Early Human Development (2007) **83**, 279–284

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/earlhumdev

Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula

Eileen E. Birch [a,b,*], Sharon Garfield [a], Yolanda Castañeda [a], Dianna Hughbanks-Wheaton [a], Ricardo Uauy [c], Dennis Hoffman [a]

[a] *Retina Foundation of the Southwest, Dallas, TX, USA*
[b] *Department of Ophthalmology, UT Southwestern Medical Center, Dallas, TX, USA*
[c] *INTA, University of Chile, Santiago, Chile*

Accepted 26 November 2006

KEYWORDS
Long-chain polyunsaturated fatty acids;
Visual acuity;
IQ;
Infant;
Infant formula;
Preschool child

Abstract

Background: While there is a large body of data on the effects of long-chain polyunsaturated fatty acid supplementation of infant formula on visual and cognitive maturation during infancy, longterm visual and cognitive outcome data from randomized trials are scarce.
Aim: To evaluate docosahexaenoic acid (DHA) and arachidonic acid (ARA)-supplementation of infant formula on visual and cognitive outcomes at 4 years of age.
Methods: Fifty-two of 79 healthy term infants who were enrolled in a single-center, double-blind, randomized clinical trial of DHA and ARA supplementation of infant formula were available for follow-up at 4 years of age. In addition, 32 breast-fed infants served as a "gold standard". Outcome measures were visual acuity and the Wechsler Preschool and Primary Scale of Intelligence — Revised.
Results: At 4 years, the control formula group had poorer visual acuity than the breast-fed group; the DHA- and DHA+ARA-supplemented groups did not differ significantly from the breast-fed group. The control formula and DHA-supplemented groups had Verbal IQ scores poorer than the breast-fed group.
Conclusion: DHA and ARA-supplementation of infant formula supports visual acuity and IQ maturation similar to that of breast-fed infants.

1. Introduction

Two long-chain polyunsaturated fatty acids (LCPs), docosahexaenoic acid (DHA) and arachidonic acid (ARA), are important structural components of membrane lipids in the

* Corresponding author. Retina Foundation of the Southwest, 9900 N. Central Expressway, Suite 400, Dallas, TX 75231, USA. Tel.: +1 214 363 3911; fax: +1 214 363 4538.
E-mail address: ebirch@retinafoundation.org (E.E. Birch).

0378-3782/$ - see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.earlhumdev.2006.11.003

central nervous system [1,2]. Beginning in the mid trimester of human fetal development and continuing throughout the second year of life, there is rapid brain growth, starting in mid gestation with the neuronal hyperplastic growth spurt, followed by synaptogenesis and myelination [3–6]. The ten-fold increase in brain size during this period is accompanied by an approximate 30-fold increase in DHA content and a 15-fold increase in ARA content [7–10]. The rapid accretion of LCPs into neural membranes is dependent prenatally on placental transfer of LCPs and, postnatally, on dietary sources and limited endogenous synthesis.

Human milk in most Western countries provides 0.2 to 0.4% of fatty acids as DHA and 0.3 to 0.7% of fatty acids as ARA (see Table 3 in Lauritzen et al. [11] for review). Some infant formulas currently sold in North America provide no DHA or ARA and those that do provide DHA and ARA vary widely in the amount provided. Infants fed formulas lacking LCPs have significantly lower LCP concentrations in cerebral cortex [12,13] and significantly lower LCP concentrations in red blood cell membranes than breast-fed infants [13–19]. Most LCP-supplemented infant formulas, which contain a range of LCP-concentrations and provide LCPs from various sources, increase red blood cell LCP concentrations to within the range of concentrations found among breast-fed infants [13–19]. It is unknown whether the varying formulations result in equivalent LCP concentrations in the retina and brain.

The link between rapid LCP accretion and the brain growth spurt has led to a number of studies on the effects of LCP supplementation of infant formula on neural function. Several randomized trials have found a specific benefit of LCP supplementation for retinal maturation, visual acuity development, or cognitive development during infancy [17,20–25]. Others have found no benefit [26–31].

Longterm outcome data from randomized trials of LCP supplementation are scarce [27,32]. No longterm visual or cognitive outcome data are available for LCP supplements derived from microalgal/microfungal sources. It is possible that, although plasma and red blood cell concentrations appear to respond similarly to LCP supplements derived from various sources, brain composition may not. The purpose of the present study was to examine the effects of microalgal/microfungal LCP supplementation of infant formula provided in the first 17 weeks of life on visual and cognitive outcomes at 4 years of age.

2. Methods

2.1. Participants

Seventy-nine healthy term infants were enrolled (1993–1995) in the randomized clinical trial (RCT) of microalgal/microfungal DHA and ARA supplementation of infant formula within the first 5 days of life; 52 were available for follow-up testing at 4 years of age. As shown in Table 1, most of the loss to follow-up occurred during the initial weeks following enrollment (prior to the 4-month visit) and was due to their pediatricians' recommendations to switch to a soy protein-based formula following symptoms suggestive of lactose or cow milk protein intolerance. Of the 68 infants who remained in the protocol to 4 months of age, 82% completed the protocol through 18 months of age and 76% completed the 4 year examination.

In addition, a non-randomized group of 40 healthy term breast-fed infants were enrolled concurrently with the randomized trial (1993–1996); 32 were available for follow-up testing at 4 years of age. As shown in Table 1, this represents 82% of the breast-fed infants who completed the protocol through 4 months of age. The breast-fed group was enrolled in order to provide a "gold standard" for comparison with formula-fed groups. The average duration of breast-feeding was 43 ± 9 weeks (range: 17–60 weeks).

Also shown in Table 1 are the demographics for the cohorts that completed testing at 4 years of age. With the exception of the breast-fed cohort, males comprised 44–55% of each cohort, which had 16–24% non-white race/ethnicity. All parents had completed high school, 62–65% of mothers had college or post-graduate degrees, and 80–84% of fathers had college or post-graduate degrees. In the breast-fed group, males comprised 53% of the cohort, which had 12% non-white race/ethnicity. All parents had completed high school, 81% of mothers had college or post-graduate degrees, and 77% of fathers had college or post-graduate degrees. Overall, the cohort followed through 4 years of age did not differ significantly from the original cohort in the proportion of males, mothers who had college or post-graduate degrees or fathers that had college or post-graduate degrees ($z < 1.6$; $p > 0.05$ for all comparisons) but did have a slightly and significantly higher proportion of white children (83.3% versus 72.2% in the original cohort; $z = 1.82$, $p < 0.05$).

All participants were born at 37 to 40 weeks postmenstrual age as determined by early sonogram, date of last menstrual period, and physical and neurodevelopmental assessment at birth. Only singleton births with birth weights appropriate for gestational age were included. All participants were born at Presbyterian Hospital of Dallas or Medical City Hospital in Dallas. Exclusion criteria were family history of milk protein allergy, genetic or familial eye disease (e.g., hereditary retinal disease, strabismus), vegetarian or vegan maternal dietary patterns, maternal metabolic disease, maternal anemia, maternal infection, presence of a congenital malformation or infection, perinatal jaundice, perinatal asphyxia, meconium aspiration and any perinatal event which resulted in placement of the infant in the neonatal intensive care unit.

Parents of eligible neonates were provided a brief information sheet about the dietary study only after hospital records noted that they had elected to formula feed. Informed consent was obtained from one or both parents 24–96 h after birth and prior to the infant's participation. This research protocol observed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center (Dallas, TX), Presbyterian Medical Center (Dallas, TX), and Medical City Columbia Hospital (Dallas, TX).

2.2. Randomization

On the day of enrollment (range = 0 to 4 days; mean ± SD = 2.1 ± 1.0 day), infants in the randomized cohort were assigned to one of 3 diets, described in the paragraph below. Each of the 3 diets was masked by two color/number codes, for a total of 6 possible diet assignments for each infant. A computerized, blocked randomization schedule was developed by the Mead Johnson Research Center and provided in sealed envelopes to the study site.

Table 1 Summary of enrollment, loss to follow-up, and demographics

	Number enrolled	Number completing 4-month feeding protocol	Number completing 18-month BSID-II testing	Number completing 4-year testing	Gender (M/F)	Race and ethnicity (white/ minority)	Maternal education (highschool/college or postgrad)	Paternal education (highschool/college or postgrad)
Control	26	23	20	19	9/10	16/3	7/12	3/16
DHA-supplemented	26	22	17	16	7/9	13/3	6/10	3/13
DHA + ARA-supplemented	27	23	19	17	9/8	13/4	6/11	3/14
Breast fed	40	38	20[a]	32	17/15	28/4	6/26	6/26

[a] cohort size limited by funding.

2.3. Diets

Study diets were Enfamil® with iron, Enfamil® with iron supplemented with 0.35% DHA (of total fatty acids), or Enfamil® with iron supplemented with 0.36% DHA and 0.72% ARA. All formulas were prepared by Mead Johnson Nutritionals (Evansville, IN) and provided approximately 15% linoleic acid (LA) and 1.5% α-linolenic acid (LNA). DHA-supplemented and DHA + ARA-supplemented formulas contained single cell oils, specifically DHASCO® and ARASCO® (Martek Biosciences, Columbia, MD). All formulas were provided in 32 oz. ready-to-feed cans and contained 2.2 g protein, 5.6 g fat and 10.3 g carbohydrate per 100 kcal; all nutrients met existing standards for commercial formula based on established Infant Formula Act requirements. Assigned diets were fed exclusively through 17 weeks of age. No solid foods were introduced before 17 weeks. Beyond 17 weeks, the infants received commercial formula (not provided by the study). At the time, the study was conducted (1993 to 1996), no LCP-supplemented formulas were commercially available in North America. As previously reported, blood lipid analyses confirmed compliance with the assigned diets [14,17,20].

2.4. General protocol

In the current follow-up phase of the study, we tested the hypothesis that infant formula lacking LCPs during the first 17 weeks of life would result in: 1. visual acuity at 4 years of age that was significantly poorer than in breast-fed infants; 2. IQ at 4 years of age that was significantly poorer than in breast-fed infants. Visual acuity and IQ were assessed at 4 years of age. All visual acuity testing was completed by research assistants who were masked to the infants' diets and other test results. All cognitive testing was completed by one of the authors (SG), a child psychologist specializing in psychoeducational testing who was masked to the infants' diets and other test results. Testing at 4 years of age was completed for 19 children in the control formula group, 16 in the DHA-supplemented formula group, 17 in the DHA + ARA-supplemented formula group, and 32 in the breast-fed group. As reported previously, these children had also provided sweep VEP acuity, growth data, blood samples, and, for a subset of infants, Bayley Scales of Infant Development (BSID-II) scores during the first 18 months of life [14,17,20,25].

2.5. HOTV visual acuity

HOTV testing was conducted for each eye using the Amblyopia Treatment Study (ATS) protocol [33] and the Electronic Visual Acuity (EVA) system developed by Moke et al [34] for the Pediatric Eye Disease Investigator Group. Four different single letters are presented ("H" "O" "T" "V"), framed with crowding bars that are spaced around the letter on the EVA display. The test protocol consists of the presentation of single-surrounded optotypes in four steps: a screening phase, a first threshold determination (phase 1), reinforcement phase, and a second threshold determination (phase2). A handheld device that used the Palm® operating system prompted the tester and controlled the order letters were presented according to the ATS protocol. Acuity testing was conducted at a test distance of 3 m.

2.6. Wechsler preschool and primary scale of intelligence

The Wechsler Preschool and Primary Scale of Intelligence, Revised (WPPSI-R) was used to assess intelligence at 4 years of age. This test has two scales. Performance IQ includes object assembly, geometric design, block design, mazes, picture completion, and animal pegs tasks. Verbal IQ includes information, comprehension, arithmetic, vocabulary, similarities, and sentence tasks. Raw scores for Performance IQ and Verbal IQ, based on the number of items successfully completed, are converted to standard scores based on the child's age at the time of testing. In addition, a Full Scale IQ standard score is computed based on both the Performance and Verbal IQ scales. Each of the standard scores, Performance IQ, Verbal IQ, and Full Scale IQ, has a mean of 100 with a standard deviation of 15.

2.7. Statistical analyses

Sample sizes for the randomized trial were based on the primary outcome measures of visual acuity at 4 and 12 months of age and the Bayley Scales Mental Development Index (MDI) at 18 months of age. For $\alpha = 0.05$ and $1-\beta = 0.80$, a sample size of 16 per diet group is sufficient to detect mean differences of one standard deviation or greater. Anticipating a 25% loss to follow-up over the original 18 month duration of the study, we recruited 26–27 infants per diet group in the randomized trial and, anticipating a potentially higher drop-out rate among breast-fed infants, we recruited 40 infants for the breast-fed group.

During the course of the study, all data were handled in a coded manner. Data analyses were conducted with analysis of variance (ANOVA) and planned comparisons among the 4 diet groups (control, DHA, DHA + ARA, and breast-fed) after verifying that the data met normality criteria.

3. Results

3.1. HOTV acuity

The average HOTV acuity, Performance IQ, Verbal IQ, and Full Scale IQ for each diet group at 4 years of age are shown in Table 2. Dietary supply of LCPs during the first 17 weeks of life had a small but statistically significant effect on HOTV acuity for the right eye (RE) ($F_{RE} = 3.12$, $p < 0.03$; $F_{LE} = 1.71$, $p = 0.17$). Planned comparisons showed that the control formula diet group had poorer HOTV acuity in the right eye than the breast-fed group ($t_{RE} = 2.96$, $p < 0.004$) as well as lower right eye acuity than the DHA group ($t_{RE} = 2.24$, $p < 0.03$). Left eye acuity of the control formula group did not differ significantly from any of the other diet groups.

3.2. Wechsler preschool and primary scale of intelligence

Although both of the LCP-supplemented formula groups and the breast-fed group averaged Performance IQ scores that were 4 points higher than the control diet group, these differences were not statistically significant ($F = 0.479$, $p = 0.70$). Dietary supply of LCPs during the first 17 weeks of life had a significant effect on Verbal IQ at 4 years of age ($F = 5.15$, $p < 0.003$). Planned comparisons showed that both the control formula diet group and the DHA diet group had Verbal IQ scores poorer than the breast-fed group ($t = 3.69$, $p < 0.0004$; $t = 2.45$, $p < 0.02$). The Verbal IQ scores of the LCP-supplemented formula groups averaged 4 to 6 points higher than the control group but this difference was not statistically significant. The Full Scale IQ of LCP-supplemented formula diet groups averaged 4 to 6 points higher than the control group but this trend was not statistically significant ($F = 2.52$, $p = 0.06$).

Only 3 children in the control formula group (16%) scored in the "Above Average" category on Full Scale IQ; i.e., had an IQ $\geq$ 115. Above average performance on Full Scale IQ was noted in 4 children in the DHA group (27%), 4 children in the DHA + ARA group (24%), and 12 children in the breast-fed group (44%). The prevalence of above average performance in the control formula diet group was significantly lower than in the breast-fed diet group (z-test for proportions; $p < 0.025$); neither of the LCP-supplemented diet groups differed significantly from the breast-fed group.

3.3. Outcomes assessed during infancy and at 4 years

Neither sweep VEP acuity at 17 weeks of age nor at 52 weeks of age was predictive of HOTV acuity or IQ outcome at 4 years of age ($-0.16 < r < 0.11$; $0.20 < p < 93$). The Bayley Scales of Infant Development (BSID-II) MDI scores at

Table 2 Visual and cognitive outcomes at 4 years of age (mean ± SE)

	Randomized			
	Control	DHA	DHA + ARA	BF
	$N = 19$	$N = 16$	$N = 17$	$N = 32$
Acuity (logMAR) RE	0.076 ± 0.022[a,b]	0.023 ± 0.019	0.034 ± 0.017	0.017 ± 0.013
Acuity (logMAR) LE	0.052 ± 0.016	0.016 ± 0.018	0.026 ± 0.017	0.007 ± 0.013
Performance IQ	104.2 ± 2.7	108.1 ± 3.8	108.6 ± 3.3	108.4 ± 2.5
Verbal IQ	98.8 ± 2.6[a]	102.7 ± 4.1[a]	104.5 ± 2.9	112.6 ± 2.3
Full scale IQ	101.0 ± 2.6	105.9 ± 3.9	107.5 ± 3.1	111.2 ± 2.3

a = significantly poorer than BF.
b = significantly poorer than DHA.
logMAR = log minimum angle of resolution in minutes of arc.

18 months of age were significantly negatively correlated with both HOTV acuity ($r_{RE}=-0.35$, $p=0.007$; $r_{LE}=-0.30$, $p=0.005$); that is, higher MDI scores were associated with lower logMAR values (better visual acuities). In addition, the BSID-II MDI scores at 18 months of age were significantly positively correlated with IQ at 4 years of age ($r_{\text{Perf IQ}}=0.38$, $p=0.003$); $r_{\text{Verbal IQ}}=0.40$, $p=0.002$; $r_{\text{Full IQ}}=0.46$; $p<0.0001$); that is, higher MDI scores were associated with higher IQ. BSID-II PDI scores at 18 months were not predictive of HOTV acuity or IQ at 4 years of age ($-0.24<r<0.12$; $0.07<p<0.83$).

4. Discussion

Children who were fed formula containing no DHA or ARA during the first 17 weeks of life in a randomized trial had significantly poorer visual acuity and verbal IQ at 4 years of age than children who were breast-fed for an average of 43 weeks. Children who were fed formula containing 0.36% of fatty acids as DHA and 0.72% as ARA during the first 17 weeks of life had visual acuity and Verbal IQ scores that did not differ significantly from breast-fed children at 4 years of age.

Potentially, there are many differences between a non-randomized breast-feeding group and formula-fed groups in addition to the nutrient composition of human milk. Maternal variables associated with the decision to breast-feed include IQ, education level, socioeconomic status, maternal-infant interaction, and the act of breast-feeding itself. Nonetheless, a breast-fed group is commonly used as a "gold-standard" when evaluating new infant formula ingredients. That the control diet group, lacking a dietary source of DHA and ARA during the first 4 months of life, had significantly poorer Verbal IQ than the breast-fed group at 4 years speaks directly to the importance of these nutrients during infancy for long-term cognitive development.

Differences in HOTV acuity among diet groups, although statistically significant, are subtle. From the poorest mean visual acuity of 0.076 logMAR (right eye of the control group) to the best mean visual acuity of 0.007 logMAR (left eye of the breast-fed group), the Snellen equivalent range is 20/24 to 20/20, a little less than one line on an eye chart. None of the children scored more poorly than a Snellen equivalent of 20/40; i.e., all acuity scores were within the normal range for 4-year-olds [35]. The differences among diet groups, then, may reflect subtle differences in the phospholipid structure of the neural membranes in the retina and/or visual cortex as a result of early dietary differences in LCP supply. Differences in the ability to name or match letters may reflect differences in cognitive maturity among the diet groups.

All of the diet groups' mean IQ scores ranged from a low of 98.8 (Verbal IQ of the control group) to 112.6 (Verbal IQ of the breast-fed group); i.e., none of the groups had borderline or intellectually deficient mean scores. Nonetheless, both the control formula diet group and the DHA diet group had Verbal IQ scores that were significantly poorer than the breast-fed group and the prevalence of above average performance in the control formula diet group was significantly lower than in the breast-fed diet group.

Neither sweep VEP acuity at 17 weeks of age nor at 52 weeks of age was associated with IQ outcome at 4 years of age. This was a surprising finding because sweep VEP acuity at 17 weeks was predictive of BSID-II MDI score at 18 months of age in the same cohort (Birch et al., 2000) and, in the current study, BSID-II MDI score at 18 months was predictive of Performance IQ, Verbal IQ, and Full IQ at 4 years of age. This finding may indicate a greater influence of visual development on cognitive evaluation at 18 months relative to the more complex cognitive assessment conducted at 4 years, where tasks require the integration of multiple sensory systems.

For the same randomized cohort of children, we previously reported that LCP-supplementation of infant formula resulted in better sweep VEP visual acuity during the first year of life and better Bayley Scales Mental Development Index scores at 18 months of age than control formula. The current finding that only LCP-supplemented children had acuity and IQ similar to that of breast-fed infants further supports the need to supplement infant formula with LCPs. Our IQ results differ markedly from those of Auestad et al. [32] who found no differences in IQ between breast-fed infant and those fed control or LCP-supplemented formulas. The amount of LCP-supplementation in the Auestad et al. study [32] was one-half to two-thirds the amount provided in this study so it is possible that higher levels of LCP-supplementation are required to see an effect on IQ.

Our finding that the DHA group also had poorer Verbal IQ than the breast-fed group suggests that a dietary supply of both DHA and ARA are important for the developing brain. Further studies of LCP-supplementation levels, including long-term follow-up, will be needed to clarify the optimal level of LCP-supplementation for cognitive and visual maturation.

Acknowledgements

This project was supported by NIH grant HD22380. Milk-based infant formulas were generously provided by the Mead Johnson Nutritional Center (Evansville, IN). We are grateful for the collaboration of the staff of the Margot Perot Women's and Children's Hospital newborn nursery and postpartum care at Presbyterian Medical Center (Dallas, TX) and of the staff of the newborn nursery and postpartum care at Medical City (Dallas, TX). We appreciate the continuing pediatric support of Pediatric Associates, Woodhill Pediatric Associates, Clinical Associates, and North Dallas Pediatrics.

References

[1] Giusto N, Pasquare S, Salvador G, Castagnet P, Roque M, Ilincheta de Boschero M. Lipid metabolism in vertebrate retinal rod outer segments. Prog Lipid Res 2000;39:315–91.
[2] Sastry P. Lipids of nervous tissue: composition and metabolism. Prog Lipid Res 1985;24:69–176.
[3] Garey L. Structural development of the visual system of man. Hum Neurobiol 1984;3:75–80.
[4] Huttenlocher P, De Courten C. The development of synapses in striate cortex of man. Hum Neurobiol 1987;6:1–9.
[5] Blakemore C. Mysteries in the making of the cerebral cortex. In: Bock G, Cardew G, editors. Development of the Cerebral Cortex. West Sussex, England: John Wiley & Sons; 1995. p. 1–20.
[6] Dobbing J, Sands J. Comparative aspects of the brain growth spurt. Early Human Dev 1979;3:79–83.

[7] Svennerholm L. Distribution and fatty acid composition of phosphoglycerides in normal human brain. J Lipid Res 1968;9:570–9.
[8] Svennerholm L, Vanier M. The distribution of lipids in the human nervous system. 3. Fatty acid composition of phosphoglycerides of human foetal and infant brain. Brain Res 1973;50:341–51.
[9] Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992;120:S129–38.
[10] Martinez M, Mougan I. Fatty acid composition of human brain phospholipids during normal development. J Neurochem 1998;71:2528–33.
[11] Lauritzen L, Hansen H, Jorgensen M, Michaelsen KF. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 2001;40:1–94.
[12] Farquharson J, Cockburn F, Patrick W, Jamieson E, Logan R. Infant cerebral cortex phospholipid fatty-acid composition and diet. Lancet 1992;340:810–3.
[13] Makrides M, Neumann M, Byard R, Simmer K, Gibson R. Fatty acid composition of brain, retina, and erythrocytes in breast-fed and formula-fed infants. Am J Clin Nutr 1994;60:189–94.
[14] Birch EE, Birch DG, Hoffman DR, Uauy R. Dietary essential fatty acid supply and visual acuity development. Investig Ophthalmol Vis Sci 1992;33:3242–3.
[15] Carlson S, Rhodes P, Rao V, Goldgar D. Effect of fish oil supplementation on the n-3 fatty acid content of red blood cell membranes in preterm infants. Pediatr Res 1987;21:507–10.
[16] Carlson S, Ford A, Werkman S, Peeples J, Koo W. Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. Pediatr Res 1995;39:882–8.
[17] Hoffman D, Birch E, Birch D, et al. Impact of early dietary intake and blood lipid composition of long-chain polyunsaturated fatty acids on later visual development. J Pediatr Gastroenterol Nutr 2000;31:540–53.
[18] Innis SM, Nelson CM, Rioux MF, King DJ. Development of visual acuity in relation to plasma and erythrocyte w-6 and w-3 fatty acids in healthy term gestation infants. Am J Clin Nutr 1994;60:347–52.
[19] Koletzko B, Schmidt E, Bremer H, Haug M, Harzer G. Effects of dietary long-chain polyunsaturated fatty acids on the essential fatty acid status of premature infants. Eur J Paediatr 1989;148:669–75.
[20] Birch E, Garfield S, Hoffman D, Uauy R, Birch D. A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. Dev Med Child Neurol 2000;42:174–81.
[21] Willatts P, Forsyth J, DiModugno M, Varma S, Colvin M. Influence of long chain polyunsaturated fatty acids on infant cognitive function. Lipids 1998;33:973–80.
[22] Willatts P, Forsyth J, DiModugno M, Varma S, Colvin M. Effect of long chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age. Lancet 1998;352:688–91.
[23] Marin M, Rey G, Pedersoli L, Rodrigo M, de Alaniz M. Dietary Long-Chain Fatty Acids and Visual Response in Malnourished Nursing Infants, vol. 63. Prostoglandins Leukot Essent Fatty Acids; 2000. p. 385–90.
[24] Carlson S, Ford A, Werkman S, Peeples J, Koo W. Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. Pediatr Res 1996;39:882–8.
[25] Birch E, Hoffman D, Uauy R, Birch D, Prestidge C. Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. Pediatr Res 1998;44:201–9.
[26] Lucas A, Stafford M, Morley R, et al. Efficacy and safety of long-chain polyunsaturated fatty acid supplementation of infant formula milk: a randomised trial. Lancet 1999;354:1948–54.
[27] Makrides MMN, Simmer K, Gibson R. A critical appraisal of the role of dietary long-chain polyunsaturated fatty acids on neural indices of term infants: a randomized, controlled trial. Pediatrics 2000;105:32–8.
[28] Auestad N, Monalto M, Hall R, et al. Visual acuity, erythrocyte fatty acid composition, and growth in term infants fed formulas with long chain polyunsaturated fatty acids for one year. Pediatr Res 1997;41:1–10.
[29] Auestad N, Halter R, Hall R, et al. Growth and development in term infants fed long-chain polyunsaturated fatty acids: a double-masked, randomized, parallel, prospective, multivariate study. Pediatrics 2001;108:372–81.
[30] Scott D, Janowsky J, Carroll R, Taylor J, Auestad N, Montalto M. Formula supplementation with long-chain polyunsaturated fatty acids: are there developmental benefits? Pediatrics 1998;102:E59.
[31] Jorgensen M, Holmer G, Lund P, Hernell O, Michaelsen K. Effect of formula supplemented with docosahexaenoic acid and gamma-linolenic acid on fatty acid status and visual acuity in term infants. J Pediatr Gastroenterol Nutr 1998;26:412–21.
[32] Auestad N, Scott D, Janowsky J, et al. Visual, cognitive, and language assessments at 39 months: a follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. Pediatrics 2003;112:177–83.
[33] Holmes JM, Beck RW, Repka MX, et al. The amblyopia treatment study visual acuity testing protocol. Arch Ophthalmol 2001;119:1345–53.
[34] Moke P, Turpin A, Beck R, et al. Computerized method of visual acuity testing: adaptation of the Amblyopia Treatment Study visual acuity testing protocol for children. Am J Ophthalmol 2001;132:903–9.
[35] Pediatric Eye Disease Investigator Group. A randomized trial of patching regimens for treatment of moderate amblyopia in children. Arch Ophthalmol 2003;121:603–11.