

SHORT REPORT

Long term effects of long chain polyunsaturated fats in hyperphenylalaninemic children

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Blood fatty acid status and visual function of 20 treated hyperphenylalaninemic (HPA) children, randomly allocated into two groups to receive supplementation of either long chain polyunsaturated fatty acids (LCPUFA), including docosahexaenoic acid (DHA), or placebo for 12 months, have been investigated three years after the end of the treatment. Although in the LCPUFA group blood DHA levels and P100 wave latency improved at the end of supplementation, they had returned to baseline after three years.

Hyperphenylalaninemic (HPA) children affected by congenital deficiency of phenylalanine (Phe) hydroxylase are treated with a low Phe diet with the object of protecting brain development from potentially damaging high levels of Phe in blood. The resulting diet is deficient in whole animal foods, which are rich in Phe. Since animal foods are sources of long chain polyunsaturated fatty acids (LCPUFA) including docosahexaenoic acid (DHA) and arachidonic acid (AA), HPA children show depressed levels of these molecules in circulating and erythrocyte lipids.¹ Nevertheless, minor developmental abnormalities are common in HPA children despite good dietary compliance.² As LCPUFA may have a role in neurotransmission,³ their dietary lack could contribute to the suboptimal development of HPA patients.

Trials of dietary supplementation with LCPUFA have been performed in HPA children, with positive results during the supplementation period.^{4,5} At the end of 12 months LCPUFA supplementation, an increase of levels of DHA in circulating lipids and a decrease of P100 wave latencies at 15' checkerboard pattern, as a marker of improved neurofunctional efficiency, were observed.⁴ Whether the biochemical and/or functional effects of dietary LCPUFA may persist beyond the supplementation period is still unknown. HPA may represent a model to investigate medium and long term effects of supplemented LCPUFA after the end of supplementation periods, since patients are kept under strict clinical and metabolic control. These observations could also be of help in defining the optimal requirements for LCPUFA in the paediatric age group. We have re-examined 20 HPA patients three years after the end of a supplementation period with LCPUFA to check possible postsupplementation effects on blood fatty acid status and visual function at visual evoked potentials (VEPs).

SUBJECTS AND METHODS

In a double blind placebo controlled trial, 20 children treated by diet for HPA in our department and attending the primary school had randomly been allocated to receive either LCPUFA or placebo (olive oil) supplements for 12 months. The study protocol with entry criteria and methods, including the type of LCPUFA supplementation, has been detailed previously.⁴ In the present study all children were examined for blood fatty

acid status and VEPs three years after the end of the 12 month supplementation period. To study blood fatty acids we included in the analysis plasma total lipids (TL), triglycerides (TG), phospholipids (PL), cholesterol esters (CE), and erythrocyte total lipids (Ery), phosphatidylethanolamine (EryPE), and phosphatidylcholine (EryPC) separated with thin layer chromatography and analysed with high resolution capillary gas chromatography. Visual function was investigated with pattern reversal (P) and flash VEPs with three stimuli—a stroboscopic flash, a large pattern, and a small pattern, respectively. Plasma Phe was monitored monthly by means of Guthrie's card. Descriptive data are reported as mean (SD) and median. Comparisons between LCPUFA supplemented and unsupplemented groups were performed by the non-parametric Mann-Whitney U test. Within group comparisons were performed by the Wilcoxon test. Significance of multiple comparisons was adjusted using the Bonferroni correction. All values of $p < 0.05$ were considered to indicate statistical significance (two tailed test).

RESULTS

Age (13.5 ± 2.4 v 13.6 ± 2.8 years) and demographic characteristics were comparable in the LCPUFA unsupplemented and supplemented groups, respectively.⁴ The means of the individual average blood Phe levels were similar in the two groups, both during the three years follow up (431 ± 178 v 456 ± 201 $\mu\text{mol/l}$) and at the time of final analysis (468 ± 297 v 449 ± 168 $\mu\text{mol/l}$) in the unsupplemented versus the supplemented children, respectively. The two study arms were well balanced for all blood DHA and visual variables (table 1). At the end of supplementation both blood fatty acid status and visual function improved in the LCPUFA supplemented group. Three years after the end of supplementation blood DHA and visual variables recovered to baseline values (except for Ery), and no significant differences between unsupplemented and supplemented groups were observed (table 1). Wide intersubject variability in DHA variables was observed, with the coefficient of variation (percentage ratio of standard deviation to mean) ranging from 28% to 114% in the LCPUFA supplemented group and 28% to 98% in the unsupplemented group, respectively. Blood arachidonic acid values did not differ between the two groups at any time point (data not shown).

DISCUSSION

In the present study the improvements of the blood fatty acid status and functional performance of VEPs, observed at the end of LCPUFA trials,^{4,5} were no longer evident three years after the end of supplementation. Results suggest that the

Abbreviations: CE, cholesterol esters; DHA, docosahexaenoic acid; Ery, erythrocyte total lipids; EryPE, phosphatidylethanolamine; HPA, hyperphenylalaninemic; LCPUFA, long chain polyunsaturated fatty acids; Phe, phenylalanine; PL, phospholipids; TG, triglycerides; TL, total lipids; VEP, visual evoked potential

Table 1 DHA as weight% of total fatty acids in plasma TL, TG, PL, and CE, and erythrocyte lipids, PC, and PE followed by latencies (ms) of P100 wave

Variable	Baseline		End of supplementation		3 years after the end of supplementation	
	Unsupplemented	Supplemented	Unsupplemented	Supplemented	Unsupplemented	Supplemented
	Plasma TL DHA	1.2±0.4 (1.2; 0.5-2.0)	1.1±0.3 (1.0; 0.7-1.8) ^a	1.1±0.3 (1.1; 0.8-2.0)	2.3±1.1 (1.7; 1.2-3.9) ^b	1.0±0.2 (1.0; 0.5-1.4)
Plasma PL DHA	1.7±0.5 (2.0; 0.8-2.3)	1.6±0.3 (1.6; 1.1-2.1) ^a	1.6±0.4 (1.4; 0.9-2.5)	3.1±1.6 (2.2; 1.5-6.1) ^b	1.4±0.4 (1.4; 0.7-2.0)	1.3±0.3 (1.5; 0.9-2.0) ^a
Plasma TG DHA	0.1±0.1 (0.1; 0.0-0.3)	0.2±0.2 (0.1; 0.0-0.1) ^a	0.3±0.3 (0.1; 0.1-1.1)	0.6±0.5 (0.3; 0.1-1.5) ^b	0.2±0.1 (0.1; 0.0-0.4)	0.2±0.1 (0.2; 0.1-0.5) ^a
Plasma CE DHA	0.2±0.1 (0.2; 0.1-0.6)	0.2±0.0 (0.2; 0.1-0.4) ^a	0.2±0.1 (0.2; 0.1-0.6)	0.5±0.2 (0.3; 0.2-0.9) ^b	0.2±0.1 (0.2; 0.0-0.5)	0.1±0.1 (0.2; 0.0-0.3) ^a
Ery DHA	1.1±0.5 (0.9; 0.5-2.4)	1.3±0.6 (1.1; 0.5-2.3) ^a	1.5±0.5 (1.6; 0.8-2.5)	2.8±1.5 (2.0; 1.2-5.1) ^b	1.3±0.6 (1.3; 0.6-2.5)	1.6±0.7 (1.2; 0.9-3.1) ^a
EryPC DHA	0.3±0.1 (0.3; 0.1-0.7)	0.4±0.1 (0.3; 0.1-0.6) ^a	0.3±0.2 (0.3; 0.1-0.7)	0.8±0.2 (0.8; 0.5-1.3) ^b	0.6±0.2 (0.2; 0.1-3.0)	0.4±0.2 (0.3; 0.2-1.0) ^a
EryPE DHA	1.8±0.7 (1.9; 0.5-2.7)	2.2±1.0 (2.1; 0.3-3.7) ^a	1.3±0.9 (1.1; 0.3-2.2)	3.7±1.7 (3.5; 1.5-6.1) ^b	1.8±0.9 (1.5; 0.6-3.5)	2.1±0.8 (2.0; 1.1-3.9) ^a
Pattern reversal VEP						
60'	109±6 (111; 99-119)	106±3 (108; 106-112)	109±8 (108; 96-125)	104±4 (102; 100-112)	106±5 (106; 97-113)	101±3 (101; 95-106)
15'	116±8 (115; 100-133)	113±8 (115; 96-128) ^a	118±11 (115; 101-132)	107±6 (108; 98-115) ^b	110±7 (110; 100-122)	108±5 (107; 102-122)
Flash VEP						
1Hz-2J	123±8 (124; 112-136) ^a	122±9 (121; 111-142) ^a	114±8 (111; 104-130)	113±10 (112; 100-128)	107±10 (107; 94-123) ^b	104±10 (106; 80-115) ^b
2Hz-1J	124±11 (121; 105-142) ^a	121±11 (120; 104-138) ^a	121±8 (118; 110-138)	111±12 (109; 97-131) ^b	111±10 (111; 94-122) ^b	114±5 (116; 104-126)

Data are expressed as mean±SD (median; min-max). LCPUFA supplemented v unsupplemented: p<0.05 for DHA in plasma TL, PL, CE, EryPC, EryPE, pattern reversal VEPs at 15', and flash VEPs at 2Hz-1J at the end of the supplementation period. Different superscripts (a, b, c) indicate significant within group differences between time points.

positive effects on fatty acids status and visual function of a 12 month LCPUFA supplementation period to treated HPA children may weaken over time after the end of the supplementation. Although this conclusion may be consistent with a pharmacological-like effect of circulating LCPUFA levels on neural performance, caution should be exercised owing to the small size of the studied population and the relatively high intersubject variability. It may be hypothesised that the neurotransmission processes could be helped by a high availability of LCPUFA (particularly DHA) in circulating lipids. Indeed, the question of the persistence of any biochemical and/or functional effect of dietary LCPUFA remains open, particularly in the case of healthy infants.³ HPA patients might offer a unique model to assess the effects over time of specific fat supplementation, as they do have no other opportunity to acquire LCPUFA with their diet. To what extent supply of LCPUFA could benefit HPA children needs to be examined in long term longitudinal and powered trials.

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