Published in final edited form as:

Prostaglandins Leukot Essent Fatty Acids. 2017 June ; 121: 52–56. doi:10.1016/j.plefa.2017.05.005.

Docosahexaenoic acid (DHA) and arachidonic acid (ARA) balance in developmental outcomes☆

John Colombo[c], **D. Jill Shaddy**[a], **Elizabeth H. Kerling**[a], **Kathleen M. Gustafson**[b], and **Susan E. Carlson**[a,*]

[a]Department of Dietetics and Nutrition, University of Kansas Medical Center, Kansas City, KS, USA

[b]Department of Neurology, University of Kansas Medical Center, Kansas City, KS, USA

[c]Schiefelbusch Institute for Life Span Studies/Department of Psychology, University of Kansas, Lawrence, KS, USA

Abstract

The *DHA Intake and Measurement of Neural Development* (DIAMOND) trial represents one of only a few studies of the long-term dose-response effects of LCPUFA-supplemented formula feeding during infancy. The trial contrasted the effects of four formulations: 0.00% docosahexaenoic acid (DHA)/0.00% arachidonic acid (ARA), 0.32% DHA/0.64% ARA, 0.64% DHA/0.64% ARA, and 0.96% DHA/0.64% ARA against a control condition (0.00% DHA/0.00% ARA). The results of this trial have been published elsewhere, and show improved cognitive outcomes for infants fed supplemented formulas, but a common finding among many of the outcomes show a reduction of benefit for the highest DHA dose (i.e., 0.96% DHA/0.64% ARA, that is, a DHA: ARA ratio 1.5:1.0). The current paper gathers and summarizes the evidence for the reduction of benefit at this dose, and in an attempt to account for this reduced benefit, presents for the first time data from infants' red blood cell (RBC) assays taken at 4 and 12 months of age. Those assays indicate that blood DHA levels generally rose with increased DHA supplementation, although those levels tended to plateau as the DHA-supplemented level exceeded 0.64%. Perhaps more importantly, ARA levels showed a strong inverted-U function in response to increased DHA supplementation; indeed, infants assigned to the formula with the highest dose of DHA (and highest DHA/ARA ratio) showed a reduction in blood ARA relative to more intermediate DHA doses. This finding raises the possibility that reduced ARA may be responsible for the reduction in benefit on cognitive outcomes seen at this dose. The findings implicate the DHA/ARA balance as an important variable in the contribution of LCPUFAs to cognitive and behavioral development in infancy.

☆Mead Johnson Nutrition provided support for the parent DIAMOND study and for investigators at both Dallas and Kansas City sites to follow the long term development of these children, results of which are reviewed here.

*Correspondence to: Department of Dietetics and Nutrition, University of Kansas Medical Center, Mail Stop 4013, 3901 Rainbow Blvd., Kansas City, KS 66160, USA.

Declaration of interest: SEC and JC have given lectures on LCPUFA and these results for Mead Johnson Nutrition; JC has been a consultant to Mead Johnson Nutrition on studies of infant and child cognitive development. The other authors declare no conflicts of interest. All authors read and approved the final manuscript.

Keywords

Arachidonic acid; Docosahexaenoic acid; Infant development

1. Introduction

Long chain omega-3 (mainly 22 carbon) and omega-6 fatty acids (mainly 20 and 22 carbon) are found in all cell membranes where their presence influences the functional performance of organs and tissues. Much of the extant literature on supplementation of formula in infancy with long-chain polyunsaturated fatty acids (LCPUFAs) has focused on the addition of single compounds, such as docosahexaenoic acid (DHA), but the metabolism of these nutrients is intricately linked [1] and many authors have stressed the importance of the balance or ratio of the omega-3 and omega-6 acids in chronic disease, brain function, and human development at various phases of the lifespan [2–4].

The goal of this paper is to discuss the potential role of the balance between DHA and arachidonic acid (ARA) in light of the results of cognitive outcomes from a long-term follow-up of a randomized clinical trial that provided 0.64% of total fatty acids as ARA in combination with a varied concentration of DHA (0.32, 0.64 or 0.96% of total fatty acids) throughout the first year of life in infants born at term. When expressed in terms of the DHA: ARA ratio, the supplemented conditions reflect ratios of 0.5:1 (0.32% DHA/0.64% ARA), 1:1 (0.64% DHA/0.64% ARA), and 1.5:1 (0.96% DHA/0.64% ARA). Thus, the highest dose provided a ratio favoring DHA beyond that which has been typically provided in formula [5], although DHA: ARA ratios of 2:1 have been documented in breast milk [6].

We will briefly gather and summarize what has been reported from that trial, but our focus in this report is previously unreported data on red blood cell (RBC) phospholipid DHA and ARA levels in children from this trial, and the relation of those levels to cognitive performance from infancy through later childhood. The analysis of DHA and ARA levels was prompted by an unexpected but consistent finding that appeared in many of our outcomes. While supplementation with DHA and ARA from birth to 12 months conferred benefits in physiology, attention, and cognition out to 5 years of age, we observed a consistent reduction of benefit with formula containing the highest dose of DHA and thus, the highest DHA/ARA ratio. The conduct of this study was preceded by a study of brain regional fatty acid composition in infant baboons fed three of the 4 formulas fed in the human trial [7], which also becomes highly relevant to the examination of the balance between DHA and ARA in understanding the effects of supplementation on cognitive outcomes.

1.1. The DIAMOND trial

The *DHA Intake and Measurement of Neural Development* (DIAMOND) trial [8] is the only dose response trial of DHA and development reported to date. The DIAMOND study was conducted at two sites in the US (Dallas, TX and Kansas City, KS) and was funded by Mead Johnson Nutrition, Evansville, IN. The primary outcome of the study was visual acuity at 12

months of age, but enrollment at each site exceeded that estimated for the primary outcome to permit independent study of infant and child cognitive development at each site.

The amount of ARA in human milk typically exceeds that of DHA. The choice of ARA concentration in the DIAMOND trial was based on a median concentration of ARA in milk samples from around the world. The different concentrations of DHA reflected the range found in human milk except that the lowest concentration reflected the median milk DHA worldwide, a concentration shown previously to enhance visual acuity of term infants [9]. It is well known that the DHA content of human milk is highly dependent upon the DHA intake of the mother. To reiterate, all infants supplemented with DHA received 0.64% ARA. Consequently, the long term effects of supplementation on cognition and brain structure/function must be attributed to DHA and ARA. Where effects of DHA and ARA supplemented groups differ, the results reflect how their balance in the diet early in human development is relevant.

The initial trial into which infants were enrolled is described in detail elsewhere [8]. Briefly, 343 term infants who weighed between 2490 and 4200 g at birth were enrolled and randomly assigned to one of the 4 formulas described above between September 2002 and September 2004. The formulas provided either 0, 17, 34 or 51 mg DHA/ 100 kcal. The three formulas that contained DHA provided 34 mg ARA/ 100 kcal. The study was a randomized, double-blind study, and randomization was performed by Mead Johnson Nutrition biostatisticians. The study remained double-blinded until the last child reached 6 years of age. Random-number generation for each site included a list for males and one for females. Blocking kept study groups similar. Formulas were color-coded with each formula having two color codes. Parents were asked to feed formula exclusively for at least 4 months and were provided their infant's assigned formula as needed throughout the first 12 months. A total of 244 children completed the study to 12 months [8]. Of these, 81 were enrolled for long term cognitive assessment at the Kansas City site [10] and 131 children at the Dallas site [11]. RBC phospholipid DHA and ARA (g/100 g total fatty acid) were determined at 4 and 12 months of age. Here, we report primarily on the Kansas City cohort, as the major follow-up studies were run and published with those participants. The longitudinal schedule for following the Kansas City cohort is shown in Table 1.

2. Results

2.1. Non-linear supplementation dose-beneft relationship

2.1.1. Infancy outcomes—Significant effects of supplementation were seen for most measures taken in infancy. For some measures, the control condition (0.00% DHA/0.00% ARA) simply performed less well than the supplemented groups, which were clustered together and did not vary from one another. Supplemented groups were observed to have better visual acuity [12] through 12 months (combined Kansas City and Dallas cohorts), and lower heart rates [13] during the visual habituation task at 4, 6, and 9 months. However, the first indication of a reduced benefit in the highest DHA dose group (0.96 DHA/0.64% ARA, DHA: ARA = 1.5:1) was observed on sustained attention [13], a measure of the degree to which visual attention was coupled with active information processing [14]. Here, significantly improved attention relative to controls was seen in only the two middle doses.

The highest dose was not different from controls, but neither was it different from the other supplemented groups (see Fig. 1). An important point here, relevant to the other findings reported later in the paper, is that this pattern reflects a *reduced benefit* (rather than increased risk or actual harm) at the highest dose; that dose did not incur performance that was worse than controls, it merely reflected performance that was less than that seen for the intermediate levels of DHA supplementation.

2.1.2. Standardized outcomes and spatial memory—Subsequent outcomes of the study beyond the first year were reported in 2013 [15]. Among the measures taken at 18 months were the Bayley Scales of Infant Development, 2nd Edition (a global assessment of developmental status) and MacArthur-Bates Communicative Development Inventory (a well-validated parent-report measure of communicative and language skills) scales. A measure of spatial memory (Delayed Response) was administered semiannually from 24 to 36 months as well. Performance of supplemented groups were not different from controls on any of these outcomes [15].

2.1.3. Executive function and problem solving—Beginning at 36 months, measures of rule learning, flexibility, and inhibition were administered at semi-annual intervals. The Dimensional Change Card Sort task involves having the child learn a sorting rule for pictures, and then observing the child's response after the rule is reversed; this assesses the child's mental adaptability and flexibility. Children became progressively better overall at this task (i.e., at both initial rule-learning and adapting to reversals), but children fed the two middle-dose formulas showed an accelerated developmental course of improvement relative to controls, and once again children assigned to the highest DHA-dose formula fell intermediate between these doses and controls. On this task, the pattern of reduced benefit emerged first at 42 months was increasingly evident at 48 and 60 months of age. Stroop tasks (which require the child to learn a rule that involves inhibiting a previously-learned, or pre-potent, response) administered at the same ages were the only measure on which a distinctly linear trend was observed for any of the outcomes.

Two other executive function tasks showed clear developmental improvement but no effects of supplementation. In one of these, children were asked to follow instructions provided by a Bear puppet but ignore (i.e., inhibit responding to) instructions provided a Dragon puppet; this task was administered from 36 to 48 months. The Tower of Hanoi task, a classic problem-solving task requiring planning and execution of sequential actions toward the attainment of a goal, was administered from 42 through 72 months.

2.1.4. Verbal and composite IQ—Children were assessed on the Peabody Picture Vocabulary Test, 3rd Edition (PPVT) at 60 months, and on the Wechsler Preschool and Primary Scale of Intelligence, 3rd Edition (WPPSI) at 72 months. The PPVT yields a single score, which is often taken as a surrogate for Verbal IQ; the WPPSI yields standard scores on subscales for Verbal, Performance, and Processing Speed, as well as a Full Scale (composite) IQ score. There was a significant effect of supplementation on the PPVT, which mirrored previous findings in which the two middle DHA doses outperformed controls, but where the highest DHA dose incurred a performance that was at least as good as that of controls but not better than the one seen with intermediate levels of supplementation. By 72

months of age, differences among the four groups were diminished such that supplemented children varied in a statistically significant way from controls only when data from the three supplemented groups were pooled. However, the pattern for most of the WPPSI subscales and the WPPSI full (composite) IQ, shown for the first time in Fig. 2, continued to show the inverted-U pattern, with performance declining in the 0.96% DHA/0.64% ARA group relative to the two other supplemented doses.

2.2. Explaining the reduced beneft in the high-dose condition

The consistency of this pattern of findings in so many of our outcomes suggested that it was not a spurious result, and so it generated considerable conversations among our group. We considered the most likely candidate for this factor to be the particularly high DHA/ARA ratio in the highest DHA dose group. Indeed, a study of LCPUFA distribution in brain tissue in baboons as a response to the feeding of formulas directly analogous to the control condition (0.00% DHA), the lower DHA dose (0.32%) and the highest dose (0.96% DHA) in DIAMOND (9) consistently found that the highest dose in that study (1.00% DHA/ 0.67% ARA, also a 1.5:1 DHA/ARA ratio) was associated with a reduction of ARA in all areas of the brain assayed. Select data from that study were redrawn from tables and are shown in Fig. 3.

We sought to determine whether the same decrease in ARA might be present in the highest DIAMOND DHA dose. While brain tissue data were obviously not available for such testing in the DIAMOND sample, data from RBC phospholipid fatty acid analysis were available on both DHA and ARA at 4 and 12 months of age. We used data from all infants across the four conditions in DIAMOND and modeled the functions relating the amount of DHA in formula for each of the four conditions to RBC DHA and ARA measured at 4 and 12 months. To do this, we used curve-fitting algorithms (SPSS/IBM Statistics 23.0), and examined the degree to which these associations could be accounted for by linear and quadratic functions. In each case, the inclusion of quadratic terms in the equations accounted for significant and unique variance in the overall model; thus, the data were better represented by curvilinear rather than simpler linear functions.

In modeling RBC DHA, the quadratic term accounted for an additional 10.6% of variance ($p < 0.001$) at 4 months and 2.3% of variance ($p = 0.024$) at 12 months over that accounted for by the linear term alone. For RBC ARA, equations containing the linear term alone did not attain statistical significance at either 4 or 12 months; the quadratic term accounted for 24.7% of unique variance ($p < 0.001$) at 4 months and 14.1% of unique variance ($p = 0.001$) at 12 months. The quadratic results (including the resulting equations, and the statistical tests for significance of each of the curves derived from the SPSS curve-fitting algorithm) are shown in Figs. 4 and 5.

As Fig. 4 shows, RBC DHA increased sharply from control (0.00% DHA) to the two intermediate doses (0.32% and 0.64% DHA), but then plateaued between the 0.64% DHA formula at the highest (0.96% DHA) dose. This pattern was extremely consistent across both 4 and 12 months, with the calculated curves showing nearly perfect overlap. Thus, RBC DHA seemed to reach some level of saturation at the highest dose for this sample.

Fig. 5 speaks directly to the hypothesis concerning the involvement of ARA in explaining the reduced benefit seen at the highest formula DHA dose. Once again, at both ages, the best-fitting curve for ARA showed nearly perfect overlap at both 4 and 12 months, but the nature of the curve was somewhat different than that seen for DHA. The quadratic component of the equation for those curves were almost equivalent to the linear component, thus yielding a strongly inverted-U function; thus, at the highest formula dose for DHA in DIAMOND (0.96%), ARA actually was seen to decrease, relative to the two intermediate doses (0.32% and 0.64%). These findings are highly consistent with the brain tissue results from the baboon study (9) using very similar formulas.

3. Discussion

These results suggest that the DHA/ARA balance may be an important factor in the efficacy of DHA and ARA on cognitive and developmental outcomes in infancy and early childhood. The DIAMOND results showed significant, meaningful, and long-lasting effects of feeding DHA and ARA-supplemented formula to infants from birth to 12 months of age. However, we found that increasing DHA while holding ARA constant (which increases the DHA: ARA ratio) resulted in a reduction of ARA in the RBC phospholipids of human infants at the highest DIAMOND DHA dose. Young baboons fed formula with a nearly identical amount of DHA and ARA showed a reduction in ARA in multiple brain regions compared to formula with a 0.5:1 ratio of DHA to ARA. In the DIAMOND results, infants assigned to this highest DHA dose demonstrated cognitive performance that was indistinguishable from controls in a number of outcome measures. Although the studies are not available to address the effect of feeding a formula containing DHA without ARA in either human or baboon infants, it seems likely that the effect on membrane phospholipids and perhaps development would be even more profound than with a 1.5:1 ratio of DHA to ARA.

The results here are expressed in terms of group outcomes and means; that is, group levels of the DHA/ARA balance were broadly associated with developmental and cognitive measures. Correlations between individual ARA levels (or the DHA/ARA ratios) with individual cognitive outcomes would provide strong evidence for our hypothesis, but those correlations are too inconsistent and variable to provide either support or refutation of the position, perhaps owing to the lack of adequate power with this sample size.

Biochemical interactions between ARA and DHA are well documented in the literature. Both n-6 and n-3 LCPUFA utilize the same elongases and desaturases for synthesis from linoleic acid and alpha-linolenic acid, respectively [16]. The balance of ARA and DHA appears to influence membrane n-6 and n-3 LCPUFA balance by bypassing this biosynthetic pathway leading to a potential imbalance similar to what has been seen in this study.

Although much of the literature on brain and cognitive function has focused on DHA, ARA is implicated in CNS function as well [16–18] and n-6 LCPUFA predominate over n-3 LCPUFA in the brain of developing primates [7]. We show here that a ratio of DHA to ARA of 1.5:1 reduces red blood cell membrane ARA during brain development compared to a 1:2 or 1:1 ratio. Because our results are analogous to the decrease in ARA and other n-6 LCPUFA found in brain of developing baboons fed a 1.5:1 ratio of DHA to ARA compared

to a ratio of 1:2 (should be ref. 7), they suggest a similar decrease could occur in the brain of infants. Our studies of neurodevelopment in infants fed these same formulas suggest the balance of DHA to ARA in formula may be a factor in realization of benefits of LCPUFA supplementation in infancy and early childhood. The neurodevelopmental outcomes favor a 1:1 or 1:2 balance of DHA to ARA consistent with the conclusions from a recent review [16].

Acknowledgments

The authors' responsibilities were as follows: SEC and JC designed the parent study and the follow-up study to age 6 years; KMG was responsible for the assessment of visual acuity; DJS coordinated data collection and management for DIAMOND, and EHK coordinated the overall conduct of the trial. JC wrote the manuscript and all authors participated in the critical review of the manuscript and approve of its final version.

References

1. Innis SM. Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. J Pediatr. 2003; 143:1–8. [PubMed: 12915812]
2. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 2002; 56:365–379. [PubMed: 12442909]
3. Simopoulos AP. Evolutionary aspects of diet: the omega-6/omega-3 ratio and the brain. Mol Neurobiol. 2011; 44:203–215. [PubMed: 21279554]
4. Loef M, Walach H. The omega-6/omega-3 ratio and dementia or cognitive decline: a systematic review on human studies and biological evidence. J Nutr Gerontol Geriatr. 2013; 32:1–23. [PubMed: 23451843]
5. Lapillonne A, Groh-Wargo S, Lozano Gonzalez CH, Uauy R. Lipid needs of preterm infants: updated recommendations. J Pediatr. 2013; 162:S37–S47. [PubMed: 23445847]
6. Delplanque B, Gibson R, Koletzko B, Lapillonne A, Strandvik B. Lipid quality in infant nutrition: current knowledge and future opportunities. J Pediatr Gastroenterol Nutr. 2015; 61:8–17. [PubMed: 25883056]
7. Hsieh AT, Anthony JC, Diersen-Schade DA, Rumsey SC, Lawrence P, Li C, Nathanielsz PW, Brenna JT. The influence of moderate and high dietary long chain polyunsaturated fatty acids (LCPUFA) on baboon neonate tissue fatty acids. Pediatr Res. 2007; 61:537–545. [PubMed: 17413857]
8. Birch EE, Carlson SE, Hoffman DR, Fitzgerald-Gustafson KM, Fu VL, Drover JR, Castaneda YS, Minns L, Wheaton DK, Mundy D, Marunycz J, Diersen-Schade DA. The DIAMOND (DHA intake and measurement of neural development) study: a double-masked, randomized controlled clinical trial of the maturation of infant visual acuity as a function of the dietary level of docosahexaenoic acid. Am J Clin Nutr. 2010; 91:848–859. [PubMed: 20130095]
9. Birch EE, Castaneda YS, Wheaton DH, Birch DG, Uauy RD, Hoffman DR. Visual maturation of term infants fed long-chain polyunsaturated fatty acid-supplemented or control formula for 12 mo. Am J Clin Nutr. 2005; 81:871–879. [PubMed: 15817866]
10. Colombo J, Carlson SE, Cheatham CL, Shaddy DJ, Kerling EH, Thodosoff JM, Gustafson KM, Brez C. Long-term effects of LCPUFA supplementation on childhood cognitive outcomes. Am J Clin Nutr. 2013; 98:403–412. [PubMed: 23803884]
11. Drover JR, Hoffman DR, Castaneda YS, Morale SE, Garfield S, Wheaton DH, Birch EE. Cognitive function in 18-month-old term infants of the DIAMOND study: a randomized, controlled clinical trial with multiple dietary levels of do-cosahexaenoic acid. Early Human Dev. 2011; 87:223–230.
12. Birch EE, Carlson SE, Hoffman DR, Fitzgerald-Gustafson KM, Fu VL, Drover JR, Castaneda YS, Minns L, Wheaton DK, Mundy D, Marunycz J, Diersen-Schade DA. The diamond (DHA intake and measurement of neural development) study: a double-masked, randomized controlled clinical trial of the maturation of infant visual acuity as a function of the dietary level of docosahexaenoic acid. Am J Clin Nutr. 2010; 91:848–859. [PubMed: 20130095]

13. Colombo J, Carlson SE, Cheatham CL, Fitzgerald-Gustafson KM, Kepler A, Doty T. Long-chain polyunsaturated fatty acid supplementation in infancy reduces heart rate and positively affects distribution of attention. Pediatr Res. 2011; 70:406–410. [PubMed: 21705959]
14. Richards JE. The development of sustained visual-attention in infants from 14 to 26 weeks of age. Psychophysiology. 1985; 22:409–416. [PubMed: 4023152]
15. Colombo J, Carlson SE, Cheatham CL, Shaddy DJ, Kerling EH, Thodosoff JM, Gustafson KM, Brez C. Long-term effects of LCPUFA supplementation on childhood cognitive outcomes. Am J Clin Nutr. 2013; 98:403–412. [PubMed: 23803884]
16. Hadley K, Ryan A, Forsyth S, Gautier S, Salem N. The essentiality of arachidonic acid in infant development. Nutrients. 2016; 8:216. [PubMed: 27077882]
17. Carlson SE, Colombo J. Docosahexaenoic acid and arachidonic acid nutrition in early development. Adv Pediatr. 2016; 63:453–471. [PubMed: 27426911]
18. Carlson SE. Docosahexaenoic acid and arachidonic acid in infant development. Semin Neonatal. 2001; 6:437–449.

Abbreviations

ARA	arachidonic acid
DHA	docosahexaenoic acid
DIAMOND	DHA intake and measurement of neural development

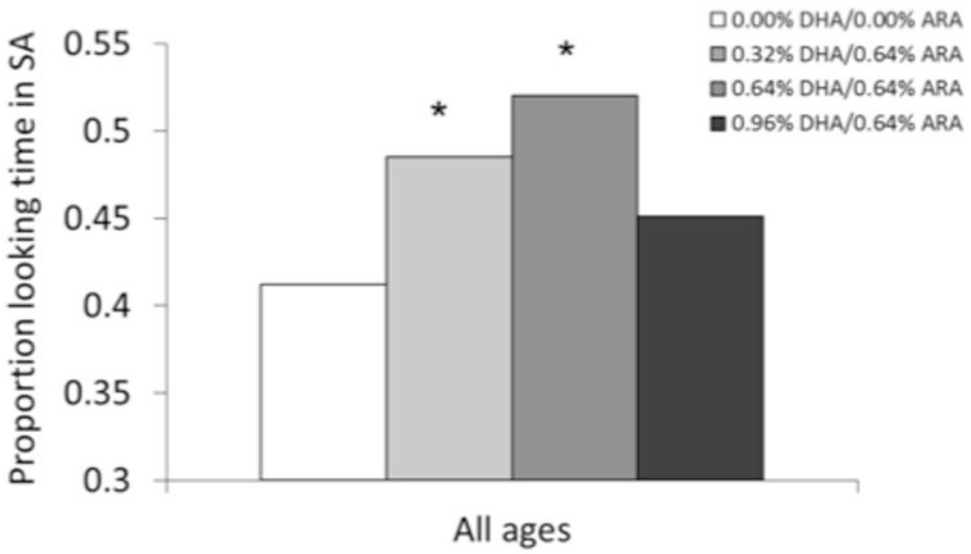

Fig. 1.
Sustained attention at 4, 6, and 9 months in the four formula groups.

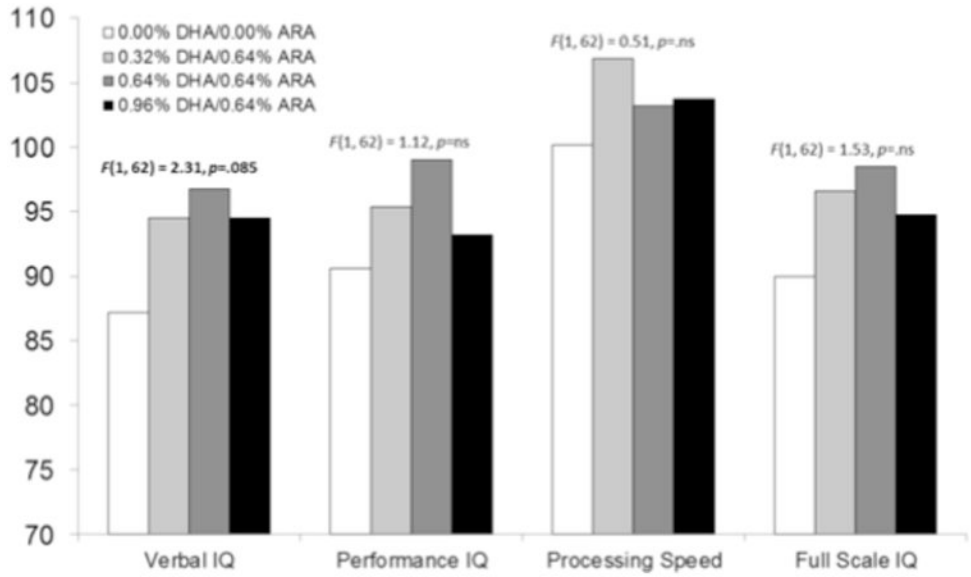

Fig. 2.

WPPSI subscale and full-scale (Composite) scores at 72 months of age in the four formula groups.

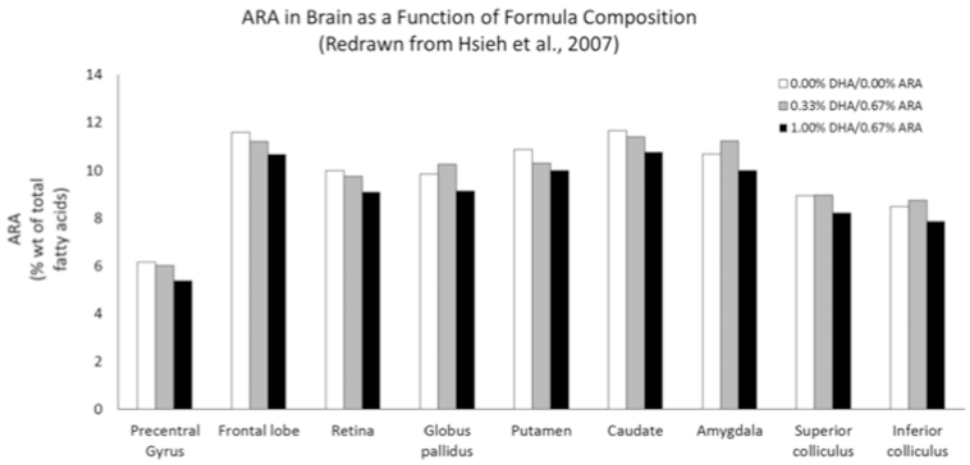

Fig. 3.

ARA in baboon brain as a function of formula composition. (Data redrawn from Heish et al. [7]). Note that, in each case, the highest DHA dose (black bar) has lowest ARA tissue concentration in brain.

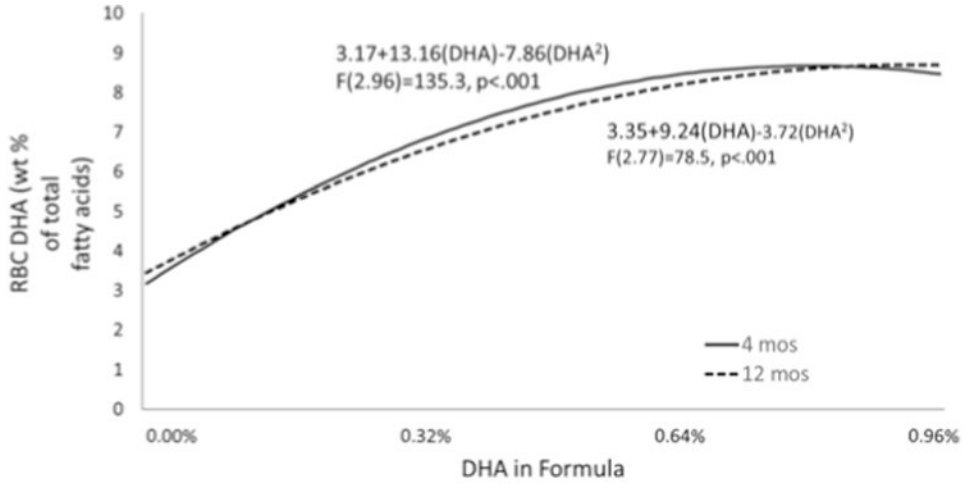

Fig. 4.
RBC DHA levels for infants in the DIAMOND trial at 4 and 12 months.

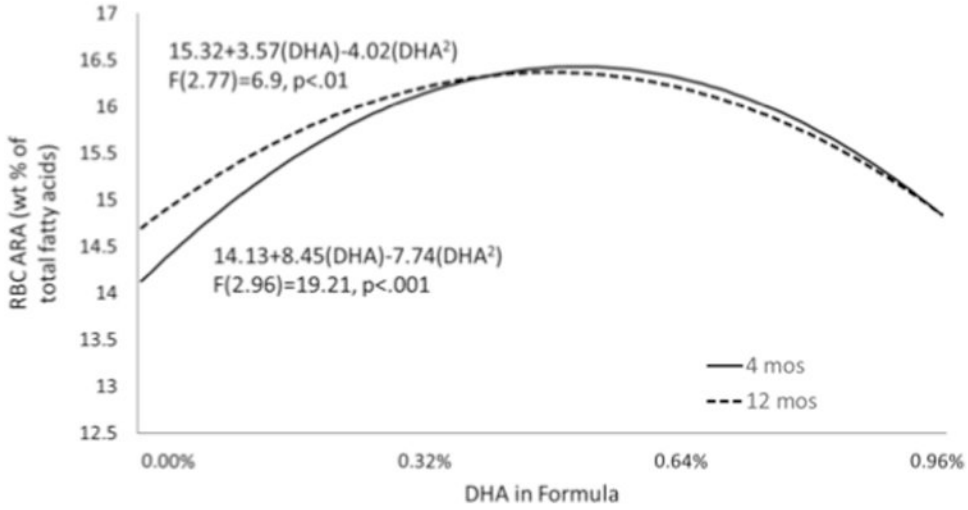

Fig. 5.
RBC ARA levels for infants in the DIAMOND trial at 4 and 12 months.

Table 1

Longitudinal schedule for follow-up of the Kansas City DIAMOND Cohort.

Assessment	Age (months)											
	4	6	9	18	24	30	36	42	48	60	66	72
Visual Habituation with Heart Rate	●	●	●									
Bayley Scales of Infant Development				●								
MacArthur-Bates Communicative Development Inventory				●								
Delayed Response (Spatial Memory)					●	●	●					
Bear-Dragon Task							●	●	●			
Dimensional Change Card Sort							●	●	●	●		
Stroop Tasks (2)							●	●	●	●		
Peabody Picture Vocabulary Test										●		
Tower of Hanoi Task									●	●		●
Wechsler Preschool and Primary Scale of Intelligence												●