

## Therapeutic effects of docosahexaenoic acid ethyl ester in patients with generalized peroxisomal disorders<sup>1-3</sup>

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**ABSTRACT** Generalized peroxisomal disorders are severe congenital diseases that involve the central nervous system, leading to severe psychomotor retardation, retinopathy, liver disease, and early death. In these disorders, peroxisomes are not normally formed and their enzymes are deficient. Characteristically, plasmalogen synthesis and  $\beta$ -oxidation of very-long-chain fatty acids (VLCFAs) are affected. We found that patients with generalized peroxisomal disorders have a profound brain deficiency of docosahexaenoic acid (DHA; 22:6n-3) and low DHA concentrations in all tissues and the blood. Given the fundamental role of DHA in neuronal and retinal membranes, a DHA deficiency of this magnitude might be pathogenic. Thus, we studied the possible therapeutic effect of normalizing DHA concentrations in patients with peroxisomal disorders. We chose the DHA ethyl ester (DHA-EE) because of its high degree of purity at daily oral doses of 100–500 mg. This article summarizes the results of treatment of 13 patients with DHA-EE, with some follow-up evidence of clinical improvement. Supplementation with DHA-EE normalized blood DHA values within a few weeks. Plasmalogen concentrations increased in erythrocytes in most patients and after DHA concentrations were normalized, amounts of VLCFAs decreased in plasma. Liver enzymes returned almost to normal in most cases. From a clinical viewpoint, most patients showed improvement in vision, liver function, muscle tone, and social contact. In 3 patients, normalization of brain myelin was detected by magnetic resonance imaging. In 3 others, myelination improved. In a seventh patient, myelination is progressing at a normal rate. These results suggest a fundamental role of DHA in the pathogenesis of Zellweger syndrome. DHA therapy is thus strongly recommended, not only to alleviate symptoms in patients with life-threatening diseases, but also to clarify remaining questions regarding the role of DHA in health and disease. *Am J Clin Nutr* 2000;71(suppl):376S–85S.

**KEY WORDS** Docosahexaenoic acid, Zellweger syndrome, peroxisomal disorders, magnetic resonance imaging, plasmalogens, very-long-chain fatty acids

### INTRODUCTION

Microbodies or peroxisomes are single membrane-bound organelles, 0.2–1  $\mu$ m in diameter, that are distributed ubiquitously in most cells in nature. Microbodies were first described

by Rhodin (1) in rat kidneys. In plants, microbodies oxidize a variety of substrates, producing hydrogen peroxide (2), which is later degraded by catalase. Because of this ability to form hydrogen peroxide, microbodies were named *peroxisomes* (2), which is now the most widely used term. In mammals, peroxisomes were considered metabolically unimportant until Goldfischer et al (3) discovered the virtual absence of these organelles in the liver and proximal tubules of the kidneys in 2 patients with Zellweger (cerebrohepato renal) syndrome. This is a fatal congenital disease, first described clinically in 1964 (4), that involves the brain, liver, retina, adrenal glands, bones, and kidneys. Constant clinical features are severe hypotonia from birth, feeding difficulty, failure to thrive, convulsions, psychomotor retardation, blindness, and death very early in infancy. The lack of peroxisomes in such a severe disease was thus considered pathogenic and the multisystemic metabolic failure was attributed to the defective peroxisomal enzymes.

Investigation in this field has been active during the past 2 decades. Today, many enzymes are known to be located in peroxisomes, several of which are related to lipid metabolism. In particular,  $\beta$ -oxidation of very-long-chain fatty acids (VLCFA; ie, fatty acids with  $\geq 24$  carbon atoms) is carried out in the peroxisomes (5). When the chain length has been reduced to 22 carbon atoms,  $\beta$ -oxidation can proceed in the mitochondrion. At the starting point of peroxisomal  $\beta$ -oxidation, the membrane-bound peroxisomal enzyme acyl-CoA synthetase (long-chain-fatty-acid-CoA ligase) activates LCFAs (fatty acids with 12–22 carbon atoms) to their corresponding acyl-CoA derivatives. It has been suggested that a different synthetase activates LCFAs and VLCFAs and that a deficiency of the latter may be the cause for the  $\beta$ -oxidation defect in X-linked adrenoleukodystrophy (6), another peroxisomal disorder with apparently a single enzyme

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<sup>2</sup>The docosahexaenoic acid ethyl ester used in this study was obtained with a grant from the Biomedical Tests Materials Program (NIH/DOC), Bethesda, MD. Some extra amounts of docosahexaenoic acid ethyl ester were kindly provided by Harima Chemicals (Japan) and Pronova Biocare (Norway).

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deficiency. A difference between mitochondrial and peroxisomal  $\beta$ -oxidation is that the former requires carnitine for the passage of fatty acids across the inner mitochondrial membrane; in the latter, carnitine only facilitates the output of the  $\beta$ -oxidation end products. Another difference relates to the enzymes involved in the  $\beta$ -oxidation cycle. In the mitochondrion, the double bond in the fatty acyl group is hydrated and dehydrogenated in the second and third  $\beta$ -oxidation steps by 2 different enzymes (a hydratase and a dehydrogenase). In peroxisomes, these same reactions are catalyzed by a single, bifunctional enzyme.

Other lipids oxidized in peroxisomes are long-chain dicarboxylic acids, pristanic acid, prostaglandins, and the side chain of cholesterol (7). The  $\alpha$ -oxidation step in the degradation of phytanic acid seems also to be a peroxisomal reaction (8). Besides these catabolic functions, peroxisomes carry out important anabolic reactions. In particular, the first 2 steps of plasmalogen biosynthesis are carried out in peroxisomes (9), catalyzed by the enzymes glycerone-phosphate *O*-acyltransferase (dihydroxyacetonephosphate acyltransferase) and alkyldihydroxyacetonephosphate synthetase (alkylglycerone-phosphate synthase). Plasmalogen synthesis is completed in the endoplasmic reticulum. Other peroxisomal anabolic reactions include cholesterol and bile acid biosynthesis.

In Zellweger syndrome, deficient peroxisomal  $\beta$ -oxidation leads to increases in the concentrations of saturated and monounsaturated VLCFAs, especially 26:0 and 26:1 (5, 10). Because concentrations of 22:0 are reduced (11), the ratios of 26:0 to 22:0 and of 26:1 to 22:0 are markedly increased in plasma and have diagnostic value. Plasmalogen synthesis is affected because of a defect in dihydroxyacetonephosphate acyltransferase, which can be measured in tissues (12, 13). The low concentrations of plasmalogens can be easily quantitated in erythrocytes. For diagnostic purposes, the plasmalogen decrease is most simply estimated by the ratio of the plasmalogen dimethyl acetals (DMAs) to the corresponding fatty acid methyl ester, ie, 16:0DMA to 16:0 and 18:0DMA to 18:0 (14). The defective  $\beta$ -oxidation of pristanic acid and deficient  $\alpha$ -oxidation of phytanic acid lead to the accumulation of these 2 ramified fatty acids in plasma (15, 16). Bile acid synthesis is also affected, resulting in increases in abnormal metabolites (17).

Classic Zellweger syndrome is rapidly progressive and death occurs during the first months of life. Postmortem examination of the brain reveals gliosis, neuronal heterotopias, and myelin abnormalities. There are also milder variants of the Zellweger syndrome within the group of generalized peroxisomal disorders. Neonatal adrenoleukodystrophy (18) and infantile Refsum disease (19) are the most important Zellweger variants and are characterized by mental retardation, hypotonia, progressive neurosensory deterioration with early blindness and deafness, failure to thrive, and hepatomegaly. Myelination is usually affected in generalized peroxisomal disorders. For example, in classic Zellweger syndrome myelin is never formed properly (dysmyelination). In contrast, some myelin can be formed during the first months of life in the milder forms of the disease. Whether this myelin has an abnormal composition is not known. However, our experience indicates that if a patient with a generalized peroxisomal disorder lives long enough, brain demyelination will most likely appear.

Classically, treatment of patients with peroxisomal disorders has consisted of trying to correct some of the metabolic abnormalities. Thus, attempts have been made to decrease the concentrations of VLCFAs and of phytanic acid through fat-restricted diets, similar to those used to treat X-linked adrenoleukodystro-

phy (20). Plasmapheresis was used in one patient to decrease the concentrations of phytanic acid in the blood (21). To compensate for the deficit in plasmalogen synthesis, plasmalogen precursors were given (22), and administration of cholic acid was recommended as a means of both inhibiting the synthesis of abnormal metabolites and providing normal bile acids (23). Neither of these treatments, however, produced clear clinical improvements in the few patients in whom they were tested.

We discovered a dramatic deficiency of docosahexaenoic acid (DHA; 22:6n-3) in the brain, retina, liver, kidneys, and blood of patients with peroxisomal disorders (24-26). DHA is a polyunsaturated fatty acid (PUFA) localized in brain phospholipids and the photoreceptor cells of the retina and that seems to play a crucial role in these tissues (27, 28). A DHA deficiency of the degree found in patients with peroxisomal disorders might, therefore, be an important cause of the neurologic and visual involvement in these patients. Thus, we have assayed the possible therapeutic effects of DHA ethyl ester (DHA-EE) in patients with peroxisomal disorders since 1991 (29-32). The encouraging results obtained, both biochemically and clinically, seem to confirm the crucial role of DHA in the pathogenesis of these diseases. This article presents the results obtained in 1 patient with classic Zellweger syndrome and in 12 patients with milder Zellweger variants in whom marked DHA deficiency was detected in the blood.

## SUBJECTS AND METHODS

### Patients

Because peroxisomal disorders are fatal and the severe DHA deficiency in these patients may contribute to their brain damage, we did not use a randomized, double-blind study design. For ethical reasons, all patients were treated as soon as their DHA deficiency was discovered. The results discussed here are for 13 patients with generalized peroxisomal disorders, of whom 1 had the most severe form of the disease known as classic Zellweger syndrome. This male patient was 5 mo old when treatment was initiated. He was in critical condition and had frequent episodes of bronchopneumonia. He had craniofacial dysmorphism with complete diastasis of sutures, convulsions, and severe hypotonia and had to be fed by a nasogastric tube.

It is difficult to classify the other 12 patients because the diagnosis of peroxisomal disorders is mainly dependent on disease severity, which is partly dependent on age. Patients with infantile Refsum disease live longer and are less severely affected than patients with neonatal adrenoleukodystrophy. In patients with neonatal adrenoleukodystrophy, the leukodystrophy can be detected by magnetic resonance imaging (MRI) of the brain but, again, detection depends on the age of the patient. In very young children, MRI scans are difficult to interpret because hypomyelination or a delay in myelination cannot be clearly distinguished from demyelination. When present, demyelination is not evident until 2 y of age or later. Thus, classification based solely on clinical examination may be misleading. A patient seeming to be mildly affected and with a normal MRI scan and in whom infantile Refsum disease is diagnosed may later develop leukodystrophy of the type found in neonatal adrenoleukodystrophy and start to deteriorate rapidly. Classification based on complementation analysis (33) has not solved the problem either because there is much overlap



between the different clinical forms, with some extreme diagnoses belonging to the same complementation group.

Because of the difficulty in diagnosing these disorders, we simply classified our patients with generalized peroxisomal disorders into 2 wide diagnostic groups: those with classic Zellweger syndrome and those with Zellweger variants in which some phenotypes are more severe than others. Shown in **Table 1** for the 13 patients studied is each patient's initial clinical diagnosis by a physician, in addition to the patients' ages, clinical findings, and duration of treatment.

### Treatment

The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983. The therapeutic protocol was approved by the ethical committee of our institution and each case was supervised by the Spanish Ministry of Health as a special, expeditious form of therapeutic trial called "Compassionate Use of a New Drug" (in which rapid approval is given by the Ministry of Health for clinical trials involving patients with life-threatening illnesses). The DHA-EE was diluted in pure olive oil and distributed into individual monodose vials that were sealed under nitrogen. Each vial contained between 50 and 500 mg DHA-EE in a total volume of 2 mL. This total volume was found to be easily given and was not rejected by the children. Diets were not restricted during the treatment period but nutrition was as complete as possible for each patient's age. Only lean meat and poultry were provided, but fatty fish and standard dairy products were permitted. A commercial formula with DHA and arachidonic acid contents similar to those in breast milk (0.3% and 0.44% of total fat, respectively; Milupa, Friedrichsdorf, Germany) was given to all small infants not being breast-fed. The intake of solid food in the other patients made the use of such a formula unnecessary.

### Biochemical methods

The total fatty acid and plasmalogen composition of plasma and erythrocytes was studied by capillary column gas chromatography. The fatty acid methyl esters and plasmalogen DMAs were obtained by direct transesterification (34) of red blood cell and plasma lipids with hydrogen chloride-methanol as described previously (35). This method resulted in high recoveries of all the compounds studied, including PUFAs, VLCFAs, and DMAs. In the particular case of erythrocytes, however, special care was taken to perform the analyses as soon as possible and we avoided freezing the cells because disruption of erythrocyte membranes leads to substantial decreases in PUFAs, mainly DHA.

An aliquot of the benzene phase containing the fatty acid methyl esters and DMAs (1–3  $\mu$ L) was analyzed directly by capillary column gas chromatography with a gas chromatograph (model 5890; Hewlett-Packard, Palo Alto, CA) and a 30-m, 0.25-mm internal diameter RTX-2330 column (Restek, Bellefonte, PA). The carrier gas was helium, at a column head pressure of  $\approx$ 105 kPa. Samples were analyzed at split ratios of 15:1 to 50:1 and the column was programmed to change temperature from 140°C to 200°C by 3°C/min or by using a 2-step program: from 140°C to 180°C at 4°C/min and from 180°C to 200–210°C at 2 or 2.5°C/min, depending on the batch and state of the column. These programs separated the whole spectrum of fatty acids: VLCFAs, PUFAs, and plasmalogen DMAs.

To verify the separation and purity of the peaks, especially of the VLCFAs, another capillary column (25 m, 0.25 mm internal diam-

eter) with a different polarity (BPX70; SGE, Victoria, Australia) was also used. This column also separated all the compounds studied, with a temperature program of 140–210°C at 4°C/min and a column head pressure of  $\approx$ 105 kPa (carrier gas: helium). Injector and detector temperatures were 260°C in all cases. Detector response linearity was periodically checked with quantitative standard mixtures, and variation in detector response was found to be <10% for the major fatty acid methyl esters. Currently, we prefer to use 2 internal standards with widely different molecular weights (13:0 and 23:0 or 27:0) because linearity can vary at any time, especially after the injection of erythrocyte samples.

Peaks were measured with D-2500 and D-2000 computer integrators (Hitachi Ltd, Tokyo) and identified by comparison of peak retention times with those of pure standards and of mixtures of known composition. When necessary, the identification of a peak was confirmed by mass spectrometry of the DMAs, methyl esters, or picolinyl esters (36) with a mass-selective detector (model 5970B; Hewlett-Packard). Spectra were obtained at an ionization potential of 70 eV. In addition to the fatty acid analyses, blood concentrations of cholesterol, aspartate aminotransferase (EC 2.6.1.1), alanine aminotransferase (EC 2.6.1.2), and  $\gamma$ -glutamyltransferase (EC 2.3.2.2) were measured periodically with a Mega autoanalyzer (Merck, Darmstadt, Germany).

### Statistical analysis

Statistical analyses were performed with STATVIEW II (Abacus Concepts, Berkeley, CA).

## RESULTS

The duration and result of treatment in all 13 patients is summarized in Table 1. The patient with classic Zellweger syndrome died early of a pulmonary disease and thus was treated for only 3 mo. In a 3-y-old girl (patient 10) in whom treatment was initiated late (when the child was already in a vegetative state complicated by frequent episodes of bronchopneumonia), DHA-EE could be given for only 6 wk (29). The other 10 patients were treated for periods of from 8 mo to 6 y. All patients experienced some clinical improvement, the most consistent being improvements in liver function and vision.

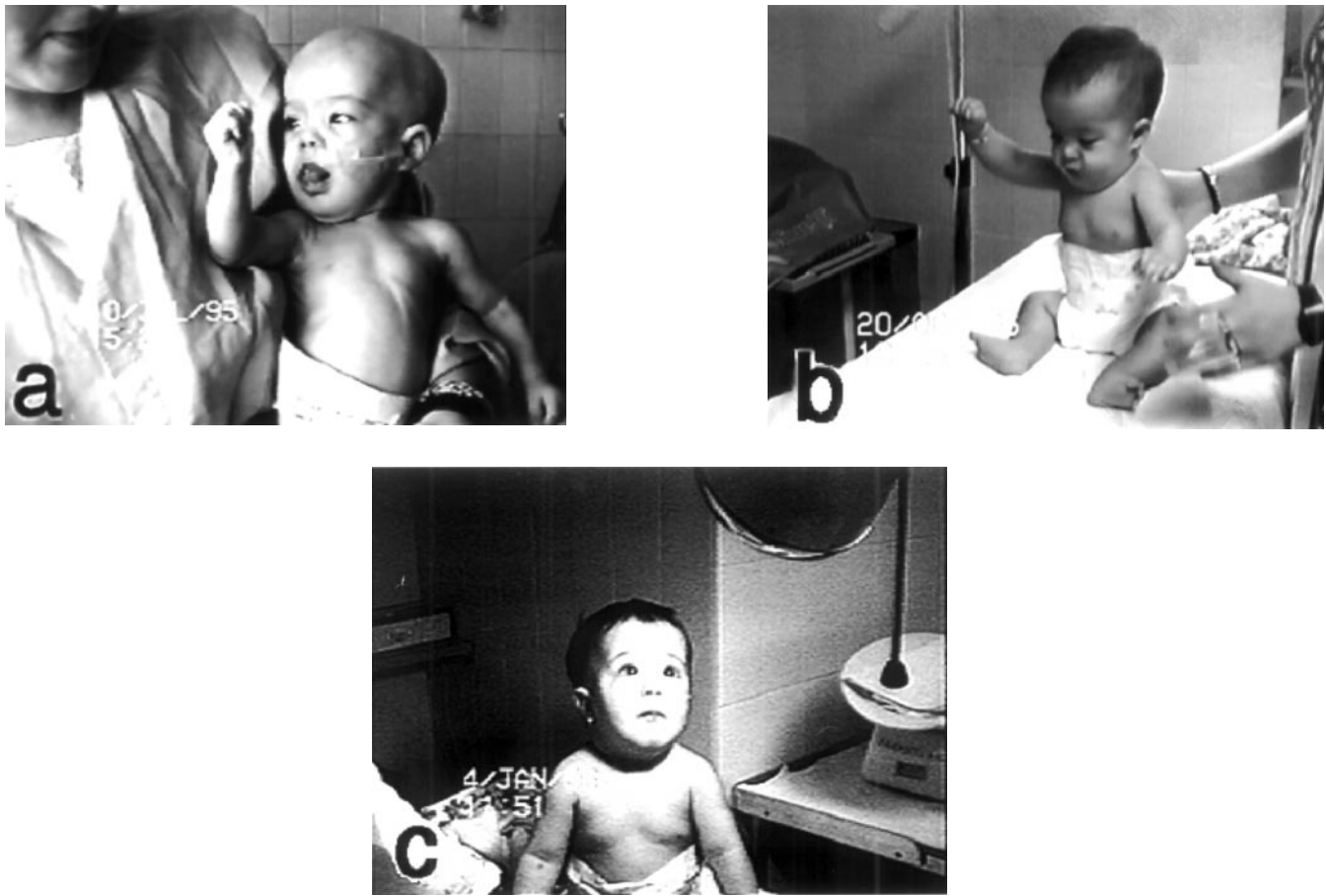
Spectacular responses were observed in 2 undernourished children with marked failure to thrive (patients 2 and 5). On initiation of treatment (at 5 and 9 mo of age, respectively), these patients gained weight quickly and their psychomotor development accelerated. Liver function improved dramatically in both patients. Before treatment was initiated, the youngest of these patients (**Figure 1A**) had severe hepatic involvement, with marked jaundice and cholestasis, and required continuous drip feeding through a nasogastric tube to avoid hypoglycemia. This child weighed only 3900 g. After a few days, the child could be fed normally and started to gain weight quickly; in a few weeks, the activity of her liver enzymes had returned virtually to normal (**Figure 2**). After 3 mo of treatment with DHA-EE, the child's muscle tone and strength improved and she could sit unsupported (**Figure 1B**). Her body weight doubled within 6 mo. Apparently blind before the treatment, the child could follow a light source 5 mo later (**Figure 1C**). The other patient also experienced dramatic improvements in body weight, vision, hearing, and psychomotor development during the first year of treatment (30). Unfortunately, this child later died unexpectedly of fulminant septicemia.

**TABLE 1**

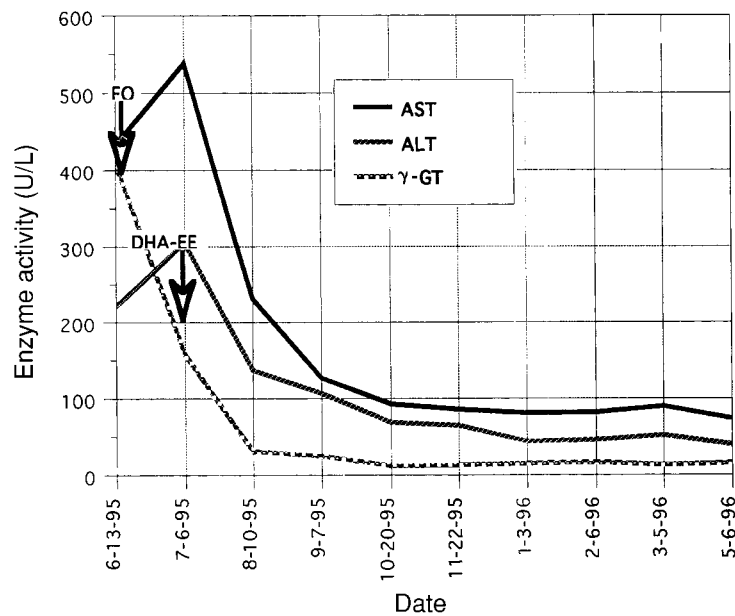
Summary of the clinical findings and evolution in patients treated with docosahexaenoic acid ethyl ester for various lengths of time<sup>1</sup>

Patient	Age	Diagnosis	Initial clinical status	Pretreatment MRI findings	Pretreatment vision	Daily dose <i>mg</i>	Duration of treatment	Clinical evolution	MRI evolution
1, male	5 mo	Classic ZS	Severe hypotonia, convulsions, sensorially nonresponsive, and hepatosplenomegaly	Neuronal heterotopias, polymicrogyria-pachygyria, and dysmyelination	No response, even to strong light	100–400	3 mo	Only biochemical improvement, died shortly from bronchopneumonia	No MRI follow-up
2, female	5 mo	IRD	Severe liver involvement, marasmus, hypoglycemia, and continuous drip fed	Within normal limits	No response to light, no tracking of objects, strabismus, and abnormal eye movements	200–400	3 y	Dramatic improvement of liver function and nutritional status, good muscle tone, tracks light, starts walking	Normal myelin progression
3, male	8 mo	ZS	Liver cirrhosis, hypotonia, severe psychomotor delay, and nasogastric tube fed	Hypomyelination	No response to light, no tracking of objects, nystagmus, and strabismus	200–600	3 y	Visual and biochemical improvement, better muscle tone	Myelin normalization
4, female	8 mo	NALD	Severe hypotonia and liver involvement, hypoglycemia, and psychomotor retardation	Within normal limits	No response to light, no tracking of objects, nystagmus, and strabismus	200–400	2 y, 5 mo	Slight psychomotor progress and biochemical improvement, starts tracking light	No MRI follow-up
5, female	9 mo	ZS	Severe hypotonia and undernutrition, psychomotor retardation, and hepatomegaly	Within normal limits	Very poor, delayed response to light, nystagmus, and strabismus	100–300	1 y, 5 mo	Dramatic clinical and biochemical improvement, died unexpectedly of fulminant septicemia	No MRI follow-up
6, female	9 mo	ZS	Hypotonia, developmental and psychomotor delay, and hepatomegaly	Hypomyelination	No response to light or eye pursuit and conjugated deviation of the eyes	200–400	4 y	Visual, psychomotor, and biochemical improvement; starts walking	Myelin normalization
7, male	15 mo	NALD	Axial hypotonia, mental retardation, and hepatosplenomegaly	Hypomyelination	No response to light, nystagmus, and abnormal eye movements	200–400	8 mo	Visual, psychomotor, and biochemical improvement	Myelin improvement
8, male	15 mo	IRD	Axial hypotonia, hypoglycemia, mental retardation, and hepatomegaly	Hypomyelination and demyelination	Poor eye contact, nystagmus, and abnormal eye movements	200–400	3 y	Visual, psychomotor, and biochemical improvement	Myelin improvement
9, male	16 mo	IRD	Severe sensorial damage, psychomotor retardation, and hepatosplenomegaly	Hypomyelination	No response, even to strong light, and conjugated deviation of the eyes	200–400	2 y	Slight psychomotor progress, biochemical improvement	Marked myelin improvement
10, female	3 y, 4 mo	NALD	Terminal, vegetative state; areflexia; and tetraplegia	Hypoplasia of corpus callosum and demyelination	Totally nonresponsive, even to strong light	250	6 wk	No clinical response, biochemical improvement, died of respiratory infection	No MRI follow-up
11, female	5 y	IRD	Spasticity, polyneuropathy, mental retardation, and hepatomegaly	Active demyelination and cortical atrophy	Response to strong light and very poor tracking of objects	200–500	4 y	Visual, psychomotor, and biochemical improvement	Demyelination halted, slight remyelination
12, female	5 y, 7 mo	NALD	Severe liver involvement, tetraplegic, vegetative state, fed by gastrostomy	Demyelination and cortical atrophy	No response, even to strong light, and nystagmus	200–400	2 y, 5 mo	Slightly more alert, dramatic liver improvement, biochemical improvement	No MRI follow-up
13, male	6 y, 7 mo	NALD	Liver cirrhosis, failure to thrive, spasticity, and psychomotor retardation	Massive demyelination of forebrain, pons, and cerebral peduncles	Poor, slow eye pursuit, and nystagmus	250–500	7 y	Improvement of vision, hearing, and spasticity in upper limbs; biochemical improvement	MRI stabilization

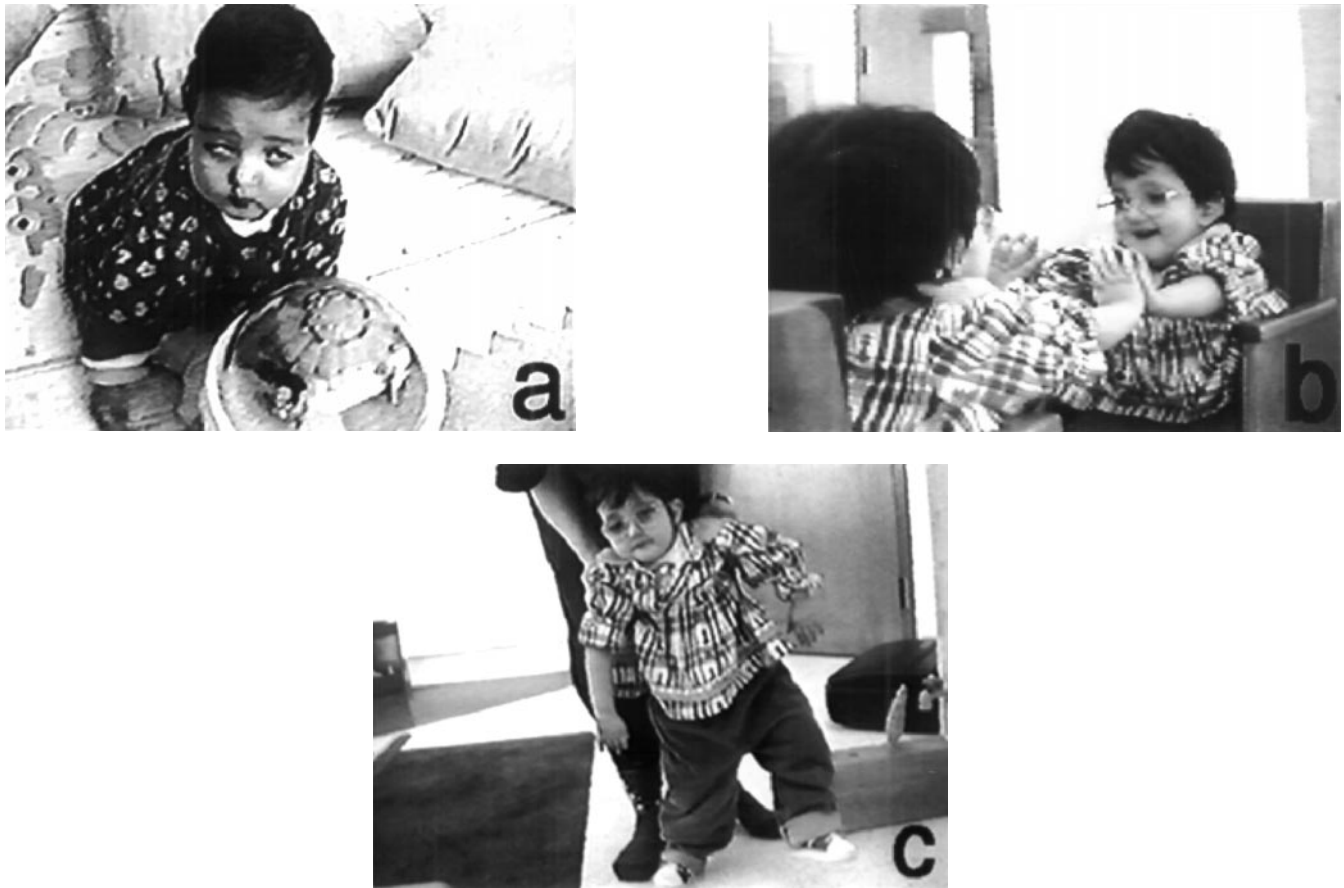
<sup>1</sup>Age is that at the start of treatment. The diagnosis is that made by the physician who referred the patient. The dose of docosahexaenoic acid ethyl ester given was adjusted according to docosahexaenoic acid concentration changes in the blood within the range indicated. Doses <50 mg were given only during the first days of treatment. MRI, magnetic resonance imaging; ZS, Zellweger syndrome; IRD, infantile Refsum disease; NALD, neonatal adrenoleukodystrophy.



**FIGURE 1.** A patient with infantile Refsum disease (patient 2 in Tables 1 and 2) before (A) and after 3 mo (B) and 5 mo (C) of treatment with 200 mg docosahexaenoic acid ethyl ester/d. The nutritional status and liver function (see also Figure 2) of the patient improved dramatically. Although she cannot track objects, the child can now see light and bright color.



**FIGURE 2.** Liver enzyme activity of a patient with infantile Refsum disease (patient 2 in Tables 1 and 2) throughout treatment with docosahexaenoic acid ethyl ester (DHA-EE). Fish oil was provided for  $\approx$ 2 wk until DHA-EE was available. The arrows show the dates when treatment with fish oil (FO) and treatment with DHA-EE were initiated. AST, aspartate aminotransferase; ALT, alanine aminotransferase;  $\gamma$ -GT,  $\gamma$ -glutamyltransferase.



**FIGURE 3.** A patient with a mild Zellweger variant (patient 6 in Tables 1 and 2). At 8 mo of age (A), the child could not see the toys or even strong light (note the conjugate deviation of the eyes upward). At 15 mo, she could follow a light source and at 19 mo, she tracked objects. At 3 y of age, she could look at herself in the mirror (B) and walk a few steps with help (C).

The other 8 patients also improved, more or less markedly, during treatment. Some of these results are reported elsewhere (31). Especially noteworthy was the visual and auditory improvement of a girl (patient 11) who was already 5 y old when treatment was started. The initial MRI scans in this patient showed active demyelination. The patient walked with difficulty and fell down often. After 2 y of DHA therapy, however, the patient fell down less often and could get up from the floor unsupported. MRI scans showed not only that the demyelination process had been halted but also a tendency toward remyelination (32).

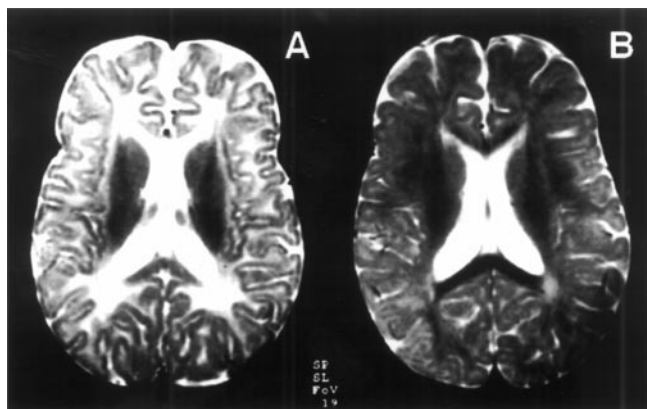
Another patient with a milder Zellweger variant (patient 6), who was apparently blind and had conjugated deviation of the eyes (**Figure 3A**), started to track light after 6 mo of treatment with DHA-EE and fixed on objects after 10 mo of treatment. She can now look at herself in the mirror (**Figure 3B**). Her muscle tone also improved and she recently began to walk (**Figure 3C**).

A significant finding in patient 6 and in 2 other patients was a normalization of brain myelin as shown by MRI (**Figure 4, A and B**). One of the other patients was an infant with a severe Zellweger variant (patient 3) in whom classic Zellweger syndrome had been diagnosed on the basis of his craniofacial dysmorphism and clinical status. At 8 mo, this patient had severe liver disease, was virtually blind, and needed to be fed by a

nasogastric tube. After a few weeks of DHA-EE treatment, however, the child could be fed normally. In a few months, his liver function improved markedly and he showed improvements in vision, muscle tone, and social contact (31). After 2 y, his weight leveled off but his growth did not. Later, it was shown that much of his weight problem was due to chronic tonsillitis that interfered with his nutritional state. After his tonsils were removed the child started to thrive and gain weight quickly. At 8 and 16 mo of age, this patient's brain was clearly hypomyelinated (**Figure 5A**). At 3 y of age, however, his brain myelin was normal for his age (**Figure 5B**).

In a third patient (patient 9) who began treatment late (at 16 mo of age), MRI showed a marked delay in myelination and some possible areas of demyelination (**Figure 6A**). After only 10 mo of DHA-EE treatment, MRI scans showed marked remyelination, especially of the frontal lobes (**Figure 6B**).

The most significant biochemical changes in the patients studied are summarized in **Table 2**. Treatment with DHA-EE normalized erythrocyte DHA concentrations within a few weeks. This was more or less expected but other findings were not. In parallel with DHA normalization, plasmalogen concentrations increased, even in the patients with classic Zellweger syndrome. In patients with slightly decreased red blood cell plasmalogen concentrations, plasmalogen ratios (16:0DMA to 16:0 and 18:0DMA to 18:0) returned to normal with treatment and the correlation



**FIGURE 4.** Magnetic resonance imaging scans of a patient with a mild variant of Zellweger syndrome (patient 6 in Tables 1 and 2). Shown are T2-weighted images obtained at the level of the lateral ventricles when the patient was 9 (A) and 18 (B) mo old. At 9 mo, the cerebral white matter was scarcely myelinated, with a myelination pattern roughly corresponding to an age of 5 mo. At 18 mo (after 9 mo of treatment with docosahexaenoic acid ethyl ester), there was obvious progress in myelination of the frontal white matter. Only some unmyelinated areas were seen in the occipital lobes, corresponding to the location of the so-called terminal zones.

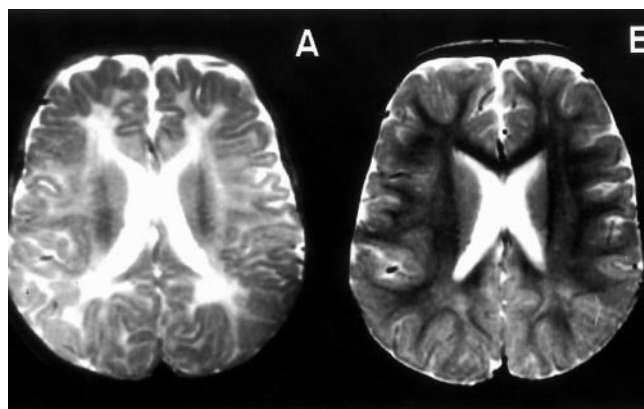
between the ratio of 18:0DMA to 18:0 and DHA concentrations was highly significant in erythrocytes (Figure 7). Note that concentrations of VLCFAs in plasma usually decreased during DHA therapy and that the ratios of 26:0 to 22:0 and of 26:1 to 22:0 fell abruptly in most cases (Figure 8 and Table 2). This is specially significant considering that DHA therapy was given in conjunction with complete, nutritious diets, resulting in much higher intakes of VLCFAs than before the treatment.

## DISCUSSION

The origin of the DHA deficiency in peroxisomal disorders is unknown. One plausible explanation for the low DHA concentrations in patients with defective  $\beta$ -oxidation of VLCFAs is provided by the proposed pathway for DHA biosynthesis (37). According to this pathway, DHA is produced by  $\beta$ -oxidation of the very-long-chain PUFA tetracosahexaenoic acid (24:6n-3). Such a mechanism of retroconversion from 24:6n-3 should explain the low DHA concentrations in patients with generalized peroxisomal disorders as well as X-linked adrenoleukodystrophy and other  $\beta$ -oxidation disorders. However, real DHA deficiency occurs only in generalized peroxisomal disorders and not in X-linked adrenoleukodystrophy (35). On the other hand, the decrease in VLCFA concentrations in patients with generalized peroxisomal disorders treated with DHA, despite normal VLCFA intake, suggests that the DHA deficiency may be the cause rather than the consequence of defective  $\beta$ -oxidation.

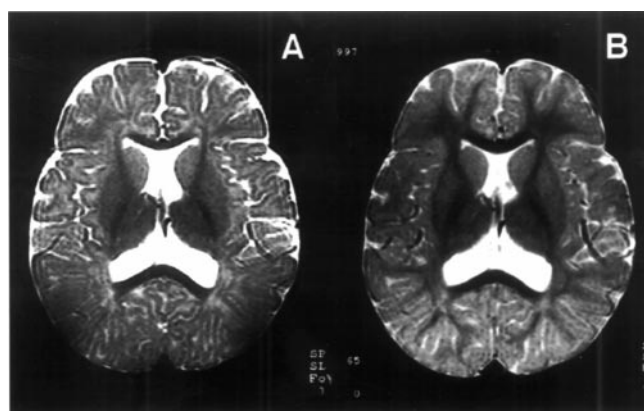
Whatever the mechanism of DHA synthesis and its deficiency in peroxisomal disorders, the relation between DHA administration and improvement in some diagnostic biochemical indexes is intriguing. It is possible that DHA is necessary for the formation of peroxisomal membranes, the activity of peroxisomal enzymes, or both. Perhaps the biogenesis of peroxisomes is linked to a normal DHA content at the molecular level.

Even more significant are the beneficial clinical effects in patients receiving DHA therapy, suggesting that DHA is involved



**FIGURE 5.** Magnetic resonance imaging scans of a patient with Zellweger syndrome (patient 3 in Tables 1 and 2). Shown are T2-weighted images obtained at the level of the lateral ventricles when the patient was 8 (A) and 36 (B) mo old. At 8 mo, note that most of the white matter was unmyelinated, with a hyperintense signal on the T2 sequence. At 36 mo (after 29 mo of treatment with docosahexaenoic acid ethyl ester), images obtained at the same level show reduced signal intensity (representing myelination) of the white matter diffusely throughout the brain, with an appearance identical to a mature brain.

in the pathogenesis of generalized peroxisomal disorders at a fundamental level. In particular, normalization of brain myelin in a disease usually leading to progressive demyelination suggests an important role for DHA in myelinogenesis, both in health and disease. Thus, given the ominous prognosis of these patients, the excellent tolerance of this treatment, and the absence of any other efficient treatments, DHA therapy is strongly recommended in patients with generalized peroxisomal disorders. Treatment



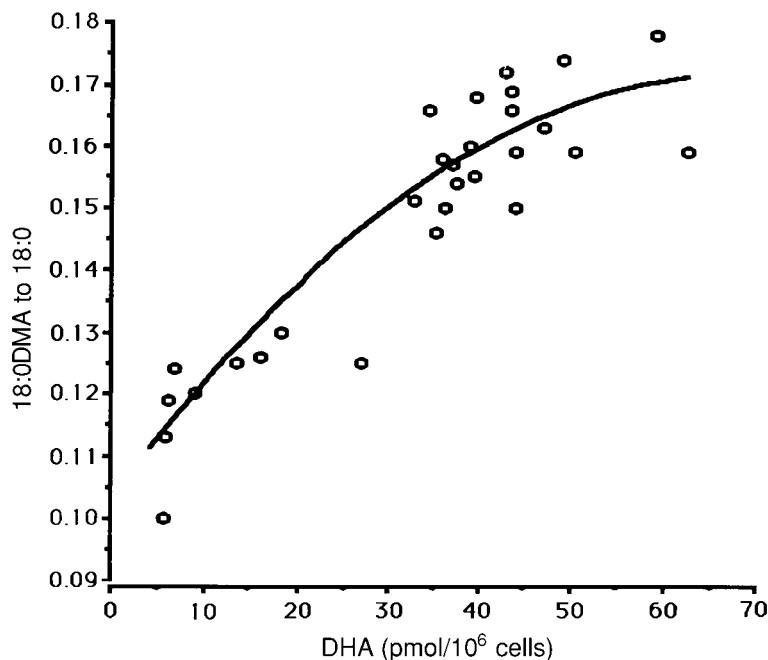
**FIGURE 6.** Magnetic resonance imaging scans of a patient with infantile Refsum disease (patient 9 in Tables 1 and 2). Shown are axial T2-weighted images obtained at the level of the lateral ventricles when the patient was 16 (A) and 26 (B) mo old. At 16 mo, myelination was scarce and was limited to the anterior and posterior limbs of the internal capsule, corpus callosum, and paracentral areas. The frontal and occipital white matter have a high signal intensity suggestive of demyelination. At 26 mo (after 10 mo of treatment with docosahexaenoic acid ethyl ester), there was significant progression in the maturation of the white matter. Low signal intensity was increased because of myelination, with fine arborization of the subcortical white matter. This feature is more obvious in the frontal lobes, with some patchy high signal intensity in the occipital regions.

**TABLE 2**

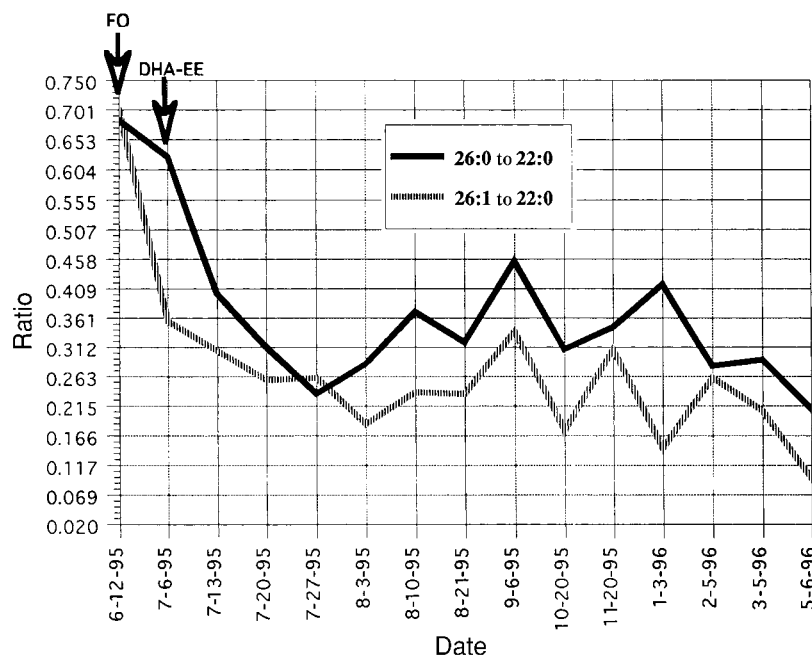
Fatty acid and plasmalogen changes in plasma and erythrocytes in 13 patients with peroxisomal disorders treated with docosahexaenoic acid ethyl ester<sup>1</sup>

	Plasma								Erythrocytes					
	18:2n-6	20:4n-6	20:5n-3	22:5n-3	22:6n-3	24:0 to 22:0	26:0 to 22:0	26:1 to 22:0	18:2n-6	20:4n-6	20:5n-3	22:5n-3	22:6n-3	18:0 DMA to 18:0
Patient 1	<i>μmol/L</i>								<i>pmol/10<sup>6</sup> cells</i>					
Pretreatment	1780.4	211.8	7.3	3.6	8.0	1.893	0.520	0.915	109.0	66.9	0.4	2.8	1.7	0.019
3 mo	2339.1	348.9	12.8	7.9	62.0	2.014	0.360	0.369	89.7	67.5	1.2	3.9	12.2	0.025
Patient 2														
Pretreatment	1875.8	201.6	5.4	2.3	2.6	2.523	0.683	0.701	114.5	80.9	1.1	2.2	1.8	0.111
19 mo	817.3	125.9	9.3	8.8	85.4	1.379	0.190	0.164	80.9	68.3	3.8	6.9	42.0	0.131
Patient 3														
Pretreatment	1126.7	147.9	6.5	8.7	2.2	2.219	0.379	0.347	97.0	57.6	1.6	6.7	1.2	0.087
32 mo	655.4	164.6	16.6	9.9	89.9	1.809	0.292	0.103	62.8	71.0	2.7	5.4	36.8	0.122
Patient 4														
Pretreatment	4696.5	543.6	24.4	18.5	26.5	1.519	0.240	0.189	111.6	66.3	1.4	9.3	5.3	0.094
15 mo	1273.2	254.5	11.8	14.9	92.9	1.293	0.180	0.125	75.4	77.8	1.7	6.7	40.4	0.144
Patient 5														
Pretreatment	957.4	277.3	15.1	15.0	19.7	2.071	0.526	0.625	74.9	95.1	3.5	12.1	9.6	0.146
1 y	1225.3	295.5	16.9	18.4	82.8	1.371	0.130	0.128	61.7	97.6	4.9	9.0	40.3	0.215
Patient 6														
Pretreatment	3511.6	272.1	16.9	16.8	16.5	1.721	0.275	0.485	124.7	67.4	1.7	10.0	6.0	0.113
2 y	842.1	267.2	36.2	15.9	134.7	1.302	0.208	0.162	60.6	75.6	4.2	4.4	40.0	0.160
Patient 7														
Pretreatment	2088.2	136.6	5.4	14.9	2.9	1.888	0.241	0.185	142.8	64.4	1.2	7.7	1.8	0.069
8 mo	1835.4	299.6	8.9	7.6	121.3	1.755	0.250	0.092	117.1	75.6	2.6	6.9	43.3	0.121
Patient 8														
Pretreatment	1803.9	297.9	12.7	22.9	11.4	1.273	0.198	0.100	90.0	79.4	1.9	11.6	3.0	0.149
28 mo	1111.6	305.7	12.1	18.4	179.6	1.535	0.146	0.130	67.9	67.2	2.5	6.6	40.5	0.152
Patient 9														
Pretreatment	1450.3	152.6	8.1	15.0	7.6	1.782	0.294	0.118	87.5	72.5	1.9	10.1	4.2	0.150
8 mo	890.3	240.5	47.6	23.4	123.0	1.427	0.142	0.048	71.6	81.7	7.2	7.9	44.1	0.146
Patient 10														
Pretreatment	2634.1	482.6	20.7	13.3	29.4	1.882	0.454	0.442	110.2	107.5	1.6	6.3	6.2	0.069
6 wk	2418.1	508.1	38.4	15.8	69.9	1.425	0.345	0.466	79.6	113.6	4.4	10.2	31.8	0.084
Patient 11														
Pretreatment	1984.7	334.1	12.6	22.1	35.7	1.548	0.195	0.033	127.6	104.6	1.6	8.5	12.9	0.130
34 mo	1413.8	199.4	34.3	13.6	88.9	1.054	0.080	0.000	80.2	81.8	6.5	10.0	41.7	0.181
Patient 12														
Pretreatment	896.2	259.4	6.3	10.4	13.2	1.961	0.426	0.312	45.9	74.3	1.1	5.1	6.1	0.123
15 mo	1062.0	224.1	16.3	21.3	86.4	1.652	0.214	0.106	61.0	77.2	2.0	6.9	36.5	0.148
Patient 13														
Pretreatment	1966.0	129.0	2.0	2.0	2.2	1.880	0.319	0.537	144.3	91.4	0.8	4.7	1.5	0.120
6 y	2149.4	206.2	13.6	26.1	151.2	1.790	0.140	0.100	92.7	72.4	3.7	11.3	44.9	0.139
Normal range	2500–3500	400–1000	10–80	35–75	100–300	0.70–0.95	<0.030	<0.005	60–85	90–110	1.5–4.0	9–14	30–45	0.160–0.230

<sup>1</sup>The 2 values given correspond to the initiation of treatment and the time at which docosahexaenoic acid concentrations were optimal. Because the variation in normal values is large as a result of nutritional differences, the range is given rather than the mean. DMA, dimethyl acetal.




**FIGURE 7.** Erythrocyte ratio of the plasmalogen dimethyl acetal (DMA) to the corresponding fatty acid methyl ester (18:0DMA to 18:0) in a patient with Zellweger syndrome (patient 6 in Tables 1 and 2) throughout treatment with docosahexaenoic acid (DHA) ethyl ester, plotted against DHA concentration.  $y = 0.999 + 0.002x - 1.478E - 5x^2$ .  $n = 32$ ;  $r = 0.925$ ;  $P = 0.0001$ .



**FIGURE 8.** Plasma ratios of 26:0 to 22:0 and of 26:1 to 22:0 in a patient with infantile Refsum disease (patient 2 in Tables 1 and 2) throughout treatment with docosahexaenoic acid ethyl ester (DHA-EE). FO, fish oil.

should be initiated as soon as possible, before irreversible damage renders any therapy useless.

Note added in proof: in the 3 y that have elapsed since these data were presented, no patients have died and 2 (patients 2 and 9) can now walk independently. 

We thank N Salem Jr and P Fair from the National Institutes of Health and the Charleston Laboratory for providing the docosahexaenoic acid ethyl ester

used in the present study. We are also greatly indebted to M Hayashi and Y Misawa (Harima Chemicals, Japan) and to O Thorstad (Pronova Biocare, Norway) for kindly providing some extra amounts of docosahexaenoic acid ethyl ester. We also thank MA Monedero of the Spanish Ministry of Health for her invaluable help and collaboration. Many other physicians contributed to the study of the patients: M Pineda, R Vidal, A Verdú, and M Roig were the neurologists; M Velasco did the audiometric tests; M Olesti and J Conill did the neurophysiologic tests; M Tallada performed the electroencephalographic studies; F Roels, M Speel, and E Nizalik performed the pathologic examina-



tion of some liver biopsy samples; GTN Besley, RBH Schutgens, RJA Wanders, NA Chamoles, M Manescau, P Ramos, C Dominguez, E Riudor, and ML Girós contributed to the biochemical diagnosis; and JJ Ortega, C Guell, A Perareda, A Llort, P Puttinger, E Naughten, PT Ward, and M Boland helped with the management of the patients. The Biomed 2 project Peroxisomal Leukodystrophy provided blood samples and data for some patients.

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