($P < 0.005$) than the NF lipids, findings consistent with a $\Delta^6$-desaturase deficiency. Although the mean value for 22:6n-3 also was 25% lower in the DF lipids, the difference was not significant ($P > 0.05$).

### $\Delta^6$-Desaturase and $\Delta^5$-desaturase mRNA levels

**Figure 5** shows representative Northern blots of $\Delta^6$-desaturase mRNA in DF and three of the NF cell lines. Densitometric analysis indicated that, relative to the GAPDH mRNA content, the DF cells contained 86% less $\Delta^6$-desaturase mRNA than the NF1 cells, 94% less than the NF3 cells, and 81% less than the NF4 cells. These findings are consistent with the reduction in $\Delta^6$-desaturase metabolic activity observed in the DF cultures.

Similar studies were done to measure $\Delta^5$-desaturase mRNA content in the DF and two of the NF cell lines, and representative Northern blots are shown in **Fig. 6**. This work was done with passages of the DF and NF cells different from those tested for $\Delta^6$-desaturase. Densitometric analysis normalized to the GAPDH mRNA intensities indicated that the DF cells contained 15% less $\Delta^5$-desaturase mRNA than the NF2 cells and 13% less than the NF3 cells. Thus, unlike $\Delta^6$-desaturase, the $\Delta^5$-desaturase content was only slightly reduced in the DF cells.

## DISCUSSION

These results demonstrate that the DF cells have a major deficiency in $\Delta^6$ desaturation. **Figure 7** illustrates the mammalian n-6 PUFA metabolic pathway (1, 4). Eight PUFA make up this pathway, ranging from 18- to 24-carbon molecules. The reactions catalyzed by the $\Delta^6$- and $\Delta^5$-desaturases and the points where 14:2n-6 and 16:3n-6 enter the pathway are indicated. Both segments of the pathway that require $\Delta^6$ desaturation, the conversion of 18:2n-6 to 20:4n-6 and the conversion of 24:4n-6 to 22:5n-6, were severely depressed in the DF cells. Likewise, 14:2n-6 was elongated only to 18:2n-6 by the DF cells, whereas appreciable amounts of 14:2n-6 were converted to 20:3 and 20:4 by the NF cells. By contrast, both cells readily converted 16:3n-6, which does not require $\Delta^6$ desaturation to proceed through the first segment of the pathway, to 20:3 and 20:4. These results further establish the presence of a deficiency in $\Delta^6$ desaturation in the DF cells. The pathway shown in Fig. 7 also is utilized by n-3 PUFA (1–3). As noted with n-6 PUFA, the n-3 PUFA conversions dependent on $\Delta^6$ desaturation, 18:3n-3 to 20:5n-3 and 24:5n-6 to 22:6n-3, were severely depressed in the DF cultures. Thus, the deficiency in $\Delta^6$ desaturation in the DF cells occurs with n-6 and n-3 PUFA at both the 18- and 24-carbon segments of the metabolic pathway.

These findings help to resolve the uncertainty as to whether human tissues contain chain length-specific $\Delta^6$-desaturases. Results obtained with rat liver microsomes demonstrated competition for $\Delta^6$ desaturation between the 18- and 24-carbon PUFA, suggesting that a single enzyme acts on both substrates (9). However, data with two human cell lines, Y79 retinoblastoma and Jurkat T cell leukemia, were interpreted to indicate that separate $\Delta^6$-desaturases act on the 18- and 24-carbon PUFA substrates (10). Therefore, the possibility that human tissues, unlike rat liver (9), have chain length-specific $\Delta^6$-desaturases could not be excluded. The cloning of FADS3, a third de-

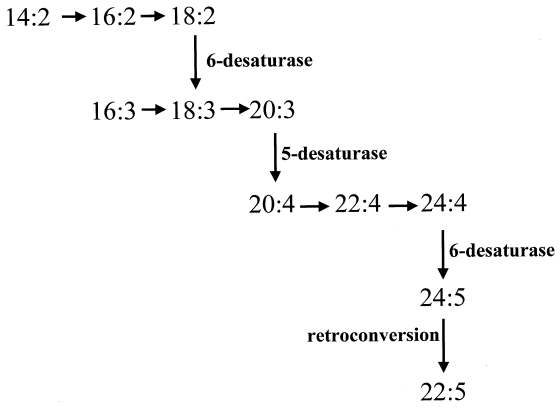


**Fig. 7.** Schematic representation of the n-6 PUFA metabolic pathway in mammalian cells. This scheme is adapted from the review by Sprecher et al. (1). The steps in the pathway where the $\Delta^6$- and $\Delta^5$-desaturases act, and the points where 14:2n-6 and 16:3n-6 enter the pathway, are shown. This pathway also is utilized by n-3 PUFA (1, 2).

saturase gene located in the same region of human chromosome 11q12-q13.1 as the $\Delta^5$- and $\Delta^6$-desaturases (11), further suggested that two human $\Delta^6$-desaturases may exist. This possibility seems unlikely in view of the present finding that the desaturation of 18:2n-6, 18:3n-3, 24:4n-6, and 24:5n-3 is reduced to a similar extent in an inherited disease. The most probable explanation is that human tissues, like rat liver, contain only one $\Delta^6$-desaturase. This is consistent with the conclusion of Marquardt et al. (11) that FADS3 is unlikely to represent a second human $\Delta^6$- or $\Delta^5$-desaturase.

The presence of a $\Delta^6$-desaturase deficiency explains the decreases in 20:4n-6 and DHA observed in the patient's plasma and in her cultured fibroblasts. It also explains the symptomatic improvement produced by the dietary lipid supplements. Black currant seed oil contains 15–19% 18:3n-6 (26). Because 18:3n-6 has a $\Delta^6$ double bond, it does not require $\Delta^6$ desaturation for conversion to 20:4n-6 (1). Previous studies demonstrated that cultured cells with low $\Delta^6$-desaturase activity are able to convert [1-$^{14}$C]18:3n-6 to 20:4n-6 (27), and this was confirmed in the DF cultures (Fig. 2C). Therefore, the patient was apparently able to produce enough 20:4n-6 from the 18:3n-6 contained in the black currant seed oil to obtain some symptomatic improvement.

Black currant seed oil also contains 3–4% stearidonic acid (18:4n-3), an n-3 PUFA that, like 18:3n-6, contains a $\Delta^6$ double bond (26). However, an additional $\Delta^6$ desaturation step, the conversion of 24:5n-3 to 24:6n-3, is necessary to produce DHA from 18:4n-3 (1–3). DHA is the n-3 PUFA that is highly enriched in the brain and retina, and it is required for normal neural and retinal function (28–34). Because of the $\Delta^6$-desaturase deficiency, the patient apparently was unable to synthesize enough DHA from the 18:4n-3 contained in the black currant seed oil to satisfy the functional requirement. This probably explains why the addition of fish oil capsules, which contain 10–16% DHA (35), produced further symptomatic improvement.

We cannot fully explain why 30% less [1-$^{14}$C]16:3n-6 and 50% less [1-$^{14}$C]18:3n-6 were converted to 20:4n-6 by the DF cultures. Because the sum of the radiolabeled 20:3 and 20:4 produced from [1-$^{14}$C]16:3n-6 and [1-$^{14}$C]18:3n-6 was the same in the DF and NF cultures, chain elongation cannot be the cause. Furthermore, there was only a 10% reduction in [1-$^{14}$C]20:3n-6 conversion to radiolabeled 20:4n-6 in the DF cells, a finding consistent with the 15% decrease in $\Delta^5$-desaturase mRNA content that was observed in these cells. This small decrease may occur because the $\Delta^5$- and $\Delta^6$- desaturase genes are located in reverse orientation within about 10,000 bp of each other on human chromosome 11q12-q13.1 and are coordinately regulated (8, 11). Although the reduced $\Delta^5$-desaturase activity in the DF cultures may contribute to the decreased conversion of 16:3n-6 and 18:3n-6 to 20:4n-6, it appears to be too small to be responsible for the entire effect.

To our knowledge this is the first observation of an abnormality in human PUFA metabolism shown to be associated with a deficiency in the expression of the $\Delta^6$-desaturase gene. A previous report indicated that a defect in $\Delta^6$ desaturation occurs in the Sjögren-Larsson syndrome (19), an autosomal recessive disease caused by a deficiency in the oxidation of fatty alcohols to fatty acids (36, 37). The evidence of a $\Delta^6$ desaturation defect in this syndrome was based entirely on GLC analysis of plasma fatty acids (19). Although the clinical picture in our patient was not consistent with the Sjögren-Larsson syndrome, we thought it necessary in view of the previous report to determine whether she might have this metabolic defect. Therefore, the fatty aldehyde dehydrogenase activity of the DF cultures was measured (36, 37). The value obtained, 12,600 pmol/min/mg protein, is within the normal range and 20 times higher than those obtained in Sjögren-Larsson syndrome fibroblasts.[3] This indicates that the deficiency in $\Delta^6$ desaturation in our patient is unrelated to the Sjögren-Larsson syndrome and constitutes a distinct genetic abnormality.

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[3] This assay was performed by W. B. Rizzo (Department of Pediatrics, Virginia Commonwealth University, Richmond, VA). Dr. Rizzo also provided the data for 22 normal control subjects and 70 Sjögren-Larsson syndrome fibroblasts.

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