

Visual Follow-Up in Peroxisomal-Disorder Patients Treated with Docosahexaenoic Acid Ethyl Ester

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PURPOSE. To assess the possible beneficial effects of docosahexaenoic acid (DHA) ethyl ester on visual function in DHA-deficient patients with peroxisome biogenesis disorders (PBDs).

METHODS. A total of 23 patients were studied, of whom 2 had classic Zellweger syndrome and 1 had a D-bifunctional protein (DBP) deficiency. Most of the PBD patients could be followed up, but for only nine of them was there ophthalmic baseline data to enable a full evaluation of the visual effects of the treatment. A daily dose of 200 mg of DHA ethyl ester was given to all patients. Clinical examination, visual evoked potentials (VEPs), and electroretinogram (ERG) were obtained in all cases.

RESULTS. Nystagmus disappeared very quickly in all the patients. The retinal appearance remained stable in all but one. Visual acuity was maintained without deterioration in all the patients. The electrophysiological examination showed a general improvement in retinoneural function, better documented in those patients who had undergone a baseline examination, but also in two children whose ERG continued to improve many years after the treatment was initiated.

CONCLUSIONS. The visual improvement obtained with DHA therapy emphasizes the deleterious role that a DHA deficiency plays on the retina, especially in PBD patients, with retinas virtually devoid of DHA. These data, together with those reported previously, indicate that the DHA deficiency is an important pathogenic factor in peroxisomal disorders and should always be corrected. Treatment with DHA ethyl ester, given as early as possible, is strongly recommended, before the damage becomes irreversible. (*Invest Ophthalmol Vis Sci.* 2010;51:2277-2285) DOI:10.1167/iops.09-4020

Generalized peroxisomal disorders are a group of extremely severe infantile diseases, whose prototype is Zellweger syndrome.¹ Characteristically, no peroxisomes can be found in the cells of these patients,² because of defective protein import into the peroxisomal matrix,³ which leads to abnormal perox-

isome assembly. As a consequence, empty “ghosts”⁴ are often found in the cells of these patients instead of normal peroxisomes. For this reason, the conditions are also known as peroxisomal biogenesis disorders (PBDs).⁵ There are milder variations of Zellweger syndrome, known as neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD), but all share the same clinical picture, albeit with different degrees of severity. Patients with classic Zellweger syndrome have the most severe brain abnormalities, with neuronal migration defects and dysmyelination. These children have been very ill since birth, with severe hypotonia, feeding difficulties, and uncontrollable seizures. The sensorineural functions are always deeply affected and the children soon become totally blind and deaf. The disease is multisystemic, with many other abnormalities in the liver, kidneys, bones, and adrenal glands. Patients with the classic Zellweger syndrome phenotype die very early, usually during the first year of life. It is difficult to distinguish between the two other, milder variants, which should be classified as a unique NALD/IRD entity within the Zellweger spectrum. These patients may live much longer, although the prognosis is always ominous.

From the biochemical point of view, 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.⁶ The first two steps of plasmalogen synthesis are catalyzed by peroxisomal enzymes (dihydroxyacetone phosphate [DHAP] acyltransferase and alkyl-DHAP synthetase)⁷; thus, plasmalogen levels are low in Zellweger syndrome and are practically absent in the classic form of the disease. Other lipid abnormalities include accumulation of phytanic acid in plasma and cultured skin fibroblasts⁸ and defective bile acid synthesis.⁹

In 1987, we found a patient with Zellweger syndrome who had extremely low levels of docosahexaenoic acid (DHA, 22:6n-3) in the brain, liver, and kidneys, the main organs affected in these diseases.^{10,11} Later, we confirmed this finding in many other peroxisomal-disorder patients.¹²⁻¹⁴ In the retina, DHA was virtually absent. The fact that diseases in the Zellweger spectrum consistently produce severe neural and visual defects suggests that there may be a relationship between these abnormalities and their DHA deficiency. The brain is very rich in DHA, and the retina, in particular, has extraordinarily high DHA levels, mainly localized in the phospholipid bilayer of rod outer segments (ROS), where it amounts to approximately 50% of ROS membrane phospholipids,¹⁵ and to 30% of total retinal ethanolamine phosphoglycerides.¹⁶ In contrast, the Zellweger retina has less than 1%.¹³ In the brain, DHA concentration may be reduced to as little as 20% of normal. It would therefore not be surprising to find abnormal visual and neurologic functions in peroxisomal-disorder patients, even if DHA deficiency was the only abnormality.

Indeed, DHA is considered essential for the visual process. A close relationship between DHA and rhodopsin has long been suggested.¹⁷ Recently, specific interactions of DHA with rhodopsin have been demonstrated,¹⁸ where DHA packs

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Supported by the organizations and laboratories that have generously provided DHA-EE of very high purity: Fish Oil Test Materials Program, of the National Institutes of Health/ADAMHA (Alcohol, Drug Abuse and Mental Health Administration)/U.S. Department of Commerce, Grants no. 215A, 215B, 215C, Bethesda, MD; Pronova Biocare, Lysaker, Norway; Harima Chemicals, Ibaragi, Japan, and Bizen Chemical Company, Okayama, Japan.

Submitted for publication May 21, 2009; revised October 25, 2009; accepted November 6, 2009.

Disclosure: **M.T. Noguer**, None; **M. Martínez**, None
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tightly to certain specific regions in rhodopsin, probably affecting its stability and kinetics.¹⁹ Among polyunsaturated fatty acids (PUFAs), DHA seems to specifically modulate the interactions of interphotoreceptor retinoid-binding protein with 11-*cis*-retinal,²⁰ the retinoid which associates with opsin in the ROS to form rhodopsin. It has been postulated that DHA is specifically involved in visual transduction by increasing the efficiency of G protein-coupled signaling in the ROS,²¹ a mechanism similar to that in neural tissues.

Research on different, DHA-deficient experimental animals has consistently found visual abnormalities resulting in altered electroretinograms (ERGs),^{22,23} with greater a-wave amplitudes corresponding to animals with higher blood DHA levels.²⁴ From studies performed in the developing human retina, it can be concluded that there is a clear correlation between the DHA levels in blood and ERG and visual evoked potential (VEP) performances.²⁵⁻²⁷ The human infant, especially if premature, is born with suboptimal DHA levels in the brain and retina. If not supplied with enough DHA postnatally, the preterm retina will have a reduced DHA content.¹⁶ Such a DHA-deficient retina will try to compensate with an increase in 22:5n-6 (docosapentaenoic acid, DPA), a similar PUFA with just one less double bond which, nevertheless, will not be able to perform DHA functions in the visual transduction cascade. In the case of classic Zellweger syndrome, even this compensatory increase in DPA cannot occur, because the synthesis of both DHA and DPA is currently believed to be dependent on the same peroxisomal route.²⁸ In fact, we found that the DPA levels in the Zellweger retina were very low, too.¹⁵

In retinitis pigmentosa, some studies have found low blood DHA levels²⁹⁻³¹ that were corrected by enriching the diet with a DHA or omega-3 supplement. Supplementation seemed to have a positive clinical effect on the patients. In peroxisomal disorders of the Zellweger spectrum, retinal degeneration is common, often resembling retinitis pigmentosa, but sometimes acquiring the appearance of leopard spot retinal pigmentation.³² Pathology studies have demonstrated a marked degeneration of photoreceptor cells throughout the retina, with loss of retinal nerve fibers and ganglion cell layers.³³ There is optic nerve degeneration with optic atrophy.³⁴ It must be taken into account that blindness in these disorders is of a double, sensorineural origin, since demyelination of the visual tracts usually accompanies the retinal abnormalities. Other ocular abnormalities found in peroxisomal disorders include cataracts, corneal opacifications, and glaucoma. Some patients have been reported to have myopia. Nystagmus is the rule.³⁵ The natural course of the disease is rapid progression to total blindness. Disregarding what may be the exact origin of the retinal degeneration in these patients, we can be sure that a retina with such low DHA levels as we found in Zellweger syndrome cannot work properly. As happens with the brain, the normal retina continually tries to keep its DHA content constant, taking it preferentially from the diet and recycling it from the rod outer segments when they are renewed.³⁶ The pigment epithelium is in charge of this important task, which emphasizes once more the importance of DHA in retinal function. In Zellweger syndrome, obtaining DHA in this way cannot happen since the amount of DHA available is far too small, even for tissues with such high affinity for DHA as the retina.

In an attempt to normalize their brain and retinal DHA levels, we have been treating peroxisomal-disorder patients with DHA ethyl ester (DHA-EE) since 1991 and have published the results in several papers.³⁷⁻⁴³ Although visual improvement has been detected in most cases, for technical reasons it has not been possible until now to substantiate the clinical changes with a complete ophthalmic and electrophysiological

examination. In this article, we present a complete visual study in 23 patients, examined during the past 5 years. To our knowledge, this is the first publication on ophthalmic follow-up in patients with peroxisomal disorders who were treated with DHA ethyl ester. During the preparation of this work, a paper appeared online⁴⁴ reporting a substantial VEP improvement in most of the patients treated with a different DHA preparation, but no ERG data were presented.

MATERIALS AND METHODS

Patients

It is necessary to classify the 23 patients, to evaluate the results of the study properly. Enrolled were 14 boys and 9 girls, with an age range between 3 months and 12 years. They were heterogeneous, not only in phenotype but also in the timing of treatment and ophthalmic examination. For ethical reasons, we did not reject any patient for treatment, regardless of the severity of the clinical picture. Some patients started the treatment long before we could perform the visual evaluation, whereas others could only be examined once. Our research adhered to the tenets of the Declaration of Helsinki. Informed parental consent was obtained for every patient and the treatment was given with the authorization and supervision of the Spanish Ministry of Health.

Of the 23 patients, one had a D-bifunctional protein (DBP) defect and the rest had PBDs, although in one of them, it was biochemically and clinically more similar to a DBP defect, and there was no DHA or plasmalogen deficiency. This patient's disease was later found to be due to a new genetic defect. Among the 21 typical PBD patients, two had classic Zellweger syndrome, and 19 were classified within the NALD/IRD spectrum, albeit with widely different degrees of severity.

To minimize the differences, we grouped the patients as follows:

Group A: nine patients within the NALD/IRD spectrum, for whom we had baseline examination data.

Group B: 10 NALD/IRD patients who had begun the treatment sometime before the present study.

Group C: two patients with classic Zellweger syndrome.

Group D: one patient with a DBP defect and one with an atypical PBD, clinically and biochemically resembling a DBP deficiency.

Because of the short time since they had started the treatment or because of their clinical picture, some patients were examined only once. On the other hand, we had data from several examinations in some patients who started the treatment years ago, but for whom we lacked baseline data. Only in nine patients has a baseline examination (before or at the beginning of the treatment), plus a second (or more) follow-up ophthalmic evaluation, been performed.

Therapeutic Protocol

It is important to emphasize that in all the patients treated, DHA supplementation was accompanied by a normal diet, to provide all the nutrients necessary for a growing child, including fat. The DHA derivative used was always ethyl ester (degree of purity 90%-97%). A mixture of pure DHA-EE in 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 normal dose was 200 mg of DHA-EE, usually given once daily. 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 the small infants. A whole-milk formula enriched in DHA and arachidonic acid (AA, 20:4n6), 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. Green leaves (very rich in phytol, a precursor of phytanic acid) and herbivore fatty meat (high in phytanic acid and very long-chain fatty acids [VLCFA]) were restricted as a precaution. Whole dairy products were allowed. Liposoluble vitamins A and D were supplemented in regular infant doses. Vitamin K was given in

TABLE 1. Ophthalmic Follow-up of Patients in Group A

Patient	Visual Acuity				Nystagmus				Posterior Segment				VEP		ERG	
	I		II		I		II		I		II		I		II	
	Light perception only	Light perception, fix and follow targets	Light perception only	Light perception, fix and follow targets	Yes	No	Poorly pigmented	Macular dystrophy	Flash Normal P-100	Not tested	High a voltage (a/b>1)	Not tested	High a voltage (a/b>1)	Flash Normal P-100	Stable	Attenuated and delayed a- and b-waves
KW 2004/05 2005/04	Light perception only	Light perception only	Light perception only	Light perception only	Yes	No	Poorly pigmented	Macular dystrophy	Flash Normal P-100	Not tested	High a voltage (a/b>1)	Not tested	High a voltage (a/b>1)	Flash Normal P-100	Stable	Less a-wave amplitude, b-wave stable
OA 2004/07 2006/09	Light perception, fix and follow targets	Light perception, fix and follow targets	Light perception only	Light perception, fix and follow targets	Yes	No	RE rect.fold LE RD	RE ret. Fold LE RD	Flash Normal P-100	Stable	Attenuated and delayed a- and b-waves	Stable	Attenuated and delayed a- and b-waves	Flash Normal P-100	Stable	Stable
MM 2004/07 2008/05	Light perception, fix and follow targets	Yes	No	RE rect.fold LE RD	RE ret. Fold LE RD	Flash Normal P-100	Stable	Attenuated and delayed a- and b-waves	Stable	Attenuated and delayed a- and b-waves	Flash Normal P-100	Stable	Stable			
PK 2003/05 2008/05	Light perception, fix and follow targets	Light perception, fix and follow targets	Pigassou* 0.3	Light perception, fix and follow targets	No	No	RE poorly pigmented LE incipient RD	RD R/LE	Pattern Normal P-100	Enlarged latency in macular response	Extinguished	Attenuated a- and b-waves can be registered	Extinguished	Pattern Normal P-100	Enlarged latency in macular response	Attenuated a- and b-waves can be registered
HP 2005/06 2008/06	Light perception, fix and follow targets	Light perception, fix and follow targets	Pigassou 0.3	Light perception, fix and follow targets	Yes	No	RD & maculopathy R/LE	Stable	Flash Normal P-100	Flash	Extinguished	Attenuated a- and b-waves can be registered in photopic response	Extinguished	Flash Normal P-100	Stable	Stable
AC 2003/07 2007/05	Light perception, fix and follow targets	Light perception, fix and follow targets	Pigassou 0.3	Light perception, fix and follow targets	No	No	Normal	Normal	Flash Poorly defined Pattern Small amplitude but normal P-100 latency	Flash Better morphology, normal P-100 latency	Attenuated photopic and scotopic a- and b-waves	Stable	Attenuated photopic and scotopic a- and b-waves	Flash Poorly defined Pattern Small amplitude but normal P-100 latency	Flash Better morphology, normal P-100 latency	Stable
AB 2003/07 2007/05	Light perception, fix and follow targets	Yes, oscillatory	No	Salt & pepper	RD R/LE	Flash Abnormal morphology	Pattern Attenuated and delayed macular response	Attenuated and retarded photopic and scotopic a- and b-waves	Stable	Attenuated and retarded photopic and scotopic a- and b-waves	Flash Abnormal morphology	Pattern Attenuated and delayed macular response	Stable			
JJ 2007/09 2008/09	Light perception only	Light perception, fix and follow targets	Light perception, fix and follow targets	Light perception, fix and follow targets	Yes, pendular	No	Poorly pigmented optic discs	Hypoplastic optic discs Abnormal macular pigmentation	Flash Small amplitude; long P-100 latency	Flash Stable	Atypical morphology retarded a- and b-waves	Better morphology, still attenuated a- and b-waves, normal latencies	Atypical morphology retarded a- and b-waves	Flash Small amplitude; long P-100 latency	Flash Stable	Better morphology, still attenuated a- and b-waves, normal latencies
OD 2008/04 2008/10	Light perception only	Light perception, fix and follow targets	Light perception, fix and follow targets	Light perception, fix and follow targets	Yes, oscillatory	No	Hypoplastic optic disc, heterogeneous pigmentation, some spicules	Stable	Flash P-100 normal amplitude and latency	Flash Stable	Attenuated photopic response	General improvement in morphology and latency (mainly in scotopic response)	Attenuated photopic response	Flash P-100 normal amplitude and latency	Flash Stable	General improvement in morphology and latency (mainly in scotopic response)

I, baseline examination; II, last examination (the dates for both are given under each patient's code, in year/month); RD, retinal degeneration; Ret, retina.
* Pigassou vision optotypes (simple drawings of familiar things used to estimate visual acuity in young children <3 years of age).

TABLE 2. Ophthalmologic Follow-up of Patients in Group B with More Than One Visual Examination

Patient	Visual Acuity		Nystagmus		Posterior Segment		PEV		ERG	
	I	II	I	II	I	II	I	II	I	II
MW 2003/11 2008/03	Light perception, fix and follow targets Pigassou* 0.03	Light perception, fix and follow targets Pigassou 0.05	No	No	RE: RD LE: normal	Stable	Flash Normal amplitude and latency	Stable	Delayed a- and b-waves	a- and b-wave normalized latencies
APC 2004/06	Pigassou* 0.03	Pigassou 0.05	No	No	RD	Stable	Flash Small amplitude normal P-100 latency Physiologic Pattern	Flash Physiologic Pattern Physiologic	Poorly defined	Attenuated a- and b-waves can be defined
AC 2004/06	Pigassou 0.1	Pigassou 0.05	No	No	RD and macular dystrophy	Stable	Flash Physiologic Pattern Very retarded P-100	Flash Physiologic Pattern Better defined, increased amplitude and shorter latency of P-100	Extinguished	Attenuated but identifiable a- and b-waves
MD 2004/05 2006/04	Light perception only	Light perception only	No	No	RD and macular dystrophy	Stable	Flash Physiologic	Flash Stable	Poorly defined	Identifiable a- and b-waves

I, baseline examination; II, last examination (the dates for both are given under each patient's code, in year/month); RD, retinal degeneration.
* Pigassou vision optotypes (simple drawings of familiar things used to estimate visual acuity in young children under three years of age); Ret, retina.

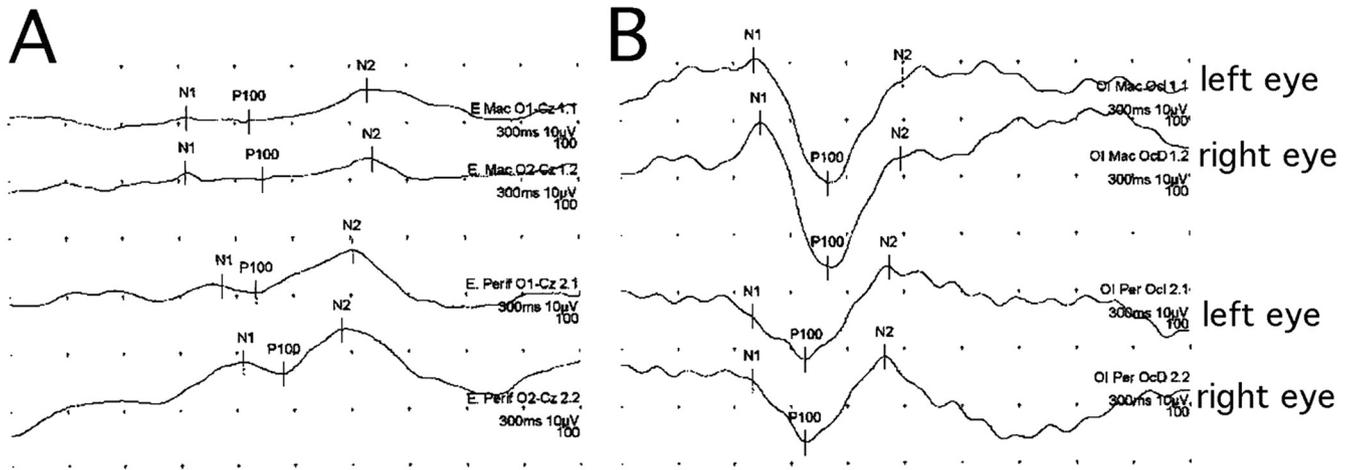


FIGURE 1. Patient AC: macular VEP in an NALD/IRD patient without signs of retinal degeneration in the posterior segment. (A) At 3 years 7 months of age. (B) At 6 years of age. Note the improvement in VEP morphology and amplitude. He remains clinically stable. His visual acuity is 0.3 (Pigassou's method).

daily doses of 3 to 10 mg/d, depending on the results of coagulation tests. Vitamin E was provided in larger doses (50–200 mg/d), as an antioxidant.

Ophthalmic Examination

Visual examination had to be adapted to the children's different ages and clinical condition. Some of our patients were in a poor general state, and most of them had difficulty cooperating with the procedure, and so we tried to make the examination as short as possible. In general, assessment of visual acuity is difficult in small children and even more so in the mentally retarded. We could not measure acuity by the standard charts used in adults or normal older children, but by Pigassou's procedure.⁴⁵ This method was devised for small children (<3 years of age). Instead of the common letter optotypes, Pigassou's chart shows familiar drawings, often used in nurseries, which the child can more easily identify. These drawings are of different sizes, and visual acuity is approximately estimated by decimal scores going from 0.05 to 0.8.

The items used for visual examination were as follows:

- Visual behavior observation or visual acuity measurement.
- External ocular motility evaluation.
- Pupil motility.
- Anterior segment examination.
- Cycloplegic refraction.

Posterior segment examination.

Visual electrophysiology (the equipment used was a Medelec Synergy, [Oxford Instruments, Oxford, UK], with a HP71 computer and a Vectra monitor [both from Hewlett Packard, Palo Alto, CA]).

For VEPs and ERGs, we used the following parameters:

Flash VEP: 100 summarized stimulations with a white flash of 0.7-J intensity and 1-Hz temporal frequency with a $\pm 10\%$ variability.

Pattern VEP: 100 summarized stimulations of alternative screen in two modalities, 15' and 60', at the same frequency as flash. Pattern VEPs were only performed in cooperative patients.

ERG: Because of the special condition of our patients, stimulation for ERG was performed with filter interposition, for long, medium, and short wave lengths, as follows: (1) four stimulations with the three kinds of interposed filter, in mesopic conditions; (2) four stimulations with the short-wave-length filter in scotopic conditions; and (3) a unique stimulation with white flash in scotopic conditions.

RESULTS

Biochemical Changes

Blood fatty acid analyses were performed periodically during the treatment, both in plasma and erythrocytes. At baseline,

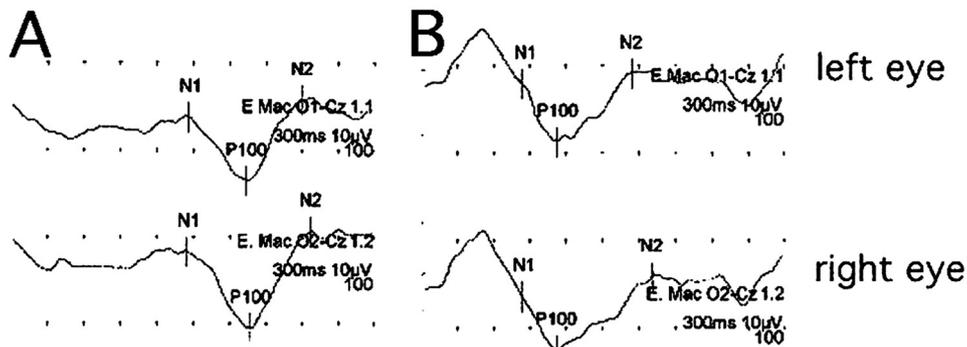


FIGURE 2. Patient HP: macular VEP in an NALD/IRD patient with a few pigment spots in peripheral retina and a visual acuity of 0.3 with Pigassou optotypes. (A) At 3 years 3 months of age, macular VEP showed very enlarged latencies. (B) At 4 years 4 months old there was total normalization of latencies in the macular PEV. The patient remains clinically stable.

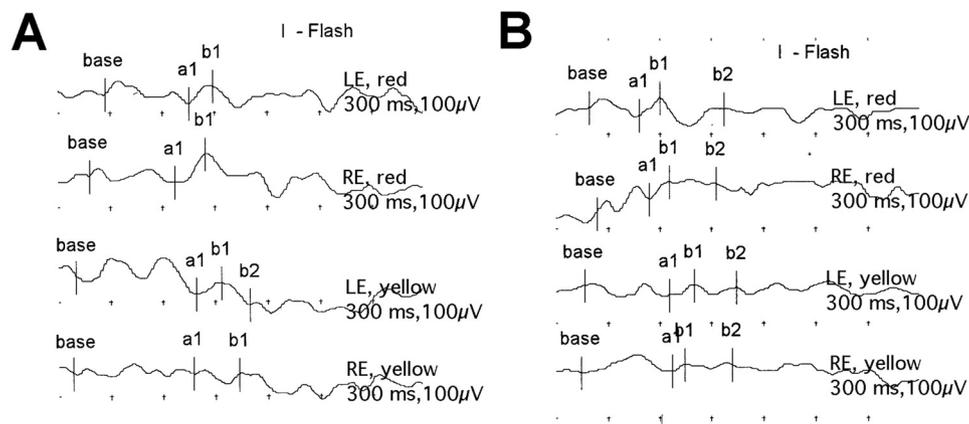


FIGURE 3. Patient JJ: ERG in an NALD/IRD patient with hypoplastic optic disc and poorly pigmented retina in both eyes. (A) At 6 months of age. (B) At 18 months of age. Notice the improved latencies of a- and b-waves in the ERG photopic responses.

the PBD patients had a marked DHA deficiency, except for the patient with DBP deficiency and the patient with atypical PBD. In the latter two patients, erythrocyte plasmalogen levels were normal. In the rest, they were decreased to a lesser or greater extent. In all DHA-deficient cases, without exception, blood DHA levels normalized and even optimized, in just a few weeks of treatment with DHA-EE. As previously reported in other patients,^{37,40,42,43} after DHA normalization, plasmalogen ratios increased in erythrocytes in most PBD cases. Little if any plasmalogen increase was detected in the two patients with classic Zellweger syndrome. As previously reported, plasma VLCFA levels always tended to decrease, even though there were no fat restrictions in the diet.⁴⁰⁻⁴³

Clinical Follow-up

It was not possible to follow up all our patients because of the different reasons already mentioned. Only about half the children could be examined more than once, nine of them belonging to group A. We will mainly focus on this group, whose ophthalmic changes are summarized in Table 1. Besides, some patients in group B continued to show some visual improvement, even after many years of starting the treatment. Those patients are depicted in Table 2. Others showed stabilization. None developed blindness after starting the treatment. The two patients with classic Zellweger syndrome in group C were too young for a correct visual evaluation and died before a second examination could be performed, as did the DPB-deficient child in group D. Other patients discontinued the treatment with DHA-EE and never came again for follow-up.

Among the initial abnormalities of patients in group A, three patients presented initial pigment retinal degeneration (just a few pigmented spots in equatorial retina). Two patients had hypoplastic optic discs (small, poorly delimited, and gray) and one patient had unilateral persistent hyperplastic posterior vitreous (PHPV; a white, dense membrane inserted in the optic disc and extending to the retrolental area). One boy had monocular superior eyelid ptosis, which had to be surgically corrected. Five patients had marked hypermetropia (over +5 D). None had myopia. No anterior segment or pupil motility abnormalities were detected. Pendular and oscillatory nystagmus, which was a very common initial sign, disappeared with the treatment in all cases, including the four patients in Table 2, who had presented this sign long before our present report.

Evaluation of vision is quite difficult in these patients, in some of whom autism adds to visual impairment, making visual behavior difficult to interpret by simple clinical examination.

That is why we tried to obtain more objective information from the electrophysiological study of our patients. Some of them responded only to light. Others could also track objects. Table 1 summarizes the changes before and after taking the DHA-EE treatment, in those patients who were followed up from the beginning of the therapy (group A).

Electrophysiological Follow-up

Figures 1 and 2 show the VEP improvement in two young children who started the treatment at 3 and 6 years, respectively, and Figures 3 to 6 show the ERG changes in four other patients. The ERGs of two of the youngest patients are presented in Figure 3 and 4. These children started the treatment at 6 and 7 months of age, respectively, and their visual improvement was clear just a few months later, both clinically and electrophysiologically.

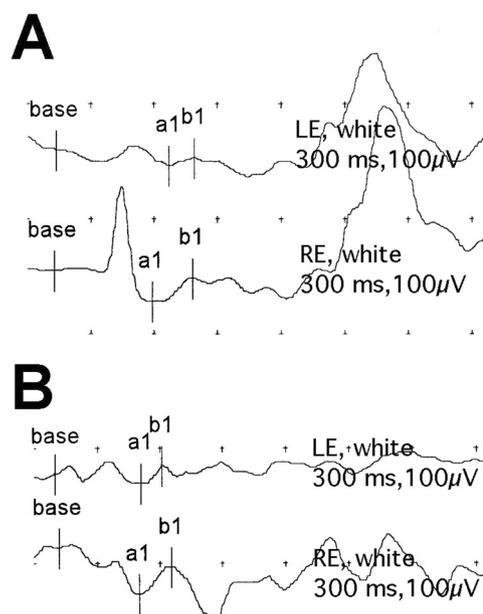


FIGURE 4. Patient OD: scotopic ERG in an NALD/IRD patient with a few pigment spots in peripheral retina. (A) At 5 months of age. (B) At 11 months of age. Note the ERG positive evolution in just 6 months of treatment with DHA-EE, with an evident improvement in morphology and latency of a- and b-waves.

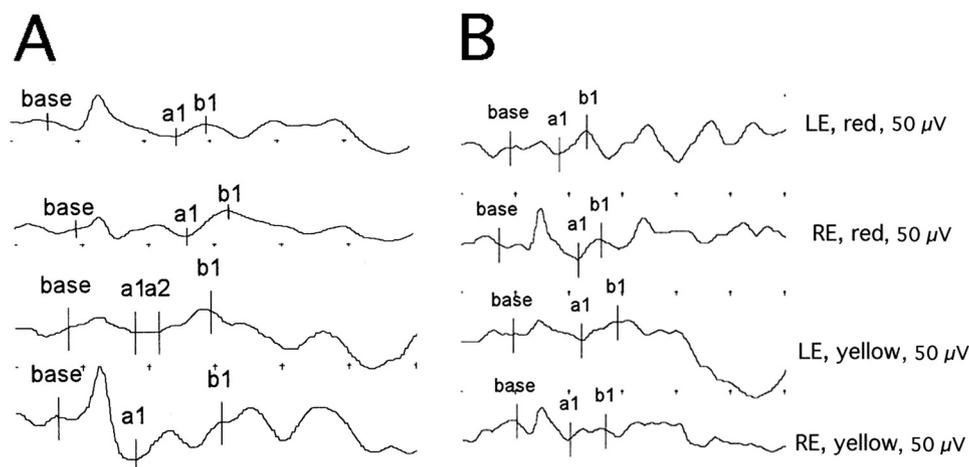


FIGURE 5. Patient MW: ERG evolution in an NALD/IRD patient who had macular dystrophy in his right eye. His left eye appeared normal. (A) At 6 years of age, having received DHA therapy since the age of 5 months (his previous ERG was reported to be extinguished). (B) At 9 years of age, this boy's ERG continues to improve, showing better tracing morphology, greater amplitude, and latency normalization.

From group B, Figures 5 and 6 show the long-term ERG follow-up in two children who started the treatment 9 and 6 years ago, respectively, before the first tracings shown in (A) in all the figures, and who continue to take DHA-EE today. The baseline ERGs of these patients, performed in a different hospital, were reported to be extinguished. Although we cannot obviously compare our electrophysiological data with those performed with different equipment by different experts, it is clear that both patients continue to make visual progress after many years of treatment.

DISCUSSION

The present data corroborate the importance of DHA in the visual process, in general, and in peroxisomal disease, in particular. Because of the fundamental role of DHA in the visual transduction cascade, a high, constant content of this PUFA should always be maintained in photoreceptor cells. This level is normally assured by the preferential DHA incorporation and recycling from the retinal pigment epithelium.³⁶ When this regulatory mechanism is not efficient because DHA is not

supplied or cannot be synthesized, the DHA deficiency is highly deleterious to the visual process, as has been repeatedly demonstrated in experimental animal models. Peroxisomal disorders represent the extreme of such a DHA-deficient condition. In these diseases, the DHA levels are much lower than those found in any other circumstance, and the retina is practically devoid of it.¹³ Such a DHA deficiency in membrane phospholipids correlates with the neurologic and retinal malfunction that these patients present.

Retinal degeneration, nystagmus, pupil motility defects, corneal opacities, cataract, and a hypotrophic optic nerve have usually been associated with peroxisomal disorders. In our study nystagmus was the rule, but we have not found corneal opacities and only one patient developed cataract until now. Strabismus was not very common either. We were surprised at the severe hypermetropia present in our patients (over +5 D) which, to our knowledge, has not been described in peroxisomal disorders. Even though in some cases it could have been due to the anteroposterior axis diminution caused by maculopathy, most of the patients did not have this pathologic effect. Pupil defects were not frequent, either.

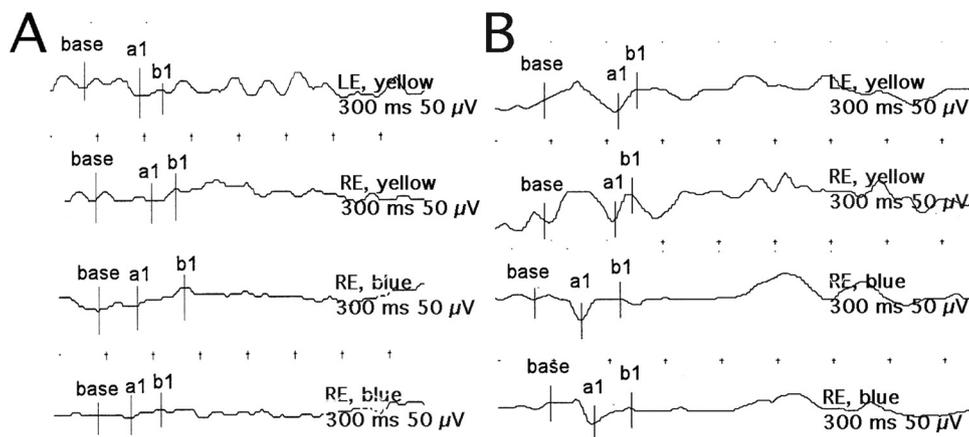


FIGURE 6. Patient APC: ERG tracings in an NALD/IRD patient with a few pigment spicules in her retinas and very scarce spots with a leopard skin-like appearance. Her visual acuity was 0.03 to 0.05 (Pigassou's chart). Like the patient in Figure 5, this girl started DHA therapy long before the first ERG shown here was performed (at 17 months). (A) At 5 years 7 months of age. (B) At 7 years of age. The greater amplitude of the a-wave shown in (B) indicates a long-term effect of the treatment.

The most frequent and disabling visual effect in peroxisomal deficiencies is retinal degeneration. Photoreceptor degeneration and apoptosis lead to blindness. In the general population, retinal degeneration has a slow evolution with good central vision until medial age, but in peroxisomal deficiency, evolution is dramatic with a quick visual deterioration. It seems that most of the patients pass from stage II (vascular narrowing and equatorial pigmentation) to stage III (macular dystrophy) very quickly, because of the presence of early maculopathy in some cases, even before peripheral pigmentary dystrophy appears. In fact, there is always a general retinal dysfunction, although pigmentary changes appear only later in many cases. These may adopt the appearance of leopard skin-like spots,³² a finding that is taken as diagnostic of NALD. However, only one of our patients in group B, who presented with pigmentary retinal degeneration, had a few rounded, pigmented spots in the pre-equatorial retina, reminiscent of leopard skin.

The ophthalmic results obtained with the DHA therapy in this study are encouraging, because of the clear amelioration in several cases and the clinical stabilization in the rest. This course is not the spontaneous one of the disease, which rapidly evolves to total blindness in most cases. Nystagmus disappeared in all of our patients. Although it was difficult to evaluate vision in such mentally and visually affected patients, in those who cooperated, it was found that they kept the same visual acuity—those who presented maculopathy, as well as the ones without this effect. Retinal appearance remained stable in all patients except one. Most important, the electrophysiological examination showed a general improvement of retinoneural function, most evident in six of the patients.

Even though the number of patients was not high (especially those for whom we had a baseline examination) and the follow-up time was short, the results obtained in this study, added to our previous positive experience in many other patients, encourage us to strongly recommend treatment with DHA-EE in peroxisomal-disorder patients within the NALD/IRD spectrum. Patients with classic Zellweger syndrome or DBP deficiency, on the other hand, show little if any response to the treatment, and no visual improvement was detected. Their disease is far too advanced from birth to make any medical intervention effective. Logically, it is in the youngest patients with relatively mild Zellweger variants in whom the DHA-EE is most effective, both neurologically as well as visually. When making a prognosis about the treatment, it must always be considered that DHA accretion in the brain is maximum at the end of gestation and during the first months of life, and that in the retina, DHA starts accumulating even earlier. Therefore, we should start DHA therapy as soon as possible if we want to obtain the maximum benefit for these children.

Acknowledgments

The authors thank the Department of Clinical Electrophysiology in the Corachán Clinic for providing all the necessary equipment for the electrophysiological evaluation of the patients.

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