

Visual evoked potentials and dietary long chain polyunsaturated fatty acids in preterm infants

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Abstract

The influence of dietary long chain polyunsaturated fatty acid (LCP) supply, and especially of docosahexaenoic acid (DHA), on evoked potential maturation, was studied in 58 healthy preterm infants using flash visual evoked potentials (VEPs), flash electroretinography (ERG), and brainstem acoustic evoked potentials (BAEPs) at 52 weeks of postconceptional age. At the same time, the fatty acid composition of red blood cell membranes was examined. The infants were fed on breast milk (n=12), a preterm formula supplemented with LCP (PF-LCP) (n = 21), or a traditional preterm formula (PF) (n = 25). In the breast milk and PF-LCP groups the morphology and latencies of the waves that reflect the visual projecting system were similar; in the PF group the morphology was quite different and the wave latencies were significantly longer.

This could mean that the maturation pattern of VEPs in preterm infants who did not receive LCP was slower. Moreover, a higher level of erythrocyte LCP, especially DHA, was found in breast milk and PF-LCP groups compared with the PF group. ERG and BAEP recordings were the same in all three groups.

These results suggest that a well balanced LCP supplement in preterm formulas can positively influence the maturation of visual evoked potentials in preterm infants when breast milk is not available.

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Keywords: long chain polyunsaturated fatty acids, visual evoked potentials, brainstem acoustic evoked potentials, breast milk, formula, flash electroretinogram.

During the third trimester of human fetal growth and the first four to six postnatal months, long chain polyunsaturated fatty acids (LCP) and especially docosahexaenoic acid (DHA), accumulate in the brain and retina. This coincides with the rapid maturation of the nervous system and photoreceptors.¹⁻³

Preterm infants may be LCP deficient because of both the lack of intrauterine nutrition in the last months of pregnancy and the relative immaturity of the enzymes necessary to elongate and desaturate their essential fatty acid precursors.⁴ An LCP supplement, and especially DHA, in preterm formulas

improves maturation of rod photoreceptor function and visual acuity in early postnatal development.⁵⁻⁸

As far as we know, an evaluation of the influence of infant diet on neurophysiological maturation of flash visual evoked potentials (VEPs) and low rate brainstem acoustic evoked potentials (BAEPs) has never been carried out. We therefore performed a prospective controlled single blind study on preterm infants to evaluate the effect of LCP, either incorporated into the formula or present in breast milk, on the maturation of flash VEPs, flash electroretinography (ERG), and low rate BAEPs. Moreover, we analysed the fatty acid composition in red blood cell membranes as an index of neural and retinal tissue composition.

Methods

Sixty six preterm infants were enrolled in the study: they were less than 33 weeks of gestational age, of appropriate weight, and with no malformation that could interfere with somatic and/or psychomotor development. They had no neurological, visual, acoustic or gastroenterological illnesses. None had experienced perinatal asphyxia. All of them had normal fundus oculi. By the 10th day of life all of them had received at least 50% of their caloric requirement through enteral feeding. They were preferentially fed on breast milk whenever available (breast milk, n=17); otherwise they were randomly assigned to a formula for preterm infants enriched with LCP (Preaptamil with Milupan, Milupa AG, Friedrichsdorf, Germany) (PF-LCP, n=23), or a traditional formula for preterm infants (PF, n=26) (table 1). Infants assigned to the breast milk group received at least 75% of their own mother's milk. The LCP enriched formula was given to integrate breast milk when necessary. Infants assigned to the formula groups received less than 25% of their caloric intake from breast milk. These feeding regimens had to be continued until 52 weeks of postconceptional age. At that time 58 infants had remained in their allocated diet group: breast milk, n=12 (6 of them 100% breast milk); PF-LCP, n=21 (19 of them 100% formula); PF, n= 25 (19 of them 100% formula), and the results refer to them. The three groups were comparable in weight and gestational age (table 2).

Parental informed consent was obtained for each infant before enrolment in the study, and the study was approved by the local ethics committee.

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Table 1 Calorie, protein, and lipid composition of breast milk, LCP supplemented preterm formula (PF-LCP), and traditional preterm formula

	Breast milk		PF-LCP		Preterm formula	
Energy value (Kcal/100 ml)	70		70		70	
Proteins (g/100 ml)	1.1		2		2	
Lipids (g/100 ml)	4.4		3.4		3.4	
Fatty acids (% of total lipids)	*		*		*	
Saturated	52.0		51.6		61.8	
18:1 n-9	34.9	27.8 (1.5)	28.7	30.4	17.09	12.1
18:2 n-6	10.0	12.1 (1.2)	10.8	12.2	19.4	18.6
18:3 n-3	0.83	0.66(0.2)	0.73	0.40	0.9	0.25
20:2 n-6	0.35		0.15			
20:3 n-6	0.34	0.35(0.2)	0.12	0.24		0.02
20:4 n-6	0.39	1.23(0.7)	0.44	0.35		0.01
20:5 n-3	0.13		0.05	0.08		0.04
22:4 n-6	0.06	1.01(0.5)	0.01	0.23		0.02
22:5 n-3	0.06	0.30(0.1)	0.07	0.11		0.01
22:6 n-3	0.16	1.22(0.7)	0.30	0.23		

* Data published by the milk company.

† Analysis performed by the research group. Breast milk (SD) from five samples; PF-LCP and preterm formula: one sample.

Table 2 Mean (SD) values of gestational age, birthweight, postnatal growth velocity to age 52 weeks postconception, and erythrocyte membrane LCP at 52 weeks postconception in 58 preterm infants according to diet

	Breast milk (n=12)	PF-LCP (n=21)	Preterm formula (n=25)	P value
Gestational age (weeks)	31.8(1.7)	31.1(1.2)	31.3(1.2)	NS
Birthweight (g)	1590 (406)	1583 (310)	1463 (273)	NS
Weight gain (g/day)	28.2(4.0)	29.8(5.2)	30.4(5.0)	NS
Length gain (cm/week)	0.8(0.1)	0.9(0.1)	0.9(0.1)	NS
Head circumference gain (cm/week)	0.6(0.1)	0.6(0.1)	0.6(0.1)	NS
Arachidonic acid (%)	10.4(2.1)	10.1(2.5)	10.0(2.1)	NS
DHA (%)	2.4(1.1)	2.5(0.8)	1.3(0.7)	0.0001
Total n=6 (%)	29.3(3.4)	30.8(4.3)	33.7(4.6)	0.016
Total n=3 (%)	3.9(1.5)	3.3(1.1)	2.4(1.1)	0.005

DHA = docosahexaenoic acid.

Growth (weight, length, head circumference) and food tolerance were examined weekly until infants were 40 weeks of postconceptional age, and every two weeks thereafter.

As there were no differences among the diet groups at study entry, we did not analyse the fatty acid composition at that time. In fact, we studied the fatty acid composition of red blood cell membranes during the 52nd week. Lipids were extracted from membranes with chloroform:methanol 2 in 1, and were methyl-esterified with methanol/HCl 5% v/v for 1 hour at 70°C. Gas chromatographic analysis of methyl-esters was performed using a Carlo Erba instrument HRGC 5160 equipped with a fused silicon column SP 2340 (Supelco) of 30 m x 0.32 mm internal diameter. The temperature column was programmed between 160° to 210°C with a gradient of 8°C/minute; the gas carrier He flow was 2 ml/minute.⁹

At 52 weeks of postconceptional age, we tested visual evoked potentials (VEPs), electroretinography (ERGs), and low rate brainstem acoustic evoked potentials (BAEPs). VEPs, ERG, and BAEPs were recorded by an experienced technician using Amplaid MK15 (Ampliphon), and were always examined by the same doctor. Infants were held supine in the mother's arms in a quiet darkened room. Silver disk electrodes were fixed with collodion on the scalp and with plaster on nasion and mastoid, in accordance with the International 10-20 EEG System.¹⁰ Electrode impedance was lowered to less than 2 kOhm using a saline jelly. Each test was always repeated at least twice and an automatic artefact reject was used. VEPs and ERG were recorded to stimu-

late each eye independently. All the records of VEPs were made in a quiet waking state, according to the method of Prechtl and Beintema,¹¹ with the infant's eye opened. The stimuli were 100 white flashes delivered by a stroboscope lamp (16 x 7 cm), held 10 cm in front of patient's eye at the rate of 1 Hz and at an intensity of 1 Joule. The responses were recorded from an electrode placed on the midline occipital position, referenced to another electrode placed on the midline frontal position over 500 milliseconds. Bandpass was 1-100 Hz. We chose to use Barnett's nomenclature for VEP components¹²; components with similar morphology and latency were given the same label. We considered the latency of all waves except N1 and P1. We did not examine the amplitudes as they are reported to be very variable and less useful for clinical evaluation during VEP maturation.¹³

For ERG, the responses to 20 flashes with the same characteristics as mentioned above were recorded from an electrode placed on the bridge of the nose referenced to another electrode placed over the vertex.¹⁴ The same bandpass as for VEPs and a sweep of 15 milliseconds were used. The latencies of α and β waves and α - β amplitude were evaluated. For ERG recording, the infants were asleep to avoid muscle artefacts. For ethical reasons no patient was sedated and no mydriatic was used. In order to have the same pupil position during ERG recording infants lay supine with their head held in the axial position and the stroboscope lamp centred on their face.

BAEPs were recorded during spontaneous sleep, from the right (M2) and the left (M1)

Table 3 Prevalence of well defined waves P3, N4, and P4 of flash VEPs at 52 weeks postconception in three dietary groups

	Breast milk	PF-LCP	Preterm formula	P value
	n (%)	n (%)	n (%)	
Eyes examined	20	38	30	
P3	7(35)	19(50)	24(80)	a = NS b = 0.004 c = 0.02
N4	6(30)	14(36.8)	21(70)	a = NS b = 0.001 c = 0.001
P4	16(80)	30(78.9)	11(36.7)	a = NS b = 0.001 c = 0.001

n = Incidence of that wave in the group.
a = Breast milk vs PF-LCP.
b = Breast milk vs preterm formula.
c = PF-LCP vs preterm formula.

mastoids referenced to Cz. Each ear was stimulated independently with low rate clicks (11 Hz) at the intensity of 100 dB SPL, and the other ear was masked with a white sound at the intensity of 60 dB SPL. A bandpass of 50-5000 Hz and a sweep of 15 milliseconds were used. Latencies of I, II, III, IV and V waves were measured, along with I-III, I-V, III-V interpeak latencies.

Some of the infants were sleepy during VEP recording, and as all the infants had to be tested at the same gestational age, we could not repeat the test on another occasion: therefore, they were not included in the final analysis. Thus 44 infants completed the visual evoked potential study (10 breast milk, 19 PF-LCP, 15 PF). Likewise, some subjects were not in the ideal behavioural state during the recording of ERG and BAEPs and so were not included in the analysis: thus ERG was studied in 53 subjects (12 breast milk, 20 PF-LCP, 21 PF), and BAEPs in 52 infants (11 breast milk, 20 PF-LCP, 21 PF). The evaluation of the evoked potentials was carried out without prior knowledge of the infant's dietary group. The results were then divided into the three dietary groups and analysed statistically.

The statistical analysis was calculated using ANOVA and Student's *t* test for continuous variables, χ^2 test for dichotomous variables, and linear simple regression for correlations. All analysis were made using the Statistical Graphics System, version 6.0.

Results

Different morphological patterns of flash VEP responses were found in the three dietary groups. Figure 1 shows representative curves from one baby in each group. Early

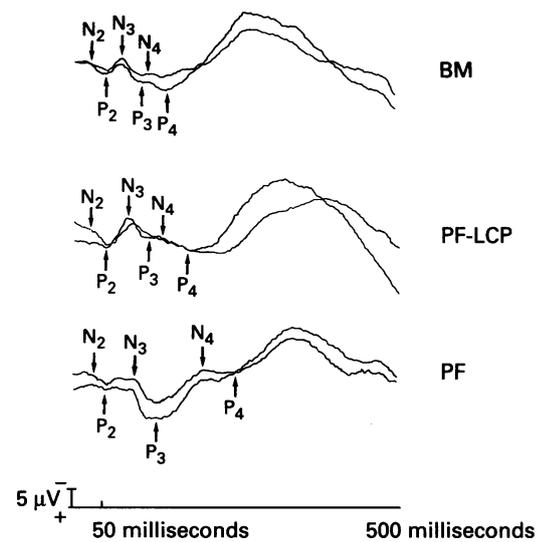


Figure 1 Morphological patterns of flash VEPs in preterm infants at 52 weeks of postconceptional age, according to diet. Curves refer to one baby from each of the three groups. All the curves have been depicted twice as a measure of repeatability. BM: breast milk; PF-LCP: supplemented preterm formula; PF: traditional preterm formula.

components—that is, waves N2, P2, and N3 whose latencies are less than 100 milliseconds—were present in almost all infants, whereas late component (100 msec) waves P3, N4, and P4 were not always well identifiable. So the examiner noted down which waves were well defined and which ones were poorly defined or completely absent (table 3). In the PF group P3 and N4 waves were mostly well defined, whereas in the breast milk group they were poorly defined or absent for the most part. The infants fed on PF-LCP showed an intermediate waveform that resembled the breast milk rather than the PF group. Wave P4 showed an opposite trend: it was well defined in the breast milk and PF-LCP groups, but poorly defined or absent in the traditional PF group.

Table 4 reports mean values and standard deviations of latencies among the three dietary groups. The latencies of waves N4 and P4 are significantly longer in the PF group than in the PF-LCP and breast milk groups ($P < 0.01$). Moreover, N4 and P4 latencies were similar in breast milk infants and in those fed PF-LCP (not significant). ERG and BAEPs did not reach significance among the three groups (table 5).

While growth parameters did not show any significant difference, the fatty acid composition of red blood cell membranes was different

Table 4 Latencies (milliseconds) of flash VEPs at 52 weeks postconception in three dietary groups

	Breast milk (Mean (SD))		PF-LCP (Mean(SD))		Preterm formula (Mean(SD))		P value
Latencies:							
N2	n = 9*	38 (4.5)	n = 18	38 (6.6)	n = 13	35 (5.3)	NS
P2	n = 10	52 (7.9)	n = 18	58 (9.3)	n = 13	53 (2.9)	NS
N3	n = 10	81 (8.1)	n = 19	84 (8.6)	n = 15	82 (9.9)	NS
P3	n = 7	106 (7.4)	n = 16	116 (9.5)	n = 13	117 (37.4)	NS
N4	n = 6	129 (15.7)	n = 14	143 (17.4)	n = 10	171 (20.8)	0.0002
95% confidence interval		(118-140)		(136-150)		(163-180)	
P4	n = 9	174 (26.2)	n = 17	178 (22.7)	n = 12	202 (19.4)	0.0117
95% confidence interval		(163-185)		(171-187)		(193-211)	

* Incidences of that wave group; the numbers refer to the right eyes (maximum: breast milk = 10, PF-LCP = 19, preterm formula = 15).

Table 5 Latencies (milliseconds) of BAEPs and latencies and amplitudes (μ V) of ERG at 52 weeks postconception in three dietary groups

	Breast milk Mean (SD)	PF-LCP Mean (SD)	Preterm formula Mean (SD)	P value
BAEPs				
Latencies:				
I	1.77 (0.24)	1.94 (0.34)	1.77 (0.18)	NS
II	2.58 (0.33)	2.77 (0.29)	2.66 (0.16)	NS
nII	3.26 (0.36)	3.47 (0.24)	3.36 (0.16)	NS
III	4.29 (0.37)	4.54 (0.36)	4.38 (0.22)	NS
IV	5.23 (0.46)	5.48 (0.35)	5.29 (0.26)	NS
V	6.29 (0.38)	6.46 (0.37)	6.28 (0.26)	NS
IPL I-III	2.52 (0.26)	2.60 (0.30)	2.61 (0.23)	NS
IPL I-V	4.52 (0.37)	4.52 (0.33)	4.51 (0.26)	NS
IPL III-V	2.00 (0.27)	1.92 (0.31)	1.90 (0.25)	NS
ERG				
Latencies				
a	17.8 (1.8)	17.8 (1.6)	17.3 (2.0)	NS
b	49.4 (6.8)	49.4 (6.4)	47.1 (3.0)	NS
Amplitude				
a-b	15.5 (6.6)	14.8 (4.6)	16.0 (6.8)	NS

IPL: interpeak latency.

BAEPs: brainstem acoustic evoked potentials.

ERG: flash electroretinogram.

in the three dietary groups. In fact, we found a significantly higher concentration of LCP, and especially DHA ($P < 0.0001$), in the breast milk and PF-LCP groups than in the traditional formula fed infants (table 2). We also studied the association between the DHA concentration in red blood cell membranes and the latencies of waves N4 and P4 in the study population as a whole, and we found a significant inverse correlation between these parameters (N4: $r = -0.31$, $P < 0.02$; P4: $r = -0.33$, $P < 0.007$).

Discussion

The correlation between LCP dietary intake and visual function in preterm newborn infants has been the focus of increasing research in recent years. In particular, DHA has a fundamental role in the modulation of the fluidity of membranes and the transmission of neuroelectrical signals in the photoreceptors and in the brain synapsis.^{5, 3, 16-20} Studies on diet and maturation of ERG and visual acuity in preterm infants have been published. Birch *et al*⁷ evaluated the ERG obtained by different conditions of stimuli (wave length, intensity, and frequency), and the visual acuity tested by pattern-reversal VEPs and forced-choice preferential looking. They found that preterm infants fed on breast milk or on a DHA enriched formula have a better ERG maturation at 36, but not at 57, weeks of postconceptional age compared with infants fed on a traditional formula, and they have a better visual acuity at both 36 and 57 weeks of postconceptional age. Carlson *et al*⁸ have obtained similar results by studying visual acuity through the Teller Acuity Card procedure.

A further aim of our study was to investigate the flash VEPs and low rate BAEP maturation in preterm newborn infants in relation to LCP dietary intake. Flash VEPs is a well consolidated technique, particularly suitable for the study of the maturation of optic pathways and visual cortex in infants and young children as no active collaboration is requested.^{3, 21} BAEPs using a low stimulus rate mainly reflect the functional state of brainstem white matter.²²

Other studies have shown that the development of the evoked potentials is related to gestational age: the number of waves increases with increasing age and, at the same time, the latency of all components progressively decreases. At two to four months from term, flash VEP morphology is similar to that of an adult, but latencies are longer.¹³ BAEP waveform is complete at 42 to 43 weeks of postconceptional age; the latency maturation reaches values that are similar to an adult's at 18 to 24 months from term.²²⁻²³

Our data show that not only the age but also the LCP dietary supply influences flash VEP maturation. Even though physiological differences in flash VEP morphology have been described among individuals,¹³ we found a recurrence of three different morphological patterns that correlate with the three dietary regimens. The prevalence of a morphological pattern in the LCP supplemented group, which more closely resembles that of breastfed infants than that of traditional formula fed infants, together with the latency shortness of some late components in the breast milk and PF-LCP groups compared with the PF group, may be the expression of different visual pathway maturation due to different levels of retinal and cerebral LCP. Animal studies have shown that diet-induced changes in the red blood cell membrane fatty acid composition were paralleled by similar changes in the brain and other tissues.²⁴ Recently, an association between diet and the fatty acid composition of infant cerebral cortex has been demonstrated.^{16, 25, 26} The DHA concentration in infant cerebral cortex is greater in breastfed than in traditional formula fed infants, the accretion of cortex DHA being dependent on the length of breast feeding probably because of the supply of preformed DHA. In fact, during early infancy it seems that the brain preferentially uses the preformed long chain polyunsaturated fatty acids and not those synthesised from their precursors.²⁶ Makrides *et al*²⁵ have also demonstrated that infant erythrocyte DHA correlates with that of the brain, indicating that erythrocyte DHA may be a valid indirect marker of cerebral cortex DHA in human infants. In our study the evaluation of the red blood cell membrane composition produced a negative correlation between some VEP late component latencies and the membrane DHA composition. Thus lipid composition in red blood cells reflects the composition of the nervous system membranes, and as DHA particularly concentrates in synaptic membranes,^{6, 27} these results could mean that the more DHA membrane content there is, the better the synaptic transmission is and the shorter is the wave latency. The influence of dietary LCP on late, but not on early, VEP components at 52 weeks of postconceptional age may be explained on a neuroanatomical basis. The early components, which are related to geniculocortical connections (the primary visual system), are already present at birth, mature earlier, and remain more constant in latency compared with the other waves.^{13, 28} Therefore, diet does not seem to influence them. The late components are the

expression of the more diffusely projecting system (reticulo-cortical or thalamo-cortical connections) that is related to behavioural processes; these interconnections gradually develop, progressively increase in complexity, and mature more slowly than the primary visual system.^{13, 28} Therefore, the influence of the diet on the late VEP components may be effective for several weeks after birth, especially in preterm newborn infants. Birch *et al*⁷ did not find any significant difference induced by diet in ERG recorded at 57 weeks of postconceptional age. Our recordings did not show any difference in flash ERG maturation at this age in relation to diet either.

Finally, the lack of any relation between BAEP latencies and both dietary and red blood cell membrane LCP composition suggests little, if any, influence of LCP on the transmission of neuroelectrical signals along the white matter of the brainstem.

In conclusion, our study supports previous findings on the importance of dietary LCP for an optimal visual development of preterm newborn infants. Although long term follow up studies are needed to evaluate the persistence of such an effect at older ages, we believe that a balanced LCP enriched milk formula represents important progress in the early nutrition of preterm infants when mother's milk is not available.

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