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Docosahexaenoic acid and retinal function in children with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency

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Progressive vision loss associated with pigmentary retinopathy is a common complication of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD; McKusick 600890), occurring in at least 70% of LCHAD-deficient individuals (Gillingham et al 1999; Tyni et al 1998). Life-threatening episodes of hypoglycaemia and acidosis are largely prevented through contemporary dietary therapy for LCHADD, which may include restriction of dietary long-chain fat, supplementation with medium-chain triglycerides (MCT) and avoidance of fasting, but the development of retinal abnormalities is not prevented. The pathophysiological basis of retinal degeneration in LCHADD is unknown. We detected plasma docosahexaenoic acid (DHA) deficiency in a 1-year-old female with LCHADD as part of routine monitoring for essential fatty acid deficiencies. DHA is the most abundant fatty acid in retinal photoreceptor membrane phospholipid (Anderson et al 1974), and DHA deficiency is associated with retinal dysfunction in rhesus monkeys (Neuringer et al 1986) and in human infants (Birch et al 1997). Also, extreme DHA deficiency due to impaired synthesis is associated with severe progressive pigmentary retinopathy in Zellweger syndrome and neonatal adrenoleukodystrophy, disorders of peroxisomal biogenesis. Our hypothesis is that DHA deficiency contributes to the development of the pigmentary retinopathy associated with LCHADD.

DHA (C_{22:6n-3}) and other long-chain polyunsaturated fatty acids cannot be synthesized from short-chain fatty acids in humans and must be obtained in the diet, so they are considered essential fatty acids. Preformed DHA may be supplied by a diet rich in fish, but DHA is also synthesized from its 18-carbon precursor, α -linolenic acid (C_{18:3n-3}) (Voss et al 1991). Plasma DHA deficiency in our patient persisted despite apparently adequate dietary intake of α -linolenic acid. LCHADD had been diagnosed biochemically and treated prior to 2 months of age. Sequence analysis of the mitochondrial trifunctional enzyme α -subunit gene revealed compound heterozygosity for a G-to-C mutation at nucleotide position 1528 in one allele and a C-to-T mutation at position 1132 in the other allele (Sims et al 1995). Details of her dietary therapy have been published previously (Gillingham et al 1999); briefly, her diet between 1 and 3 years of age consisted of restriction of long-chain fatty acids to 9% of total energy, MCT supplementation (1.5 g/kg per day; total MCT + LCT intake = 20% of total energy), and safflower oil as a source of essential fatty acid precursors. During this period, our patient's plasma DHA level varied between 4.5 and 9.8 μ g/ml (normal 20–56). The plasma α -linolenic

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acid level was $6.99 \pm 3.2 \mu\text{g/ml}$ (normal 6.9–19.7), suggesting that dietary supply of DHA precursors was sufficient to support adequate DHA synthesis.

We postulate that LCHAD deficiency impairs the synthesis of DHA from α -linolenic acid. To synthesize DHA, α -linolenic acid undergoes three elongation/desaturation cycles to form a 24-carbon ω -3 fatty acid with six double bonds $\text{C}_{24:6n-3}$ followed by a single round of β -oxidation to yield DHA. Elongation and desaturation of α -linolenic acid probably occurs in the microsomal fraction of cells and the final β -oxidation step probably occurs in peroxisomes (Moore et al 1995), but evidence suggesting a role for mitochondrial β -oxidation has been presented (Infante and Huszagh 1997). In our patient, plasma $\text{C}_{22:5n-3}$ ($10.9 \pm 1.4 \mu\text{g/ml}$) was persistently elevated to over twice normal (normal 2.7–4.7), suggesting that subsequent processing of $\text{C}_{22:5n-3}$ to DHA was partially blocked. The HPLC method used to analyse plasma essential fatty acid levels is unable to resolve fatty acids longer than C_{22} ; the levels of $\text{C}_{24:5n-3}$ and $\text{C}_{24:6n-3}$, intermediates in the synthesis of DHA from $\text{C}_{22:5n-3}$, were not measured in our patient. Oral supplementation with 65 mg DHA triglyceride (DHASCO, Martek Biosciences Corp., Columbia, MD, USA) per day over 2 years completely corrected DHA deficiency in our patient (plasma DHA $49.3 \pm 5.7 \mu\text{g/ml}$). α -Linolenic acid levels did not change, but $\text{C}_{22:5n-3}$ levels decreased ($6.33 \pm 0.58 \mu\text{g/ml}$). This suggests the existence of a feedback loop suppressing further DHA synthesis once plasma DHA content had normalized. Our interpretation of the cumulative data is that elongation and desaturation of α -linolenic acid was intact but that β -oxidation of $\text{C}_{24:6n-3}$ to $\text{C}_{22:6n-3}$ was possibly impaired in our patient. This could occur in LCHADD if mitochondrial LCHAD activity plays some as yet unknown direct role in the DHA synthetic pathway. Alternatively, intracellular accumulation of LCHADD-specific metabolites such as long chain 3-hydroxyacyl-CoA species may somehow interfere with DHA synthesis in peroxisomes. Experiments designed to critically examine these possibilities are underway.

We propose that DHA deficiency plays a role in the development of LCHADD-associated pigmentary retinopathy. If this is true, then oral supplementation with DHA may prevent or significantly delay the onset of vision loss in individuals with LCHADD. To test these hypotheses, we have begun an open-label trial of DHA supplementation in children with LCHADD. Currently, we have enrolled 8 children aged 11 months to 15 years in the study. One child is homozygous for the common c.1528G $\rightarrow$ C mutation, while 6 children are compound heterozygous for c.1528G $\rightarrow$ C and for other private mutations in the trifunctional enzyme α -subunit. Clinical and biochemical evidence of LCHAD deficiency is present in the eighth child, but sequencing of the trifunctional enzyme α -subunit cDNA has failed to detect any abnormalities. Plasma essential fatty acid analyses are available in 7 subjects; plasma DHA deficiency was detected in 4 of 7 children (plasma DHA 6.7–18.7 $\mu\text{g/ml}$), while the levels were in the low-normal range in 3 subjects (23.4–38.7 $\mu\text{g/ml}$; normal 20–56). Plasma α -linolenic acid levels were normal in all children. Ophthalmological examinations and electrophysiological measurements of visual function were performed before and repeatedly after oral DHA supplementation. Fundoscopic examination prior to DHA supplementation revealed pigmentary retinal lesions in 5 of 8 children with abnormalities detected as early as 18 months age. Even in children without fundoscopic anomalies, visual evoked cortical potential (VECP) sweep response, an electrophysiological measurement of overall visual acuity, was frequently decreased. Other VECP modalities and electroretinogram (ERG) were abnormal only in 15-year-old male with severe retinal degeneration and a measured visual acuity of 20/400, suggesting that these electrophysiological parameters become abnormal only late in the course of retinal deterioration. The presence of retinal pigment or a decrease in the VECP sweep response are the most sensitive early indicators of retinal degeneration in these children.

Four children have completed one year of DHA supplementation (65 mg DHASCO/day for children <20 kg body weight or 130 mg/day for children > 20 kg). Plasma DHA levels have increased to the high-normal range in all 4 children (Table 1). VECF sweep response improved in 3 of the 4 children. In 2 of the 4 (patients 1 and 4), VECF sweep response was initially below normal but improved significantly after only 6 months of DHA supplementation. VECF sweep response was low-normal in patient 2 at the beginning of the trial but also increased on DHA. VECF sweep response in patient 3 did not change following DHA supplementation. Pre-trial plasma DHA levels in patients 3 and 4 were in the low-normal range, yet VECF sweep response improved in both children. These data suggest that plasma DHA levels are not necessarily predictive of response to oral DHA supplementation in LCHADD and therefore may not be entirely representative of the essential fatty acid content of photoreceptor membranes. DHA supplementation may enhance visual function in children with LCHADD.

Our preliminary study has revealed tantalizing evidence but not yet definitive proof of an association between DHA deficiency and chronic progressive pigmentary retinopathy in children with LCHADD. Clinical and laboratory observations in one child with LCHADD over a 4-year period have suggested that LCHAD deficiency may impair DHA synthesis, probably at the level of the final β -oxidation step in peroxisomes. Careful investigation of the DHA synthetic pathway in LCHADD using radioisotope or stable-isotope-labelled DHA precursors will be necessary to prove this hypothesis. Early results from an oral DHA supplementation trial in a small number of subjects have demonstrated positive effects upon visual function in LCHADD, but the constancy and duration of these benefits have yet to be determined. In our experience, DHA supplementation in LCHADD has not been associated with any known adverse effects. The impact of other nutritional and metabolic parameters upon retinal function in LCHADD is also largely unexplored. Long-term, detailed study of more LCHAD-deficient children will be necessary to fully evaluate our hypotheses.

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Table 1
Plasma DHA levels and VECF sweep measurements in four children with LCHADD before and after DHA supplementation

Patient (age)	Plasma DHA (µg/ml)			V ECF sweep (cycles/degree)		
	Pre-DHA	DHA X 6 months	DHA X 12 months	Pre-DHA	DHA X 6 months	DHA X 12 months
1 (3 years)	9.9	44.7	64	15	22	20
2 (5 years)	38	49.1	64.1	25	30	30
3 (5 years)	29.6	44.5	56.0	21.9	18	19.4
4 (10 years)	23.4	58.7	48	7	19	22
Normal	20–56	20–56	20–56	25–30	25–30	25–30