0031-3998/93/3304-0425$03.00/0
PEDIATRIC RESEARCH

Vol. 33, No. 4, 1993
Printed in U.S.A.

Erythrocyte Docosahexaenoic Acid Correlates with the Visual Response of Healthy, Term Infants

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ABSTRACT. Recent studies have reported that formula-fed preterm infants score lower on visual and developmental tests relative to breast-fed preterm infants. This phenomenon has been associated with the presence of docosahexaenoic acid (DHA), an omega-3 fatty acid, in breast milk and its absence from infant formula. To investigate the possibility that DHA status of healthy, term infants is also related to neuronal function of the visual pathway, we studied the erythrocyte fatty acid profiles of 16 infants at 22.3 ± 3.9 wk of age and related these to maturity of the visual pathway as assessed by visual-evoked potentials. Healthy, term infants fed breast milk had better visual-evoked potential acuity ($p < 0.05$) and higher DHA levels ($p < 0.001$) than infants who received infant formula as their major energy source. There was a positive correlation between erythrocyte DHA and visual-evoked potential acuity ($p < 0.01$). The data are preliminary and the long-term effects as yet unknown. However, our results suggest that there is an urgent need to evaluate the dietary fatty acid supply of formula-fed term infants. (*Pediatr Res* 34: 425–427, 1993)

Abbreviations

LC-PUFA, long-chain polyunsaturated fatty acid
DHA, docosahexaenoic acid
AA, arachidonic acid
EFA, essential fatty acid
LA, linoleic acid
ALA, α-linolenic acid
VEP, visual-evoked potential
MAR, minimum angle of resolution
logMAR, log of the minimum angle of resolution.

The perceived role of the dietary fat supply in infancy has notably changed over the last decade. Dietary fat has been primarily considered an exchangeable energy source required for rapid growth. However, recent studies on the effects of LC-PUFA, such as DHA and AA, suggest that the quality and composition of dietary fat may be vital for optimal growth and neuronal development during infancy.

Until the 1980s, it was widely accepted that the two precursor EFA, LA (the omega 6 precursor) and ALA (the omega-3 precursor), could be fully metabolized to AA and DHA, respectively, and hence meet all the EFA requirements of infancy (1). Support for this belief was based on the fact that most infants receiving formulas containing only LA and ALA achieved adequate growth rates.

In contrast to formula, breast milk contains a full complement of omega-6 and omega-3 LC-PUFA including DHA and AA, which formula-fed infants must synthesize from precursors. In preterm infants, biochemical studies comparing breast and formula feeding indicate 8 to 10% less AA and 13 to 48% less DHA in the erythrocyte lipids of formula-fed infants (2). We have reported similar differences in term infants (2), suggesting that infant formulas containing only LA and ALA may not be effective at meeting the full EFA requirements of healthy infants.

These biochemical changes are clinically relevant, as studies have shown that dietary fatty acid supply affects physiologic function. Preterm formula-fed infants have a measurable reduction in neuronal function of the visual pathway, as measured by electroretinogram (3), VEP, and behavioral measures of visual acuity (4) relative to preterm breast-fed infants. These physiologic parameters were related to the DHA status of these infants and corrected by the addition of DHA containing fish oil to infant formula (3, 4).

DHA and AA form a large proportion of total lipids in the brain and retina and are primarily accreted into the brain during the "brain growth spurt," which begins in the last trimester of pregnancy and continues through early postnatal life (5). Previous research has focused on the preterm infant as being at greatest risk of disturbed fat accretion.

Our primary aim was to investigate whether the dietary supply of these fatty acids is as important to term infants as it is to preterm infants. To test the hypothesis that DHA status is related to the maturity of the visual pathway of healthy, term infants, we studied the fatty acid profiles of infants fed breast milk and infant formula and related these to their VEP. The VEP is the summed cortical response to a temporal change of a visual stimulus and is often used to assess the visual function of preverbal children. We chose the VEP as our assessment of the maturity of the visual pathway because few other noninvasive, reliable, and reproducible tests were available and studies indicate that VEP acuity is used as a measure of neuronal maturation of this pathway (6).

MATERIALS AND METHODS

Subjects. Healthy infants who were approximately 5 mo of age and born at term with appropriate weight for gestational age were enrolled into the study from immunization and postnatal (Child, Adolescent and Family Health Service) clinics. Of 22 infant-mother pairs asked to participate, four declined, one was subsequently excluded on the basis of the result of the ophthalmic examination, and one could not attend the stipulated clinic

Received for rapid publication November 5, 1992; accepted December 10, 1992.
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Supported by Scotia Pharmaceuticals and Nestle Australia.

times, leaving 16 infants. Consent was obtained from the caregivers of these infants following guidelines approved by the Clinical Investigations (Ethics) Committee of Flinders Medical Centre, the Research Ethics Committee of the Adelaide Children's Hospital, and the Research Committee and the Ethics Sub-Committee of Child, Adolescent and Family Health Service. Consenting infant-mother pairs subsequently attended two hospital clinics within a week. On the first visit, mothers answered a short questionnaire detailing feeding patterns, socioeconomic status (7), and parity. The infants were weighed and measured, a blood sample was taken, and a complete ophthalmic examination was carried out. Infants with astigmatism or large refractive error were excluded from the study. On the second visit, a VEP was performed.

Diets. The formula-fed infants received one of three infant formulas, each of which had a similar fatty acid composition. Total saturates ranged from 48 to 50%, monounsaturates from 34 to 36%, LA from 12 to 15%, and ALA from 1 to 1.6% of total fatty acids. The LA:ALA ratios were similar and ranged from 9.4 to 11.3.

Both groups of infants were receiving solids, predominantly rice cereal and stewed fruit. None of the infants were receiving detectable quantities of DHA or AA from solids.

Fatty acid analysis of blood. One hundred fifty to 200 μL of whole blood were obtained from a thumb prick and transferred to tubes containing lithium heparin. Plasma and erythrocytes were separated, and lipids were extracted, methylated, and quantified by capillary gas chromatography by following procedures previously reported (2).

VEP. Infants sat with their mothers, 1 m away from a monitor that presented high-contrast black-and-white checkerboard-pattern stimuli. A constant reversal rate of 2/s was used to elicit responses to checks with squares subtending visual angles of 7, 17, 34, 69, and 137 min of arc. Responses were obtained from an active electrode placed 3 cm above the inion, a reference electrode in a midfrontal position and an inactive electrode on the forehead. Recording occurred when infants were alert and their gaze directed at the monitor. A bell was used to help maintain infants' attention. Three recordings were taken at each check size to ensure reproducibility.

Peak-to-peak amplitude was determined for each check size. The amplitude of the evoked potential diminishes as check size decreases. Extrapolation of a regression line fitting VEP amplitude to log (check size) through 0-μV amplitude gives the smallest check size that, if tested, would render a positive wave (6). This point is defined as the logMAR or VEP acuity.

RESULTS

Of the 16 infants who successfully completed the study, eight were exclusively breast-fed and eight received >70% of their nutrition from infant formula. All infants were from families with similar social class (Table 1). There was no difference in age between the two groups, and breast-fed infants had a significantly smaller MAR (*i.e.* better VEP acuity) than those who had been predominantly formula-fed (Table 1). There was no correlation between postnatal age and VEP acuity, but the range of values reported for VEP acuity was within the range reported for healthy, term infants at 5 mo of age (6).

Infants fed breast milk had a greater proportion of erythrocyte DHA and less erythrocyte LA relative to those who had received infant formula as their major energy source (Table 1). There was a significant correlation between logMAR (VEP acuity) and the proportion of DHA ($r = -0.65$, $p < 0.01$) and LA ($r = +0.66$, $p < 0.01$) in erythrocyte phospholipids (Fig. 1).

DISCUSSION

This is the first report of a positive correlation between VEP acuity of healthy, term infants and the level of erythrocyte DHA.

Table 1. *VEP acuity and erythrocyte phospholipid DHA and LA in breast-fed and predominantly formula-fed infants*

Parameter	Breast-fed infants ($n = 8$)	Formula-fed infants ($n = 8$)
Age (wk ± SD)	22.4 ± 3.7	22.3 ± 4.3
Parity (no. children ± SD)	1.7 ± 0.7	1.5 ± 0.7
Social status (social score ± SD)	5.1 ± 0.6	5.3 ± 1.2
VEP acuity (logMAR ± SD)	0.42 ± 0.29	0.74 ± 0.27*
Erythrocyte DHA (% ± SD)†	6.16 ± 0.98	3.31 ± 0.82‡
Erythrocyte LA (% ± SD)†	9.56 ± 1.15	12.58 ± 1.02‡

* $p < 0.05$.
† % of total fatty acid methyl esters.
‡ $p < 0.0001$.

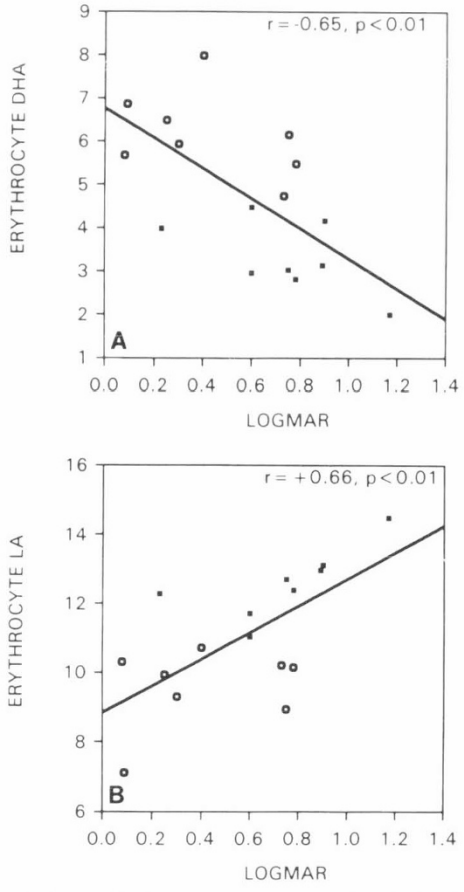

Fig. 1. *A*, Relationship between erythrocyte phospholipid DHA and logMAR. *B*, Relationship between erythrocyte phospholipid LA and logMAR. ○, infants exclusively fed breast milk; ■, infants predominantly fed infant formula. High logMAR indicates poor visual acuity, and lower values imply better acuity. LogMAR 0 = 20/20 Snellen equivalent, and logMAR 1 = 20/200 Snellen equivalent.

This suggests that term infants may be equally susceptible to altered visual/neuronal function as preterm infants (3) if there is a suboptimal dietary supply of DHA. In support of this, Birch *et al.* (4) have recently demonstrated that breast-fed term infants have better VEP and forced-choice preferential-looking acuities relative to those fed infant formula, although the quantification of fatty acid status was not performed in these infants. The long-term effect of the differences that we and others report in VEP acuity between breast- and bottle-fed infants is unknown.

In addition to containing virtually no DHA, most infant formulas contain large amounts of LA. Our data suggest that

these high levels of LA may inhibit the incorporation of DHA into erythrocyte phospholipids, because it is the higher levels of erythrocyte DHA that are associated with lower levels of erythrocyte LA. Indeed, our work with adults demonstrates that the incorporation of omega-3 LC-PUFA into erythrocytes is inhibited by diets rich in LA (8). This raises some questions regarding the nutritional adequacy of the dietary fatty acid supply of formula-fed infants. Should we add DHA to formula, or should we look for ways to correct the balance of LA to ALA and hence optimize metabolism of ALA to DHA? The inverse relationship between erythrocyte LA levels and VEP acuity suggests that this issue is important. Our previous work has shown that decreasing the LA:ALA ratio of formula from 20:1 to between 3:1 and 4:1 results in a significant improvement in the level of DHA incorporated into erythrocytes (2). It remains to be tested whether further manipulation of the LA:ALA ratio of infant formula will result in DHA levels and physiologic responses equivalent to those of breast-fed infants.

During the first 6 mo of life, there is a rapid maturation of the retinocortical sensory element of the human visual system that is reflected by an equally rapid improvement in VEP acuity (6). Studies tracking the VEP development of healthy, term infants between 2 and 7 mo of age indicate that there is an average improvement of 20% per month in VEP acuity, with adult levels being reached by 7 mo of age (6). However, these studies also indicate that at any given point in time there may be a substantial deviation from the mean VEP acuity. Our results, as well as those reported by Birch *et al.* (4), suggest that this variation may be explained by differences in LC-PUFA status of the infant, which in turn is affected by the dietary supply of EFA.

Large, long-term studies investigating the effect of dietary supply of EFA to term infants on subsequent maturation of the visual pathway are urgently needed. Evidence to suggest that breast-fed infants have a long-term intelligence quotient advantage over infants who have been fed formula has been apparent in the literature for many years (9) and has been recently supported by the work of Lucas *et al.* (10). Although these last two studies do not relate their findings to fatty acid supply, it is attractive to postulate, from data published in the last 5 y that deficiency of LC-PUFA such as DHA is potentially a nutritional factor related to these observations. We must continue to investigate ways of providing appropriate fat in infant diets and assessing its effect on developmental outcomes. This will best be achieved with programs targeting the promotion of breast feeding and additional studies investigating the optimal fatty acid composition for infant formula.

Acknowledgment. The authors thank Paul Weston for technical assistance with VEP recording and the Flinders Medical Centre blood clinic for blood collection.

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