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Restoring the DHA Levels in the Brains of Zellweger Patients

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Abstract

Patients with the Zellweger syndrome and its variants have very low levels of docosahexaenoic acid (DHA) in the brain, retina, and other tissues. Such a marked DHA deficiency could be related to the pathogenesis of peroxisomal disorders. Therefore, restoring the DHA levels in these patients can probably improve the clinical course of the disease. With this rationale, 20 patients with generalized peroxisomal disorders have been treated to date with DHA ethyl ester, at daily doses of 100–500 mg, for variable periods of time. Treatment has been always accompanied by a nutritious diet, normal for the age, in order to provide all the necessary nutrients and avoid a polyunsaturated fatty acid (PUFA) imbalance. The most constant improvement has been normalization of the DHA levels and liver function. Vision has improved in about half the patients and muscle tone has generally increased. Magnetic resonance imaging (MRI) examination revealed improvement of myelination in 9 patients. Significantly, the clinical improvement has been most marked in those patients who started the treatment before 6 mo of age. Biochemically, the plasma very long-chain fatty acids (VLCFA) 26:0 and 26:1n-9 decreased markedly despite the complete diet provided. In erythrocytes, the plasmalogen ratio 18:0DMA/18:0 increased in most cases, and sometimes even normalized. All these beneficial effects suggest that DHA deficiency plays a fundamental role in the pathogenesis of peroxisomal disease. Because DHA accretion is maximal during early brain development, it is essential to initiate the treatment as soon as possible. Otherwise, restoration of brain DHA levels and prevention of further damage will not be possible.

Index Entries: Zellweger syndrome; docosahecaenoic acid; arachidonic acid; plasmalogens; DHA therapy.

Introduction

Docosohexaenoic acid (DHA; 22:6n-3) is considered to be a most important polyunsaturated fatty acid (PUFA) in the brain and retina. Although its precise function in those tissues is still poorly understood, there is much indirect evidence that DHA must play some fundamental role in these tissues. Even from the merely quantitative point of view, the high concentrations of DHA in the brain synapses (Breckenridge,1972) and rod outer segments (ROS) (Anderson, 1974) suggest that DHA is an important PUFA in the central nervous system (CNS) and the retina. Some recent studies indicate that DHA is involved in synaptic transmission (Jones et al., 1997) and the visual process (Gibson and Brown, 1993), and the relationship between retinal DHA and photoreceptor integrity and function has largely been recognized (Neuringer et al., 1986; Bush et al., 1994; Rotstein et al., 1998).

Zellweger syndrome (Bowen et al., 1964) is the prototype of generalized peroxisomal disorders, also named peroxisomal biogenesis disorders (Lazarow and Moser, 1997). In these diseases, peroxisomes cannot be assembled normally, due to a defect in protein import into the peroxisomal matrix (Subramani, 1998). So empty "ghosts" (Santos et al., 1988) are characteristically found instead of normal peroxisomes in the cells of Zellweger patients. There are milder variants of Zellweger syndrome, like neona-

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tal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD), but all share the same clinical and biochemical picture, albeit with different severity. Biochemically, the peroxisomal enzymes involved in β-oxidation of the very long chain fatty acids (VLCFA) 26:0 and 26:1n-9 are defective, and these fatty acids increase in plasma (Moser et al., 1984). The first two steps of plasmalogens synthesis are catalized by peroxisomal enzymes (dihydroxyacetone phosphate [DHAP] acyltransferase and alkyl-DHAP synthetase) (Hajra and Bishop, 1982); thus plasmalogen levels are low in Zellweger syndrome. Other lipid abnormalities include accumulation of phytanic acid in plasma and cultured skin fibroblasts (Poulos et al., 1985) and defective bile acid synthesis (Hanson et al., 1979). From the clinical point of view, Zellweger patients have most severe brain abnormalities, with neuronal migration defects and dysmyelination. The sensorineural organs are deeply affected and the patients become blind and deaf. The disease is multisystemic and other abnormalities are found in the liver, kidneys, bones, and adrenals. Patients with the classic Zellweger syndrome phenotype die very early, usually during the first year. Patients with the NALD/IRD variants may live much longer, although the prognosis is always ominous.

In 1987, we found that patients with the Zellweger syndrome and related generalized peroxisomal disorders have extremely low levels of DHA in the brain, retina, and other tissues (Martinez, 1989, 1990, 1992b, 1992c). The fact that these patients have severe neural and visual defects suggests that there may be a relationship between these abnormalities and their DHA deficiency. With this rationale in mind, we have been treating peroxisomal-disorder patients with DHA ethyl ester since 1991 (Martinez, 1992d, 1995, 1996, 1998; Martinez et al., 1993, 1998, 2000; Martinez and Vazquez, 1998), in an attempt to normalize their brain DHA levels.

It is important to emphasize that in all the patients treated, DHA supplementation has been accompanied by a normal diet. This has been done in order to provide all the nutrients necessary for a growing child, including fat. Before reporting the results obtained with this treatment, it is necessary to briefly provide some background information on normal development.

Lipid Accretion in the Developing Human Brain

During the brain growth-spurt period (Dobbing, 1968), a series of important processes take place in the human brain. The formation of synaptic connections and myelination are reflected in the accretion of certain lipids that serve as approximate markers for these processes. Phospholipids, cholesterol, and gangliosides do not start to accrete until 32 wk of gestational age (Martinez and Ballabriga, 1978), the moment when dendritic connectivity suddenly increases (Purpura, 1975). Then, towards the end of gestation, myelination starts in the human forebrain and myelin lipids (cerebrosides and sulfatides) increase dramatically (Martinez, 1982). So the third trimester of human gestation is a specially vulnerable period from the nutritional point of view, and this period extends to the first months of life, when lipid accretion levels off.

Among fatty acids, DHA has a curvilinear accretion in the forebrain, the most rapid part of it corresponding to the perinatal period (Martinez et al., 1974). Postnatally, DHA accretion continues at a lower rate from the second month until the second year of life (Martinez, 1992a; Martinez and Mougan, 1998). These are important facts to be considered when a DHA-deficient child has to be treated with DHA. To be effective, correction of a DHA-deficiency must take place before the normal curve levels off; ideally, during the perinatal period. Given too late, DHA may fail to correct any damage due to its deficiency.

Fatty Acid Changes in the Zellweger Syndrome

The most important PUFA abnormality in Zellweger patients is DHA deficiency. In the brain of peroxisomal patients, DHA levels are often as low as 20% of the normal values (Martinez, 1992b). This is found in whole brain tissue as well as in every phospholipid fraction (Martinez, 1989; Martinez and Mougan, 1999). Phospatidylethanolamine (PE), especially, has extremely low DHA levels. On the other hand, arachidonic acid (AA) is markedly increased in PE. In the whole brain, AA contrasts with DHA in that the former is usually increased or not changed, whereas the later is drastically decreased. In some brains of peroxisomal patients, however, subnormal concentrations of AA may be found and AA is slightly decreased in the kidney and plasma of Zellweger patients (Martinez, 1992c, 1995; Martinez et al., 1994). These differences are puzzling and it is not clear whether or not AA metabolism is also affected in Zellweger patients. The increases in linoleate levels (18:2n-6) found in most tissues indicate that this may be the case. However, DHA deficiency is always much more marked and present in every organ.

Table 1
Twenty Patients with Generalized Peroxisomal Disorders, Treated with DHA Ethyl Ester for the Periods Indicated

Patient	Age	Diagnosis	Treatment duration
#1	2 mo	NALD/IRD	7 yr
#2	3 mo	NALD/IRD	2 yr
#3	5 mo	NALD/IRD	4 yr
#4	6 mo	Classic ZS	3 mo[a]
#5	7 mo	NALD/IRD	4 yr
#6	7 mo	NALD/IRD	5 yr
#7	9 mo	NALD/IRD	5 yr
#8	9 mo	NALD/IRD	17 mo[a]
#9	1 yr	Classic ZS	9 mo
#10	13 mo	NALD/IRD	6 yr
#11	14 mo	NALD/IRD	3 yr[a]
#12	15 mo	NALD/IRD	5 yr
#13	15 mo	NALD/IRD	4 yr
#14	2 yr	NALD/IRD	8 mo
#15	3 yr	NALD/IRD	18 mo
#16	3 yr	NALD/IRD	6 wk[a]
#17	5 yr	NALD/IRD	6 yr
#18	5 yr	NALD/IRD	4 yr
#19	5 yr	NALD/IRD	18 mo
#20	7 yr	NALD/IRD	9 yr

ZS, Zellweger's syndrome; NALD, neonatal adrenoleukodystrophy; IRD, infantile Refsum's disease.

[a]The patient died at the age indicated.

DHA Therapy

Patients

To date, we have treated 20 peroxisomal-disorder patients with DHA ethyl ester (Table 1), for a time varying between 9 mo and 9 yr (with the exception of one that could only be treated for 6 wk; Martinez et al., 1993). Two of the patients had classic Zellweger's syndrome and the other 18 had less severe Zellweger variants that could be diagnosed with NALD or IRD. In our experience, it is most difficult to distinguish between NALD and IRD on clinical or biochemical grounds. Patients that survive long enough may be given different diagnoses by different physicians. So we prefer to simply classify our patients as those with the classic form of Zellweger syndrome and those belonging to the NALD/IRD spectrum. For ethical reasons, no double-blind study has been attempted. Given the physiological importance of DHA in the developing brain and retina, we have always tried to correct its deficiency, diregarding age and clinical severity.

Therapeutical Protocol

The DHA derivative used has been the ethyl ester (degree of purity better than 90%). A mixture of pure DHA ethyl ester (DHA-EE) and high-quality olive oil as a vehicle was aliquoted into individual, one-dose vials, which were sealed, packed in nitrogen, and stored frozen until administration. The daily doses varied between 100 mg and 500 mg of DHA-EE, depending on age and degree of DHA deficiency, but never on a body-weight basis. This was done because the brain has the largest DHA requirements during early development; so that the youngest patients with a marked DHA deficiency received the highest doses.

The diet was as complete as possible for the age, including all nutrients, and fats other than DHA. Special care was taken with nutrition of small infants. A whole-milk formula enriched in DHA and AA (20:4n-6), in a proportion similar to that in mother's milk, was used. Solid food was introduced as soon as possible, including fruit, cereals, meat, fish, and eggs enriched with DHA. No restriction other than green leaves and the white fat in meat were made. Whole dairy products were allowed. Liposoluble vitamins A and D were supplemented in regular infant doses. Vitamin K was given in daily doses of 3–10 mg/d, depending on the results of coagulation tests. Vitamin E was provided in larger doses (50 mg–200 mg/d), as an antioxidant.

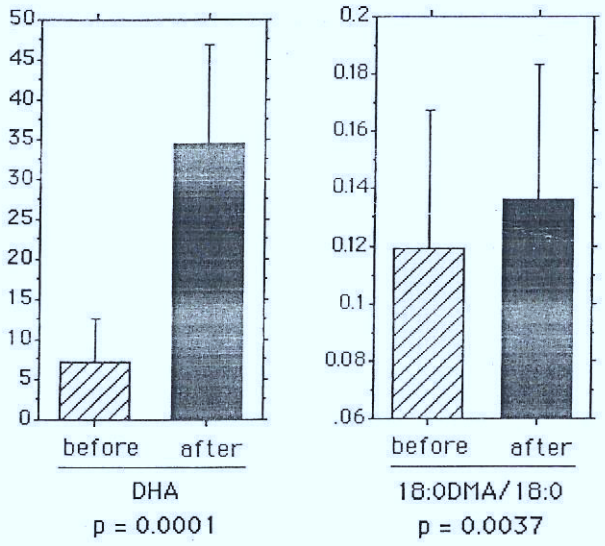

Fig. 1. Levels of DHA and the plasmalogen ratio 18:0DMA/18:0 in erythrocytes in the 18 patients with NALD/IRD, before and after DHA normalization. The two patients with classic Zellweger syndrome have not been included in the calculations because they had negligible levels of plasmalogens. The bars are the means of ±1 SD. Although the SD are quite large due to individual variation, the satistical significance for paired *t*-tests was high for both DHA ($p = 0.0001$) and the plasmalogen ratio ($p < 0.005$).

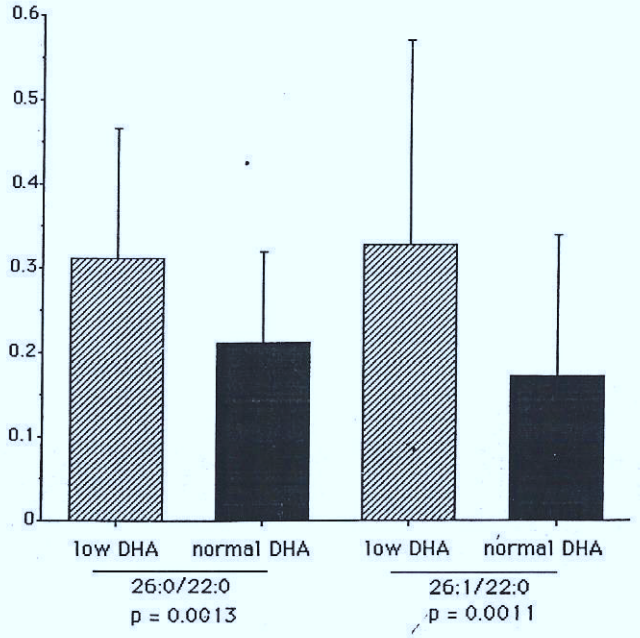

Fig. 2. Levels of the two ratios 26:0/22:0 and 26:1n-9/22:0 in plasma in the 20 patients DHA-treated, before and after normalization of the DHA levels. As in Fig. 1, paired *t*-tests were used to neutralize individual vaiation, and the satistical significance was very high in both cases.

Complete fatty acid analyses were regularly performed: every week at the beginning of the treatment, and every 1–3 mo later on. Based on the results of these tests, the DHA dosage was adjusted if necessary. Whenever a significant decrease in AA was found in erythrocytes, the diet was supplemented with some food rich in AA, such as heart, liver, and brain. This, however, was never necessary until the DHA levels had reached totally normal values.

The effects of the treatment were monitored periodically both biochemically and clinically. Special examinations included cranial magnetic resonance imaging (MRI), visual and brain stem-evoked potentials, electroencephalogram (EEG), electroretinogram (ERG) and liver echography. Routinely, liver tests (spartate aminotransferase (AST, EC 2.6.1.1), alanine aminotransferase (ALT, EC 2.6.1.2) and gamma-glutamyl transferase (gGT, EC 2.3.2.1), as well as the levels of the VLCFA 26:0 and 26:1n-9 were checked in plasma. In erythrocytes, all PUFA and plasmalogen levels were periodically quntified.

Results of the DHA Treatment

Biochemical Results

The most constant biochemical improvement was the correction of DHA deficiency in plasma and erythrocytes. DHA normalization was obtained in only a few weeks in all patients. In parallel to DHA normalization, the plasmalogen ratio 18:0DMA/18:0 increased in erythrocytes in most patients (Fig. 1). In plasma, liver tests improved constantly and rapidly, and the levels of both VLCFA, 26:0 and 26:1n-9, decreased in most patients (Fig. 2). Plasma phytanic acid levels increased at the beginning, when introducing the complete diet, but levelled off shortly afterwards and started to decrease again in a few months. Never did plasma phytanic acid reach dangerous levels despite the use of an unrestricted diet.

Clinical Results

The nutritional status improved in most patients, especially in small children with failure to thrive as a predominant clinical feature. In general, the beneficial effects of the treatment were the most marked in the youngest patients. Table 2 summarizes these effects. Three infants who started the treatment at 2, 4, and 5 mo, respectively, and who had marked liver malfunction and failure to thrive improved dramatically in a few days. In one of them (patients #1), liver function and myelination normalized very rapidly and the patient improved neurologically.

Table 2
Summary of the Biochemical and Clinical Evolution in 20 Peroxisomal-Disorder Patients Treated with DHA Ethyl Ester

Follow-up test	Improvement
DHA	20/20
VLCFA ratios	18/20
Plasmalogen ratios	12/20
Liver tests	20/20
Vision	12/20
Social contact	17/20
Muscle Tone	13/20
MRI	9/12

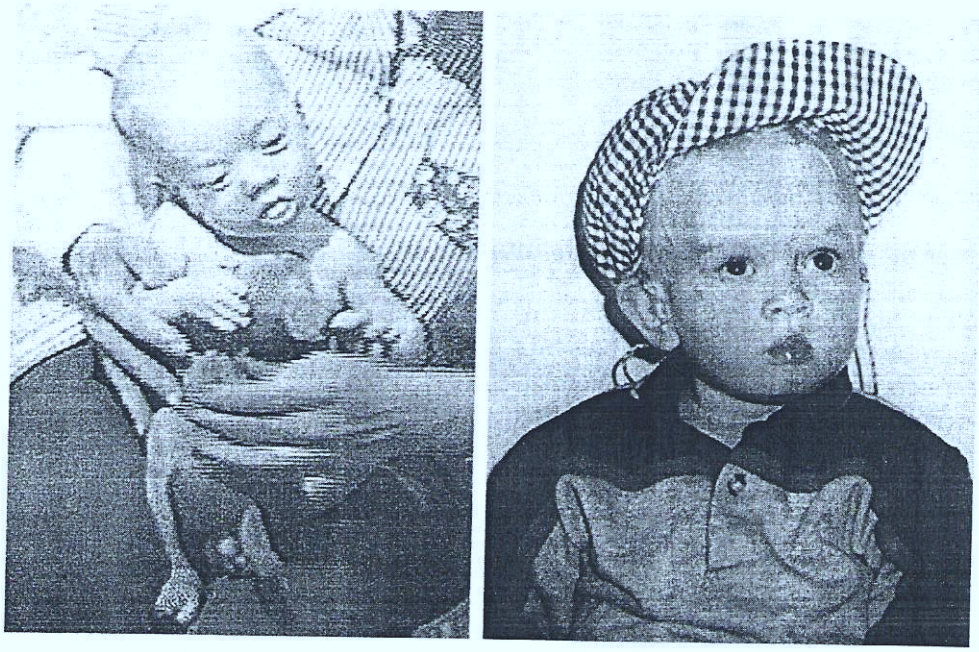

Fig. 3. Patient #2. Left, at 4 mo of age, before the DHA treatment was started. The child had severe steatorrhea, hypotonia, and failure to thrive. Right, after 1 yr of treatment with 300 mg/d of DHA ethyl ester. There was a marked improvement in liver function, growth, muscle tone, vision, and social contact.

This patient is now capable of walking normally, climbing steps, and even riding a bicycle. He is very social and communicates verbally, although with an elementary language. Another patient (patient #2) had severe steatorrhea at 4 mo of age, which virtually disappeared in 3 wk. Interestingly, this infant had been receiving a triglyceride mixture with 40–45% of DHA since 1 mo before the DHA-EE treatment and the steatorrhea persisted. Body weight, liver function, and muscle tone improved markedly with the DHA-EE in this child, and he has now a good eye contact (Fig. 3). Patient #4 had severe liver disease and marasmus at 5 mo of age. A few weeks later, her liver function, body weight, and muscle tone, and strength had improved spectacularly (Martinez et al., 2000). Another patient, who started DHA therapy at 9 mo of age and had marked hypotonia and marasmus, improved dramatically during the first year of treatment (Martinez, 1995). Unfortunately, this child unexpectedly died from septicemia a few months later.

In the patients that started the treatment after 1 yr of age, improvement was less constant, and some stabilized after an initial period of ameliora-

tion. However, neurological deterioration was only observed in one patient who initiated the treatment at 15 mo of age and had a severe form of NALD. Patients who were less affected made some progress even starting the treatment as late as at 5 yr of age (patient #17). Vision improved in that girl and demyelination halted (Martinez and Vazquez, 1998). In general, patients became more social and active, and muscle tone improved quite constantly. A clear improvement in vision was found in about half the patients and in all but one child abnormal eye movements disppeared or diminished markedly. One girl (patient #7), who was apparently blind at 9 mo of age, recovered vision progressively after some months with the DHA-EE treatment (Martinez, 1996; Martinez et al., 1998). Muscle tone and motor development has improved in this patient, and she is now walking independently.

MRI Examination

Probably the most significan changes found in the DHA-treated patients was the improvement of myelin found on MRI scans. Of the 12 patients in whom MRI follow-up could be effected, progress in myelination could be detected in 9 cases. In 4 of them, the MRI images are now normal for the age (patients #1, 3, 6, and 7). Especially significant was the cessation of active demyelination found in a 5-yr-old girl after about a year of DHA therapy. These findings have recently been published (Martinez and Vazquez, 1998; Martinez et al., 2000).

Discussion

The results of the treatment with DHA-EE suggest that DHA deficiency plays an important role in the pathogenesis of peroxisomal disease. The fact that the VLCFA decreased despite increasing their intake with a complete diet is significant, as is the increase in plasmalogens that parallels DHA normalization. The improvement in liver function and body weight was consistently present with the treatment and was so rapid in most cases that it appears to be attributable to some specific action of DHA.

It could be argued that the complete diet provided was the most important factor in making the patients thrive. This is true; but it should be remembered that such a diet is generally considered contraindicated for peroxisomal patients due to the risk of increasing their VLCFA. However, the tolerance of our patients to a normal diet was excellent and no gastrointestinal problems or increases in the VLCFA were ever found. On the contrary, when liver dysfunction and steatorrhea were present, they improved and the VLCFA decreased. This, again, suggests that DHA has some specific beneficial effect in patients with peroxisomal diseases, which counteracts the theoretical risks of providing a normal diet. The question whether or not pure DHA-EE is better than other forms of DHA administration in peroxisomal disorders remains open. Our results suggest that this may be the case, although this awaits further confirmation.

The negative correlation found between the age of the patients at the initiation of the treatment and the beneficial effects obtained are consistent with the brain developmental profiles of DHA during fetal and early postnatal life. Brain DHA accretion is maximal during the perinatal period. So, if the consequences of brain DHA deficiency have to be prevented, the treatment must be started as near birth as possible. Given too far away from this period, the beneficial effects of DHA therapy may be minimal. Only in mildly affected patients can some improvement be obtained from the treatment when given too late. On the other hand, in the most severely affected patients, especially in classic Zellweger syndrome, the brain damage is so profound, even at birth, that little can be done to improve the disease, no matter how early the treatment is started.

To summarize our personal experience with DHA therapy, we can draw some fundamental conclusions. Firstly, it is important to clarify that DHA therapy is not a drug treatment. DHA therapy is a physiological treatment, intended to correct the deficiency of the most important PUFA in the brain and retina. Secondly, DHA must be provided as early in development as possible: perinatally or, at least, during the first months of life. Because the aim of the treatment is to correct a physiological deficiency, the DHA dosage should be adapted to the developmental stage of the patient and the degree of DHA deficiency, not to body weight. However, too high DHA doses could inhibit any residual DHA synthesis that might exist. Our recommended daily dose is 200–400 mg of DHA-EE.

Thirdly, it is important to bear in mind that DHA deficiency always predominates over any marginally low AA levels that may be found. Therefore, correction of DHA deficiency is the top priority. Supplementation with high doses of AA, especially at the beginning of the treatment, should be avoided, as they will interfere with DHA normalization and cancel some of its beneficial effects. On the other