The fundamentals and practice of docosahexaenoic acid therapy in peroxisomal disorders

Manuela Martinez

Zellweger's syndrome and related peroxisomal disorders are lethal congenital diseases whose pathogenesis is still poorly understood. The low levels of docosahexaenoic acid found in these patients seem to be related to the pathogenesis of the disease. This is confirmed by the fact that correcting their docosahexaenoic acid deficiency improves the patients significantly. All treatments currently reported involve the use of low-fat diets. This review presents a totally different therapeutic approach. The aim is to correct the docosahexaenoic acid deficiency in the first place, and give the child a normal diet in order to provide all the nutrients necessary for growth and development. The biochemical and clinical improvements produced by this approach suggest that, if given very early during development, docosahexaenoic acid therapy might prevent some of the devastating consequences of peroxisomal disorders. Curr Opin Clin Nutr Metab Care 3:101–108.

Correspondence to Manuela Martinez, Laboratory of Lipids and Human Brain Development, Center for Research in Biochemistry and Molecular Biology, Maternity–Children's Hospital Vall d'Hebron, Planta 14, P. Vall d'Hebron, 119-129, 08035 Barcelona, Spain
Tel: +34 93 4894065; fax: +34 93 4894064; e-mail: mmr@hg.vhebron.es

Current Opinion in Clinical Nutrition and Metabolic Care 2000, 3:101–108

Abbreviations

AA	arachidonic acid
DHA	docosahexaenoic acid
DHA-EE	docosahexaenoic acid ethyl ester
IRD	infantile Refsum's disease
NALD	neonatal adrenoleukodystrophy
PBD	peroxisomal biogenesis disorders
PTS	peroxisomal targeting signals
PUFA	polyunsaturated fatty acids
VLCFA	very long chain fatty acids

1363-1950

Introduction

Microbodies [1] or peroxisomes [2] are small single membrane-bounded intracellular organelles, with an average diameter of 0.5 mμ, present in most eukaryotic cells. The term 'peroxisomes' comes from their ability to oxidize various substrates producing hydrogen peroxide, which is then decomposed by catalase. The importance of peroxisomes in medicine has increased over the past years, because in 1973 Goldfischer *et al.* [3] discovered that the brains, livers, and proximal tubules of the kidneys in children with Zellweger's syndrome do not have recognizable peroxisomes. Since then a new group of genetic diseases has been identified, whose common denominator is the absence of distinguishable peroxisomes. These diseases are commonly called generalized peroxisomal disorders, so named because multiple peroxisomal enzymes are defective. From the cellular point of view, however, many researchers prefer to call these diseases 'disorders of peroxisome assembly' or 'peroxisomal biogenesis disorders' (PBD) [4]. These terms come from the origin of these diseases, which is attributed to a failure in peroxisome assembly because of a defective import of newly synthesized peroxisomal proteins into the organelle matrix. PBD are lethal diseases, whose pathogenesis is still badly understood. Any therapeutic approach has thus been limited to alleviating the symptoms of these severe disorders. Several palliative therapies have been tested, mostly based on reducing some of the biochemical abnormalities, but there is still no unanimous criterion as to the most beneficial treatment for these patients. This article presents a new therapeutic approach based on the personal findings of the author, whose aim is to correct the deficiency of a most important polyunsaturated fatty acid (PUFA) (docosahexaenoic acid; DHA) without depriving the patient of any necessary nutrients. Some background information on this heterogeneous group of congenital diseases is given first.

Clinical and biochemical abnormalities in peroxisomal biogenesis disorders

There is a great heterogeneity among generalized peroxisomal disorders, both clinically and genetically. The cerebro–hepato–renal syndrome of Zellweger is the prototype [5,6]. From the clinical point of view, classic Zellweger's syndrome is a lethal disease with a life expectancy of just a few months. The central nervous system is deeply affected, involving neurons, myelin and the sensorineural organs. There is severe hypotonia, seizures since birth, and profound psychomotor retarda-

tion. Magnetic resonance imaging and post-mortem examination show neuronal heterotopias and dysmyelination. Hepatomegaly and altered liver tests are quite constant. Often, other organs are also affected, such as the kidneys, bones, and adrenal glands. There are less severe phenotypes of Zellweger's syndrome, such as neonatal adrenoleukodystrophy (NALD) [7,8] and infantile Refsum's disease (IRD) [9,10]. Patients with these variants may live for several years, and some can even reach adulthood. No clear clinical or biochemical delimitation exists between the various phenotypes, the fundamental abnormalities being basically the same, and the current view is that peroxisomal disorders form a disease continuum, with different degrees of severity [11].

The biochemical abnormalities in all phenotypes are quite similar, produced by the failure of multiple peroxisomal enzymes. There is an increase in very long chain fatty acids (VLCFA; fatty acids with more than 22 carbon atoms, mainly 26:0 and 26:1) [12,13] as a result of defective peroxisomal β-oxidation [12,14–16]. The first two steps of plasmalogen synthesis are also catalysed by peroxisomal enzymes (dihydroxyacetone phosphate acyltransferase and alkyl-dihydroxyacetone phosphate synthetase) [17,18] and are affected in PBD, so plasmalogen levels are decreased [19,20]. Bile acid synthesis is affected by defective β-oxidation cleavage of the cholesterol side chain [21,22] and abnormal intermediates accumulate (3a,7a,12a-trihydroxy-5b-cholestanoic acid and of 3a,7a-dihydroxy-5b-cholestanoic acid) [23]. Cholesterol levels are reduced by defective peroxisomal 3-hydroxy-3-methylglutaryl-coenzyme A reductase and probably other enzymes necessary for cholesterol synthesis [24,25]. Defective oxidation causes increases in phytanic and pristanic acids in the plasma and fibroblasts of peroxisomal patients [26,27]. Accumulation of pipecolic acid in the urine and plasma of these patients is usually found because of its impaired catabolism [28,29].

Cellular and molecular origin of peroxisomes

Peroxisomes lack DNA, and peroxisomal proteins are normally synthesized in free polyribosomes and imported post-translationally into pre-existing peroxisomes. The import of peroxisomal proteins is mediated by receptors for specific peroxisomal targeting signals (PTS). At least two signal sequences, PTS1 [30] and PTS2 [31], are known to target proteins to the peroxisome. At the molecular level, a group of gene products called peroxins (abbreviated Pex) are responsible for peroxisome biogenesis [32]. These peroxins are encoded for by nuclear *PEX* genes [33]. To date, only a few peroxins and *PEX* genes have been characterized. Mutations in *PEX1* are responsible for most peroxisomal disorders [34]. The final consequence of these molecular defects is a failure to import newly synthesized proteins into the peroxisome, and thus the organelle cannot be assembled. So often the peroxisomal membrane forms an empty 'ghost' devoid of peroxisomal matrix [35], which is characteristic of PBD.

The genetic classification of peroxisomal disorders has been attempted by cell fusion complementation studies [36–39], but no clear correlation between genotype and phenotype has yet been found. Indeed, at least 10 complementation groups have been defined for generalized peroxisomal disorders, most of them phenotypically heterogeneous. In particular, complementation group 1 accounts for over half of all patients, comprising the whole spectrum of phenotypes. Some recent molecular findings, however, are starting to shed some light on peroxisomal disorder classification. A point missense mutation, the G843D substitution, in the *PEX1* gene has been found to be the most common cause of peroxisomal disorders, accounting for approximately half of the patients in complementation group 1 [34,40]. This mutation seems to be correlated with the less severe phenotypes. Experimentally, it has been shown that fibroblasts from IRD patients carrying the G843D mutation are capable of forming peroxisomes when incubated at 30°C, whereas those from Zellweger's syndrome and NALD patients are not [41]. Lately, a new mutation in the *PEX1* gene, producing a frameshift in exon 13, has been correlated with the most severe phenotype of classic Zellweger's syndrome [42••,43].

Pathogenesis

It is not clear what may be the cause of such severe diseases. Based on biochemical abnormalities, it is usually taken for granted that the brain damage is caused by the accummulation of the VLCFA 26:0 in the central nervous system [11]. However, this fatty acid is a normal constituent of brain sphingolipids, which are especially enriched in the myelin sheath. Besides, in the Zellweger's syndrome brain 26:0, 26:1n-9 and 24:1n-9 are all more often decreased than increased, as a result of the loss of myelin in these patients [44]. It has been proposed that the VLCFA are situated in a different lipid compartment in the peroxisomal brain, so acting as aggressive agents [11,45]. However, this has not yet been proved. Another factor that could probably be more deleterious in the peroxisomal brain is plasmalogen deficiency. Plasmalogens are known to be mainly enriched in myelin and may play an important role there.

Some years ago we discovered that patients with generalized peroxisomal disorders have extremely low levels of DHA (22:6n-3) in the brain and other tissues [46–49], including blood [50,51]. DHA is a most abundant PUFA in the brain and retina, and is believed to play important roles in those tissues [52,53]. The

severe DHA deficiency found might thus be a most deleterious factor in the brain and retina of peroxisomal patients. Therefore, trying to normalize the DHA levels in these patients and observing whether this could produce some beneficial effects was a logical second step in our research, which was initiated in 1990 [54,55]. With this aim, we used highly pure DHA ethyl ester (DHA-EE), as a means to correct the DHA deficiency without modifying other PUFA levels. It is most important to emphasize that the aim of this new therapy is to normalize things, not to provide a drug treatment. This seems not to have been understood by most physicians now using DHA as a treatment for peroxisomal disorders. The present review tries to clarify the most important points on the subject.

Polyunsaturated fatty acid abnormalities in peroxisomal disorders

It is important to emphasize that, although DHA is constantly decreased in the brains of patients with generalized peroxisomal disorders, another important PUFA, arachidonic acid (AA, 20:4n-6) is generally not. In most peroxisomal disorders, AA is even increased in the brain [48,49]. The increase in AA and other n-6 PUFA has been checked in the different brain phospholipids [56•]. In phosphatidylamine, for example, the AA content is so high that it partly accounts for the marked increase of this phospholipid in the peroxisomal brain [19,46,56•]. This is somewhat puzzling because the AA levels are low in the plasma of peroxisomal disorder patients [50,51]. However, it must be taken into account that all plasma lipid levels are low in these patients. So when looking at percentages instead of absolute values, the AA decrease is not so marked, and DHA deficiency clearly also predominates in the plasma. In 40 plasma samples from typical generalized peroxisomal disorders, the AA levels averaged 3.61% (SD, 1.14) of the total fatty acids, a value within the lower limit of normal and approximately half the optimal AA percentage. On the other hand, DHA mean value was only 0.27% (SD, 0.25), which is between five and 10 times lower than the normal plasma total DHA concentration. Erythrocytes and kidney tissue usually have only marginally decreased AA levels and an extremely low DHA concentration. In the liver, DHA was very much decreased and AA was normal or increased. In the retina, the levels of DHA were negligible whereas the other PUFA were within normal limits. These findings clearly demonstrate that DHA is the only PUFA constantly deficient in all tissues in peroxisomal disorder patients.

What is the cause of such a marked DHA deficiency? In the Zellweger brain, the two products of classic Δ4-desaturation, 22:6n-3 and 22:5n-6 were both decreased, pointing to a defect in this enzyme. However, the existence of a putative Δ4-desaturase has lately been questioned, and a new route for DHA (and 22:5n-6) synthesis has been proposed [57,58•]. This pathway attributes DHA synthesis to peroxisomal β-oxidation of the very long PUFA tetracosahexaenoic acid (24:6n-3). This has the advantage of linking the DHA deficiency to the β-oxidation defect in peroxisomal patients. However, some facts observed in peroxisomal disorders still remain to be clarified. For example, this synthetic route does not explain why patients with X-linked adrenoleukodystrophy, who also have a defective peroxisomal β-oxidation, do not have a DHA deficiency [51]. Another unexplained fact is why 24:6n-3 does not increase at all in Zellweger patients if its β-oxidation is not possible. Instead, a relative increase in 22:5n-3 is usually observed in these patients. Besides, normalization of the DHA levels consistently produces a decrease in the VLCFA, not the other way round. So, whatever may be the mechanism for DHA deficiency, it seems to be at the very root of the disease.

Treatment of peroxisomal biogenesis disorders

Currently, it is common practice to subject peroxisomal disorder patients to low-fat diets of the type used with adult Refsum's disease patients [59]. This is done with the double aim of lowering the intakes of phytanic acid and VLCFA. Some physicians have also treated PBD patients with a mixture of trioleate-trierucate (4:1 by volume), popularly known as Lorenzo's Oil, a preparation widely used with X-linked adrenoleukodystrophy patients to decrease the levels of VLCFA [60]. Some fragmentary experience exists with other therapeutic diets. Plasmalogen precursors [61] and cholic acid [62] have been given to these patients in an effort to increase their plasmalogen levels and normalize bile acids, respectively. A patient treated with plasmapheresis [63] was reported to improve in parallel with decreases in plasma phytanate levels. Attempts have also been made to improve PBD patients by providing them with peroxisomal proliferators such as clofibrate, without success [64]. None of these treatments have shown clear evidence of improvement in the few PBD patients in whom they have been tested.

Treatment with docosahexaenoic acid

Docosahexaenoic acid accretion during normal human brain development

Before referring to DHA therapy in peroxisomal patients, it is necessary to mention briefly the PUFA changes during normal human brain development. In the fetal forebrain, the concentration of the two most important long chain PUFA, DHA and AA, and the accretion of gangliosides starts to increase very rapidly at 32 weeks of gestational age [65–67]. This very closely corresponds to the moment when an explosive growth of dendritic spines takes place in the human brain [68].

Once the perinatal period is over, the accretion of these PUFA continues at a slower speed until the second year of life [66]. Immediately after birth in the human species, myelinogenesis starts in the forebrain. This can be checked biochemically by the increase in myelin lipids (cerebrosides, sulfatides and sphingomyelin) [69,70]. Other lipids, mainly enriched in myelin, such as plasmalogens and cholesterol, also parallel myelination in an approximate way. Among the fatty acids, the very long saturated and monounsaturated ones (24:0, 24:1n-9, 26:0, 26:1n-9), which are major constituents of galactolipids, start their accretion at birth and then parallel myelination very closely, so constituting excellent myelin markers [70]. The long PUFA adrenic acid (22:4n-6) is a relatively good marker for myelin, as is the monounsaturated precursor oleic acid (18:1n-9), their accretion slopes being maximal during the time of rapid myelin formation [70].

Following the 'brain growth spurt' theory of Dobbing [71] at the biochemical level, all these things put together emphasize that the brain has a unique moment when growth and development is maximal, and any nutritional insult impinging on this period may have lasting or even irrecoverable effects [72]. Extrapolated to the situation in peroxisomal disorders, this indicates that correction of the DHA deficiency must be started very early if some kind of beneficial effect is to be obtained: at birth or even earlier if this were possible. Otherwise, perhaps we can obtain some beneficial effects but not a true recovery from the consequences of the DHA deficiency.

Protocol of docosahexaenoic acid therapy in peroxisomal disorder patients and results

First, we wanted to correct DHA deficiency and normalize the nutritional status as much as possible. A pure DHA preparation was thus chosen in order to normalize the DHA levels without altering those of other PUFA. For this reason, mixtures of n-3 fatty acids such as those present in fish oil were avoided. They contain high levels of eicosapentaenoic acid (20:5n-3), an n-3 PUFA situated before the probable enzyme block in DHA synthesis. The addition of AA and other n-6 PUFA was also avoided, because they could compete with DHA and reduce its beneficial effects.

With these ideas in mind, we have so far treated 20 peroxisomal disorder patients, two of them with classic Zellweger's syndrome. It is difficult to classify the other 18 patients because there is currently much confusion on the subject, as discussed above. Some of the patients who live longer may appear with time to have different phenotypes to different physicians. This happened with three of our patients. They seemed to have IRD before 2 years of age, but it was later realized that they should have been diagnosed with NALD. If the patients live long enough abnormal myelination is finally detected and very often adrenal function is affected. The classic Zellweger phenotype, on the other hand, can be quite clearly distinguished from the others from the beginning. Apart from the extreme severity of their clinical picture, Zellweger patients have an almost total lack of plasmalogens in their erythrocytes.

Medication

The preparation used in all patients was highly purified DHA-EE (90–95% pure), provided by the Biomedical Test Materials Program (National Institutes of Health, Bethesda, MD, USA) and Harima Chemicals (Japan). Smaller amounts were also obtained from Pronova Biocare (Norway). To avoid lipid peroxidation, the DHA-EE was divided into individual one-dose vials, made up to a total volume of 2 ml with pure olive oil and sealed in an N_2 atmosphere. The daily doses varied between 100 and 500 mg of DHA-EE, given orally once a day. For DHA dosage, the age and clinical state of the patient rather than body weight was considered, taking into account that the younger the children the higher are their DHA requirements for brain development. No other fatty acid was administered as a medication. Vitamin K was given to correct altered coagulation tests (generally 3–5 mg/day, with a maximum of 1 mg/kg/day) and vitamin E was provided as an antioxidant (50–200 mg/day). Vitamins A and D at regular daily doses were usually added, because peroxisomal patients have low levels of all liposoluble vitamins.

Diet

Since the main rationale was to normalize the PUFA status as much as possible, a low-fat diet was strictly avoided. Although no supplement of any other PUFA was added to the treatment, care was taken that the diet contained all the fatty acids that a normal child should receive during development. Otherwise, an excess of DHA could result in a fatty acid imbalance and other PUFA deficiencies may have appeared. So the diet was complete for the age in every respect. As for vegetables, only green leaves were restricted for their high content in phytanic acid. All other vegetables and fruits were permitted. Fatty meat was avoided, as it would be in any normal child. Whenever possible, maternal milk was recommended in small infants. When lactation was not possible, a whole milk formula enriched in DHA and AA in proportions similar to those found in human milk (Milupa, Germany), was given instead. All kinds of meat, fish and dairy products were permitted as was, in general, any food appropriate for the age.

Results

The biochemical results and clinical follow-up of the patients treated have appeared in successive publications

[54,55,73–77•]. Biochemically, normalization of the DHA levels in plasma and erythrocytes was obtained in all cases. Unexpectedly, plasmalogen levels increased in practically all patients. In some of them, the 18:0DMA/18:0 index (a measure of the ratio of 18:0 plasmalogen to 18:0 diacyl phospholipids in these patients) even normalized. It is important to point out that in no case did the plasma VLCFA levels increase as a result of the normal fat diet provided. On the contrary, both 26:0 and 26:1n-9 decreased in the plasma in most DHA-treated patients, often very markedly.

Table 1 summarizes the most important biochemical and clinical changes in the 20 patients treated with DHA-EE. For reasons mentioned before, the diagnoses are reduced to the 'classic' Zellweger's syndrome phenotype and the milder NALD/IRD spectrum. When one of these phenotypes predominated, it is indicated in Table 1. As can be seen in the table, only three patients have died so far. Of these, one had 'classic' Zellweger's syndrome (patient no. 4). A second patient was a severely involved, 3-year-old girl (patient no. 16), who was in a terminal, vegetative state when the treatment started and could only be treated for 6 weeks before she died from bronchopneumonia [55]. A third patient was a girl who had improved quite spectacularly for a year (patient no. 8) and suddenly died from fulminant septicaemia [50,73]. The other 17 patients survive. As a whole, this is a much longer survival time than that expected from the spontaneous course of such severe diseases. Besides, most patients have had clinical improvement, at the very least, from the point of view of their nutritional state and liver function. The latter has improved in all patients. Vision has improved in 12 patients, muscle tone in eight patients and, in general, the patients have become more alert and interactive than they were before the treatment. Most importantly, myelination has improved in nine patients, as checked by magnetic resonance imaging [75–77•]. This is the reversal of what normally happens and should be attributed to some still unknown role of DHA in myelinogenesis. As was expected from our previous nutritional studies, the beneficial effects of DHA-EE were most marked in three children who started treatment before 6 months of age. In one of them (patient no. 2), it was interesting to find normalization of intestinal absorption after a short period with DHA-EE. This 5-month-old child had been taking an n-3 triglyceride preparation since about a month before the DHA-EE treatment started and an intense steatorrhea persisted. This totally disappeared after only 3 weeks with DHA-EE. This seems to suggest that DHA-EE may be more effective than other DHA preparations in the treatment of peroxisomal disorder patients.

Conclusion

First, it must be emphasized that DHA therapy is a nutritional treatment, primarily given to normalize the DHA levels, not to obtain a drug effect. It is most important always to bear in mind that DHA deficiency always predominates over any other suboptimal PUFA

Table 1. Biochemical and clinical evolution of 20 peroxisomal biogenesis disorder patients treated with docosahexaenoic acid ethyl ester

Patient	Diagnosis	Age	Treatment duration	DHA	VLCFA	Plasmalogens	Liver enzymes	Vision	CNS	MRI
1	NALD/IRD	2 months	7 years	↑↑	↓↓↓	↑	↓↓↓	+	+	+
2	NALD/IRD	3 months	1 year	↑↑↑	↓↓	↑↑	↓↓	+	+	+
3	NALD/IRD	5 months	4 years	↑↑↑	↓↓	↑↑	↓↓↓	–	+	+
4	ZS	6 months	3 months +	↑↑	=	↑	↓	–	–	
5	NALD	7 months	4 years	↑↑↑	↓	=	↓↓	+	–	
6	ZS	7 months	5 years	↑↑↑	=	↑	↓↓↓	+	–	+
7	NALD/IRD	9 months	5 years	↑↑↑	↓	↑↑	↓↓	+	+	+
8	NALD/IRD	9 months	17 months +	↑↑↑	↓	↑	↓↓	+	+	
9	ZS	1 year	3 months	↑	=	↑	↓	–	–	
10	NALD/IRD	13 months	6 years	↑↑	↓	↑	↓	+	–	–
11	NALD	14 months	3 years	↑↑↑	=	↑	↓↓	–	–	+
12	NALD	15 months	5 years	↑↑↑	↓	=	↓↓	+	+	+
13	NALD/IRD	15 months	4 years	↑↑↑	↓↓	↑	↓↓	–	+	+
14	NALD/IRD	2 years	3 months	↑↑↑	=	↑	↓	–	–	
15	IRD	3 years	1 year	↑	=	=	↓	+	+	
16	NALD	3 years	6 weeks +	↑↑	=	↑	↓	–	–	
17	NALD/IRD	5 years	6 years	↑↑↑	↓	↑	↓	+	+	+
18	NALD	5 years	4 years	↑↑	↓	↑	↓	–	–	–
19	IRD	5 years	1 year	↑	=	↑	↑	+	+	
20	NALD/IRD	7 years	9 years	↑↑↑	↓	↑	↓	+	–	–

CNS, central nervous system; DHA, docosahexaenoic acid; RID, infantile Refsum's disease; MRI, magnetic resonance imaging; NALD, neonatal adrenoleukodystrophy; VLCFA, very long chain fatty acids; ZS, Zellweger's syndrome.
The arrows indicate the degree of increase or decrease of the corresponding parameters. For clincical response, + indicates improvement; – means non-significant change. Blank spaces mean that no second examination was performed.

values that may be found. Therefore DHA deficiency must be corrected first, and for this DHA must be given alone until its blood levels are normalized. Interference with any other PUFA given in high doses, especially with AA, may render DHA therapy ineffective, because enzyme competition will favour the n-6 over the n-3 fatty acid series. Besides, AA is not decreased, but usually increased, in the most important organ: the brain. Therefore, we should never mix AA to DHA in the treatment of patients with generalized peroxisomal disorders, at least never before the DHA deficiency has been totally corrected. In the author's personal experience, doing so may produce some undesirable biochemical effects, such as increases in the VLCFA or decreases in the plasmalogen ratios [73]. Once the DHA levels are normal, AA may be added to the diet should the levels of this PUFA be found to be below their normal lower limits. It is important to emphasize that no variation in the diet should be introduced without closely monitoring the PUFA changes.

The brain has a unique opportunity to grow and develop properly, and this is during the perinatal and early postnatal periods. Ideally, if we want to correct a DHA deficiency we should provide the DHA during those periods or, at least, as early in life as possible. If we provide the DHA too late, we may correct its deficiency but the past consequences of that deficiency may already be irrecoverable. Although DHA deficiency is not the only defect in peroxisomal disorders, it is possibly one of the most important abnormalities and is, at the least, a most serious aggravating factor. Therefore if we want to do the best for these children, their DHA deficiency must be corrected as soon as it is diagnosed. Besides DHA therapy, peroxisomal patients should receive a nutritious diet and any extra care they need. We must always remember that besides being patients, they are growing children who need everything a child would need for normal development. Even if we think that the accumulation of 26:0 and 26:1n-9 is the main pathogenic factor in peroxisomal disorders, we have shown that a complete diet does not increase the VLCFA levels of peroxisomal patients when given with DHA. Therefore, we should never deprive these children of any necessary nutrients. The dangers of doing so largely outweigh any theoretical consideration. In short, an effort should be made to treat these patients like normal children in every respect, and give them the DHA they need as soon as possible. This approach may not cure the disease but it will certainly improve the life of these patients a great deal. Logically, when provided very early during brain development, DHA therapy will be the most effective and, perhaps, some brain and visual damage may be prevented. So early diagnosis and early treatment may hopefully change the gloomy prognosis of peroxisomal disorder patients.

Acknowledgements

The author would like to thank the Biomedical Fish Oil Test Materials Program [National Oceanic and Atmospheric Administration (NOAA), National Institutes of Health (NIH), files nos. 215A, 215B, 215C] and Harima Chemicals for providing the DHA-EE used in this study. Pronova Biocare contributed some extra amounts of DHA-EE. The collaboration of the following physicians is also gratefully acknowledged: E. Naughton, A. Peltier, N. Gouffon, E. Girardin, R. Puttinger, M.T. García-Silva, M. Pineda, R. Vidal, A. Verdú, G. Lorenzo, J.M. Bertran and F. Castelló.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 Rhodin J. Correlation of ultrastructural organization and function in normal and experimentally changed proximal convoluted tubule cells of the mouse kidney. PhD thesis. Stockholm, Sweden: Karolinska Institute; 1954.

2 de Duve C, Baudhuin P. Peroxisomes (microbodies and related particles). Physiol Rev 1966; 46:323–357.

3 Goldfischer S, Moore CL, Johnson AB, Spiro AJ, Valsmis MP, Wisniewski HK, *et al.* Peroxisomal and mitochondrial defects in the cerebro–hepato–renal syndrome. Science 1973; 182:62–64.

4 Lazarow PB, Moser HW. Disorders of peroxisome biogenesis. In: The metabolic basis of inherited disease. Scriver CR, Beaudet AL, Sly WS, Valle D (editors). 7th CD-ROM edition. New York: McGraw-Hill; 1997. Records 38247–38973.

5 Bowen P, Lee CSN, Zellweger H, Lindenberg R. A familial syndrome of multiple congenital defects. Bull Johns Hopkins Hosp 1964; 114:402–414.

6 Opitz JM, Zu Rhein GM, Vitale L, Shahidi NT, Howe JJ, Chon SM, *et al.* The Zellweger syndrome (cerebrohepatorenal syndrome). Birth Defects Orig Art Ser 1969; 2:144–160.

7 Ulrich J, Hershkowitz N, Heits P, Sigrist T, Baerlocher P. Adrenoleukodystrophy: preliminary report of a connatal case, light- and electron microscopical, immunohistochemical and biochemical findings. Acta Neuropathol (Berl) 1978; 43:77–83.

8 Kelley RI, Datta NS, Dobyns WB, Hajra AK, Moser AB, Noetzel MJ, *et al.* Neonatal adrenoleukodystrophy: new cases, biochemical studies, and differentiation from Zellweger and related peroxisomal poly dystrophy syndromes. Am J Med Genet 1986; 23:869–901.

9 Scotto JM, Hadchouel M, Odievre M, Laudat MH, Saudubray JM, Dulac O, *et al.* Infantile phytanic acid storage disease: a possible variant of Refsum's disease: three cases, including ultrastructural studies of the liver. J Inherit Metab Dis 1982; 5:83–90.

10 Poll-The BT, Saudubray JM, Ogier H, Schutgens RBH, Wanders RJA, Schrakamp G, *et al.* Infantile Refsum's disease: biochemical findings suggesting multiple peroxisomal dysfunction. J Inherit Metab Dis 1986; 9:169–174.

11 Powers JM, Moser HW. Peroxisomal disorders: genotype, phenotype, major neuropathologic lesions, and pathogenesis. Brain Pathol 1998; 8:101–120.

12 Moser AE, Singh I, Brown FR III, Solish GI, Kelley RI, Benke PJ, Moser HW. The cerebro–hepato–renal (Zellweger) syndrome: increased levels and impaired degradation of very long chain fatty acids, their use in prenatal diagnosis. N Engl J Med 1984; 310:1141–1146.

13 Bakkeren JA, Monnens LA, Trijbels JM, Maas JM. Serum very long chain fatty acid pattern in Zellweger syndrome. Clin Chim Acta 1984; 138:325–331.

14 Poulos A, Singh H, Paton B, Sharp P, Derwas N. Accumulation and defective β-oxidation of very long chain fatty acids in Zellweger's syndrome, adrenoleukodystrophy and Refsum's disease variants. Clin Genet 1986; 29:397–408.

15 Suzuki Y, Orii T, Mori M, Tatibana M, Hashimoto T. Deficient activities and proteins of peroxisomal β-oxidation enzymes in infants with Zellweger syndrome. Clin Chim Acta 1986; 156:191–196.

16 Wanders RJA, van Roermund CWT, van Wijland MJA, Heikoop J, Schutgens RBH, Schram AW, *et al.* Peroxisomal very long chain fatty acid β-oxidation in human skin fibroblasts: activity in Zellweger syndrome and other peroxisomal disorders. Clin Chim Acta 1987; 166:255–263.

17 Hajra AK, Burke CL, Jones CL. Subcellular localization of acyl-coenzyme A: dihydroxyacetone phosphate acyltransferase in rat liver peroxisomes (microbodies). J Biol Chem 1979; 254:10896–10900.

18 Hajra AK, Bishop JE. Glycerolipid biosynthesis in peroxisomes via de acyl dihydroxyacetone phosphate pathway. Ann NY Acad Sci 1982; 386:170–182.

19 Heymans HSA, Schutgens RBH, Tan R, van den Bosch H, Borst P. Severe plasmalogen deficiency in tissues of infants without peroxisomes (Zellweger syndrome). Nature 1983; 306:69–70.

20 Datta NS, Wilson GN, Hajra AK. Deficiency of enzymes catalyzing the biosynthesis of glycerol-ether lipids in Zellweger syndrome: a new category of metabolic disease involving the absence of peroxisomes. N Engl J Med 1984; 31:1080–1083.

21 Pedersen JI, Gustafsson J. Conversion of 3a,7a,12a-trihydroxy-5b-cholestanoic acid into cholic acid by rat liver peroxisomes. FEBS Lett 1980; 121:345–348.

22 Krisans SK, Thompson SL, Pena LA, Kok E, Javitt NB. Bile acid synthesis in rat liver peroxisomes: metabolism of 26-hydroxycholesterol to 3b-hydroxy-5-cholenoic acid. J Lipid Res 1985; 26:1324–1932.

23 Hanson RF, Szczepanick-van Leeuwen P, Williams GC, Grabowski G, Sharp HL. Defects of bile acid synthesis in Zellweger's syndrome. Science 1979; 203:1107–1108.

24 Keller GA, Barton MC, Shapiro DJ, Singer SJ. 3-Hydroxy-3-methylglutaryl coenzyme A reductase is present in peroxisomes in normal rat liver cells. Proc Natl Acad Sci U S A 1985; 82:770–774.

25 Krisans SK. The role of peroxisomes in cholesterol metabolism. Am J Respir Cell Mol Biol 1992; 7:358–364.

26 Poulos A, Sharp P, Fellenberg AJ, Danks DM. Cerebro–hepato–renal (Zellweger) syndrome, adrenoleukodystrophy and Refsum's disease; plasma changes and skin fibroblast phytanic acid oxidase. Hum Genet 1985; 70:172–177.

27 Singh H, Usher S, Johnson D, Poulos A. A comparative study of straight chain and branched chain fatty acid oxidation in skin fibroblasts from patients with peroxisomal disorders. J Lipid Res 1990; 31:217–225

28 Danks DM, Tippett P, Adams C, Campbell P: Cerebro–hepato–renal syndrome of Zellweger: a report of eight cases with comments upon the incidence, the liver lesion, and a fault in pipecolic acid metabolism. J Pediatr 1975; 86:382–387.

29 Dancis J, Hutzler J. The significance of hyperpipecoliatemia in Zellweger syndrome. Am J Hum Genet 1986; 38:707–711.

30 Gould SG, Keller GA, Subramani S. Identification of a peroxisomal targeting signal at the carboxy terminus of firefly luciferase. J Cell Biol 1987; 105:2923–2931.

31 Swinkels BW, Gould SJ, Bodnar AG, Rachubinski RA, Subramani S. A novel, cleavable peroxisomal targeting signal at the amino-terminus of the rat 3-ketoacyl-CoA thiolase. EMBO J 1991; 10:3255–3262.

32 Distel B, Erdmann R, Gould SJ, Blobel G, Crane DI, Cregg JM, *et al.* A unified nomenclature for peroxisome biogenesis factors. Cell Biol 1996; 135:1–3.

33 Subramani S. Components involved in peroxisome import, biogenesis, proliferation, turnover, and movement. Physiol Rev 1998; 78:171–188.

34 Reuber BE, Germain-Lee E, Collins CS, Morrell JC, Ameritunga R, Moser HW, *et al.* Mutations in PEX1 are the most common cause of peroxisome biogenesis disorders. Nat Genet 1997; 17:445–448.

35 Santos MJ, Imanaka T, Shio H, Small GM, Lazarow PB. Peroxisomal membrane ghosts in Zellweger syndrome – aberrant organelle assembly. Science 1988; 239:1536–1538.

36 Brul S, Westerveld A, Strijland A, Wanders RJA, Schram AW, Heymans HSA, *et al.* Genetic heterogeneity in the cerebrohepatorenal (Zellweger) syndrome and other inherited disorders with a generalized impairment of peroxisomal functions – a study using complementation analysis. J Clin Invest 1988; 81:1710–1715.

37 Roscher AA, Hoefler S, Hoefler G, Paschke E, Paltauf F, Moser A, Moser HW. Genetic and phenotypic heterogeneity in disorders of peroxisome biogenesis – a complementation study involving cell lines from 19 patients. Pediatr Res 1989; 26:67–72.

38 Yajima S, Suzuki T, Shimozawa N, Yamaguchi S, Orii T, Fujiki Y, *et al.* Complementation study of peroxisome-deficient disorders by immunofluorescence staining and characterization of fused cells. Hum Genet 1992; 88:491–499.

39 Moser A, Rasmussen M, Naidu S, Watkins P, McGuinness M, Hajra A, *et al.* Phenotype of patients with peroxisomal disorders subdivided into sixteen complementation groups. J. Pediatr 1995; 125:755–767.

40 Portsteffen H, Beyer A, Becker E, Epplen C, Pawlak A, Kunau WH, Dodt G. Human PEX1 is mutated in complementation group 1 of the peroxisome biogenesis disorders. Nat Genet 1997; 17:449–452.

41 Imamura A, Tamura S, Shimozawa N, Suzuki Y, Zhang Z, Tsukamoto T, *et al.* Temperature-sensitive mutation in PEX1 moderates the phenotypes of peroxisome deficiency disorders. Hum Mol Genet 1998; 7:2089–2094.

42 •• Maxwell MA, Nelson PV, Chin SJ, Paton BC, Carey WF, Crane DI. A common PEX1 frameshift mutation in patients with disorders of peroxisome biogenesis correlates with the severe Zellweger syndrome phenotype. Hum Genet 1999; 105:38–44.

An important article that differentiates the classic Zellweger's syndrome phenotype from its milder variants at the molecular level.

43 Collins CS, Gould SJ. Identification of a common PEX1 mutation in Zellweger syndrome. Hum Mutat 1999; 14:45–53.

44 Martinez M. Developmental profiles of polyunsaturated fatty acids in the brain of normal infants and patients with peroxisomal diseases: severe deficiency of docosahexaenoic acid in Zellweger's and pseudo-Zellweger's syndromes. In: World review of nutrition and dietetics. Simopoulos AP (editor). Basilea: S. Karger; 1991. pp. 87–102.

45 Theda C, Moser AB, Powers JM, Moser HW. Phospholipids in X-linked adrenoleukodystrophy white matter: fatty acid abnormalities before the onset of demyelination. J Neurol Sci 1992; 110:195–204.

46 Martinez M. Polyunsaturated fatty acid changes suggesting a new enzymatic defect in Zellweger syndrome. Lipids 1989; 24:261–265.

47 Martinez M. Severe deficiency of docosahexaenoic acid in peroxisomal disorders: a defect of Δ-desaturation? Neurology 1990; 40:1292–1298.

48 Martinez M. Severe changes in polyunsaturated fatty acids in the brain, liver, kidney, and retina in patients with peroxisomal disorders. In: Neurobiology of essential fatty acids. Bazan N. (editor). New York: Plenum; 1992. pp. 347–359.

49 Martinez M. Abnormal profiles of polyunsaturated fatty acids in the brain, liver, kidney and retina of patients with peroxisomal disorders. Brain Res 1992; 583:171–182.

50 Martínez M. Polyunsaturated fatty acids in the developing human brain, erythrocytes and plasma in peroxisomal disease: therapeutic implications. J Inherit Metab Dis 1995; 18 (Suppl. 1):61–75.

51 Martinez M, Mougan I, Roig M, Ballabriga A. Blood polyunsaturated fatty acids in patients with peroxisomal disorders. A multicenter study. Lipids 1994; 29:273–280.

52 Jones CR, Arai T, Rapoport SI. Evidence for the involvement of docosahexaenoic acid in cholinergic stimulated signal transduction at the synapse. Neurochem Res 1997; 22:663–670.

53 Neuringer M, Connor WE, Lin DS, Barstad L, Luck S. Biochemical and functional effects of prenatal and postnatal omega-3 fatty acid deficiency on retina and brain in rhesus monkeys. Proc Natl Acad Sci U S A 1986; 83:4021–4025.

54 Martinez M. Treatment with docosahexaenoic acid favorably modifies the fatty acid composition of erythrocytes in peroxisomal patients. In: New developments in fatty acid oxidation. Coates PM, Tanaka K (editors). New York: Wiley–Liss, 1992. pp. 389–397.

55 Martinez M, Pineda M, Vidal R, Martin B. Docosahexaenoic acid – a new therapeutic approach to peroxisomal-disorder patients: experience with two cases. Neurology 1993; 43:1389–1397.

56 • Martinez M, Mougan I. Fatty acid composition of brain glycerophospholipids in peroxisomal disorders. Lipids 1999; 34:733–740.

This paper reveals the fatty acid composition of plasmalogens in the brain of patients with peroxisomal disorders.

57 Voss A, Reinhart M, Sankarappa S, Sprecher H. 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 1991; 266:19995–20000.

58 • Sprecher H. An update on the pathways of polyunsaturated fatty acid metabolism. Curr Opin Clin Nutr Metab Care 1999; 2:135–138.

An interesting review of the current concepts on the metabolism of long chain polyunsaturated fatty acids.

59 Dry J, Pradalier A, Canny M. Maladie de Refsum: dix ans de régime pauvre en acide phytanique et phytol. Ann Med Interne (Paris) 1982; 133:483–487.

60 Rizzo WB, Leshner RT, Odone A, Dammann AL, Craft DA, Jensen ME, *et al.* Dietary erucic acid therapy for X-linked adrenoleukodystrophy. Neurology 1989; 39:1415–1422.

61 Wilson GN, Holmes RG, Custer J, Lipkowitz JI, Stover J, Datta N, Hajra A. Zellweger syndrome: diagnostic assays, syndrome delineation, and potential therapy. Am J Med Genet 1986; 24:69–82.

62 Setchell KDR, Bragetti P, Zimmer-Nechemias L, Daugherty C, Pelli MA, Vaccaro R, *et al.* Oral bile acid treatment and the patient with Zellweger syndrome. Hepatology 1992; 15:198–207.

63 Smeitink JMA, Beemer FA, Speel M, Donckerwolcke RAMG, Jakobs C, Wanders RJA, *et al.* Bone dysplasia associated with phytanic acid accumulation and deficient plasmalogen synthesis: a peroxisomal entity amenable to plasmapheresis. J Inherit Metab Dis 1992; 15:377–380.

64 Bjorkhem I, Blomstrand S, Glaumann H, Strandvik B. Unsuccessful attempts to induce peroxisomes in two cases of Zellweger disease by treatment with clofibrate. Pediatr Res 1985; 19:590–593.

65 Martinez M, Conde C, Ballabriga A. Some chemical aspects of human brain development. II. Phosphoglyceride fatty acids. Pediatr Res 1974; 8:91–102.

66 Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992; 120:S129–138.

67 Martinez M, Ballabriga A. A chemical study on the development of the human forebrain and cerebellum during the brain 'growth spurt' period. I. Gangliosides and plasmalogens. Brain Res 1978; 159:351–362.

68 Purpura DP. Morphogenesis of the visual cortex in the preterm infant. In: Growth and development of the brain. Brazier MAB (editor). New York: Raven Press; 1975. pp. 33–49.

69 Martinez M. Myelin lipids in the developing cerebrum, cerebellum, and brain stem of normal and undernourished children. J Neurochem 1982; 39:1684–1692.

70 Martinez M, Mougan I. Fatty acid composition of human brain phospholipids during normal development. J. Neurochem 1998; 71:2528–2533.

71 Dobbing J. Vulnerable periods in developing brain. In: Applied Neurochemistry. Davison AN, Dobbing J (editors). Edinburgh: Blackwell 1968. pp. 287–316.

72 Wiggins RC, Fuller GN. Early postnatal starvation causes lasting hypomyelination. J Neurochem 1978; 30:1231–1237.

73 Martínez M. Docosahexaenoic acid therapy in DHA-deficient patients with disorders of peroxisomal biogenesis. Lipids 1996; 31:S145–152.

74 Martinez M. Docosahexaenoic acid ethyl ester as a treatment for patients with generalized peroxisomal disorders. In Lipids in infant nutrition. Huang YS, Sinclair AJ (editors). Champaign, Illinois: AOCS Press; 1998. pp. 29–38.

75 Martinez M, Vazquez E. MRI evidence that docosahexaenoic acid ethyl ester improves myelination in generalized peroxisomal disorders. Neurology 1998; 51:26–32.

76 Martinez M, Vazquez E, Garcia Silva MT, Manzanares J, Bertran JM, Castelló F, *et al.* Tratamiento de las enfermedades peroxisomales generalizadas con etil ester del ácido docosahexaenoico. Rev Neurología 1998; 28:S59–64.

77 • Martinez M, Vazquez E, García Silva MT, Manzanares J, Bertran JM, Castelló F, Mougan I. Therapeutical effects of docosahexaenoic acid in patients with generalized peroxisomal disorders. Am J Clin Nutr 2000; 71 (Suppl):376S–385S.

This article summarizes the outcome of 7 years of DHA therapy in a small group of patients with generalized peroxisomal disorders, and gives some basic concepts on this new treatment.