PEDIATRICS®
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

10-year Cognition in Preterms After Random Assignment to Fatty Acid Supplementation in Infancy

Elizabeth B. Isaacs, Sarah Ross, Kathy Kennedy, Lawrence T. Weaver, Alan Lucas and Mary S. Fewtrell

Pediatrics 2011;128;e890; originally published online September 19, 2011;
DOI: 10.1542/peds.2010-3153

10-year Cognition in Preterms After Random Assignment to Fatty Acid Supplementation in Infancy

WHAT'S KNOWN ON THIS SUBJECT: Systematic reviews of preterm infant formula supplementation with long-chain polyunsaturated fatty acids have found no cognitive effects up to 2 years of age. To date, however, studies that examined general and specific cognition >9 years after early supplementation have not been published.

WHAT THIS STUDY ADDS: No cognitive differences were found at 10 years between randomized groups. Planned gender analyses, however, revealed benefits for girls in literacy measures. Furthermore, supplementation for infants who received formula as their sole diet produced advantages in several cognitive measures.

abstract

OBJECTIVE: To test the hypothesis that long-chain polyunsaturated fatty acid (LCPUFA) supplementation in infancy would improve cognition into later childhood (after 9 years) at both general and specific levels.

METHODS: A comprehensive cognitive battery was completed by 107 formerly preterm infants (mean age: 128 months). As infants, they had been assigned randomly to receive LCPUFA-supplemented ($N = 50$) or control ($N = 57$) formula, between birth and 9 months; the docosahexaenoic acid level (DHA) in the supplemented formulas was 0.5%. In addition to randomized comparisons, we planned supplementary analyses to examine the effects of both gender and feeding group (those receiving some maternal breast milk versus those receiving none).

RESULTS: There were no significant differences between randomized diet groups on any cognitive measure. There was significant interaction between gender and supplementation; girls only showed beneficial effects of LCPUFAs on literacy. Significant interaction also occurred between feeding group and supplementation; increases of 0.7 SD in verbal IQ, full-scale IQ, and memory scores were found for the LCPUFA group, but only for infants who received only formula and no maternal breast milk.

CONCLUSIONS: The results of this post–9-year cognitive follow-up study in a randomized trial of LCPUFA-supplemented formula for preterm infants suggest no overall group effects but indicate that gender-specific and diet-specific effects may exist. The data provide some evidence that LCPUFAs are a key factor in the cognitive benefits of breast milk. Caution is advised in data interpretation because of the small groups used. *Pediatrics* 2011;128:e890–e898

AUTHORS: Elizabeth B. Isaacs, PhD,[a] Sarah Ross, DClinPsy,[a] Kathy Kennedy, MSc,[a] Lawrence T. Weaver, MD, DSc,[b] Alan Lucas, MD,[a] and Mary S. Fewtrell, MD[a]

[a]Medical Research Council Childhood Nutrition Research Centre, University College London Institute of Child Health, London, England; and [b]Department of Child Health, Faculty of Medicine, University of Glasgow, Glasgow, Scotland

KEY WORDS

long-chain polyunsaturated fatty acids, randomized trial, preterm, long-term outcome, cognition, gender effects, breastfeeding

ABBREVIATIONS

LCPUFA—long-chain polyunsaturated fatty acid
VIQ—verbal IQ
FSIQ—full-scale IQ
BSID-II—Bayley Scales of Infant Development II
CMS—Children's Memory Scale
DHA—docosahexaenoic acid

www.pediatrics.org/cgi/doi/10.1542/peds.2010-3153

doi:10.1542/peds.2010-3153

Accepted for publication Jun 28, 2011

Address correspondence to Elizabeth B. Isaacs, PhD, Childhood Nutrition Research Centre, UCL Institute of Child Health, 30 Guilford St, London WC1N 1EH, United Kingdom. E-mail: e.isaacs@ich.ucl.ac.uk

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

FINANCIAL DISCLOSURE: *Drs Lucas and Fewtrell have received research funding and performed advisory work for infant feeding manufacturers, and Dr Weaver is a member of the Infant and Toddler Forum, an educational charity funded by Danone. Drs Isaacs, Ross, and Kennedy have indicated they have no financial relationships relevant to this article to disclose.*

COMPANION PAPER: A companion to this article can be found on page e880, and online at www.pediatrics.org/cgi/doi/10.1542/peds.2010-1633.

There has been considerable interest in the role of long-chain polyunsaturated fatty acids (LCPUFAs) in the development of visual and cognitive functioning.[1–3] Three key hypotheses underpin this research, as follows. (1) Formula-fed infants, especially preterm infants, cannot synthesize LCPUFAs adequately for optimal neurodevelopment and need a dietary supply. (2) The absence of LCPUFA supplementation in previous formulas contributed to lower cognitive scores observed for formula-fed versus breastfed infants. (3) Supplementation of infant formulas with LCPUFAs improves cognitive development.

Despite some inconsistencies, data from numerous studies on the effects of LCPUFA supplementation of infant formulas on development[4–6] have driven a major public health intervention, whereby LCPUFAs are added to most formulas for the large majority of Western infants who consume formula at some point during infancy. However, 2 systematic reviews of 29 randomized controlled trials studying the effects of LCPUFAs for term[7] and preterm[8] infants concluded that there was no consistent effect of formula supplementation with LCPUFAs on cognition or visual function.

There were 2 important shortcomings in the trial results reviewed. First, the majority of studies focused on overall cognition, particularly general measures of mental and psychomotor development from the Bayley Scales of Infant Development II (BSID-II).[9] Groups that score the same on overall cognitive function tests may still demonstrate significant differences in specific aspects of cognition, however, and it is important to test for such differences, as pointed out by Cheatham et al.[10] Although some studies looked for specific effects,[11] most did not. Second, the randomized trials reviewed by Simmer et al[7,8] examined mainly children younger than 2 years and no children aged 4 years or older. To date, few data have been obtained from follow-up studies into later childhood (≥10 years),[12] particularly pertaining to specific cognitive functions. Some functions, such as literacy and numeracy, are unquantifiable in young children, and any selective influence of LCPUFAs on those functions could not be have been detected.

Here, we administered a detailed cognitive battery at 10 years to members of a preterm cohort who had been assigned randomly to receive LCPUFA-supplemented formula or control formula between birth and 9 months of age. We aimed to test the hypothesis that early LCPUFA administration would have a long-term (ie, ≥10-year) programming effect on neurodevelopmental outcomes, providing evidence to help underpin public health practice in this much-studied area of infant nutrition.

TABLE 1 Nutrient Compositions of Trial Formulas[13]

	Preterm Infant Formulas[a]	Postdischarge Formulas
Protein, g/100 mL	2.0	1.85
Casein, g/100 mL	0.77	0.72
Whey, g/100 mL	1.23	1.13
Carbohydrate, g/100 mL	7.65	7.24
Fat, g/100 mL	4.6	3.96
Energy		
kJ/100 mL	334	301
kcal/100 mL	80	72
Minerals		
Calcium, mg/100 mL	110	70
Phosphorus, mg/100 mL	63	35
Sodium, mg/100 mL	42	22
Potassium, mg/100 mL	72	78
Iron, mg/100 mL	0.04	0.65
Zinc, mg/100 mL	0.88	0.6
Vitamins		
A, μg/100 mL	100	100
D_3, μg/100 mL	2.4	1.3
K, μg/100 mL	7.0	6.0
E, mg/100 mL	10	1.5
Carnitine, mg/100 mL	1.0	1.1
Choline, mg/100 mL	5.6	5.1

[a] Values apply to both control and LCPUFA-supplemented formulas.

METHODS

Original Trial Intervention

To summarize the original trial,[13] preterm infants without congenital malformations with neurodevelopmental consequences, with birth weights of <2000 g, and with gestational ages of <35 weeks were recruited from NICUs between 1995 and 1997. Infants who were receiving at least some feedings as formula milk during hospitalization were assigned (through random permuted block allocation) to receive a LCPUFA-supplemented formula or a control formula, up to 9 months after term; preterm infant formula was used until discharge, followed by a nutrient-enriched postdischarge formula (see Tables 1 and 2 for details on the formulas). The DHA content of 0.5% in the supplemented formula (from borage and tuna oils) was high, compared with the level of 0.32 ± 0.22% reported as a worldwide mean for human breast milk[14] and the level of 0.3% typically added to preterm formulas. The infants were monitored intensively in the NICU by research nurses, who collected data on clinical progress, and made home visits after discharge. The primary cognitive efficacy outcome was neurodevelopment at corrected age of 18 months, as measured with the BSID-II.[9] Because moth-

TABLE 2 Fatty Acid Compositions of Trial Formulas[13]

	Fatty Acid Composition, g/100 g Fat	
	Control Formulas[a]	LCPUFA-Supplemented Formulas
C18:2, *n*-6, linoleic acid	11.5	12.3
C18:3, *n*-6, γ-linoleic acid	Trace	0.9
C18:3, *n*-3, α-linoleic acid	1.6	1.5
C20:5, *n*-3, eicosapentaenoic acid	0.0	0.1
C22:6, *n*-3, docosahexaenoic acid	0.0	0.5
C20:4, *n*-6, arachidonic acid	0.0	0.04

[a] Values apply to both preterm and postdischarge formulas.

ers often provided breast milk in addition to formula, a prior decision was made to group the infants according to whether the trial formula was the sole diet or a supplement to maternal breast milk.

Ten-Year Follow-up Study

Subjects

All surviving children from the original study were approached regarding participation, with ethics approval from the local research ethics committee. One hundred seven children (51 boys and 56 girls) were seen at a mean age of 128 months (range: 113–147 months); 57 children (26 boys and 31 girls) were originally assigned randomly to the control formula group and 50 (25 boys and 25 girls) to the supplemented formula group (Fig 1). Participants were seen at a children's hospital or at home for detailed cognitive assessment. Anthropometric, body composition, and blood pressure measurements were reported separately.[15] Maternal education was scored on a 5-point scale ranging from 1 (no educational qualifications) to 5 (degree/further training). Social codes ranged from 1 (high) to 7 (low). Written informed consent was obtained from the parent/guardian, and assent was obtained from the child. A sample size of 64 children per randomly assigned group (follow-up rate of 66%) would allow detection of a 0.5-SD difference in outcome variables with 80% power and 5% significance. The number we recruited at age 10 represented a rate of 45%, which was powered to detect a 0.6-SD difference.

Cognitive Measures

Testing

Tests were administered by 1 researcher, who was blinded to formula allocation. The most global score for any test was used as the main outcome measure. Any significant difference between groups in a main outcome measure was explored further through examination of subtest scores.

Intelligence

The Wechsler Abbreviated Scale of Intelligence[16] provided measures of verbal IQ (VIQ) (vocabulary and similarities subtests), performance IQ (matrix reasoning and block design subtests), and full-scale IQ (FSIQ) (all 4 subtests).

Neuropsychological Assessment

The Neuropsychological Test for Children,[17] a standardized neuropsychological assessment, provided scores for memory and language domains. The tower subtest from the attention/executive domain also was administered.

Additional Memory Measure

Because hippocampal function in particular has been associated with LCPUFA status,[18] we included a test of association learning (for which the hippocampus is thought to be important[19]), namely, the word pairs subtest

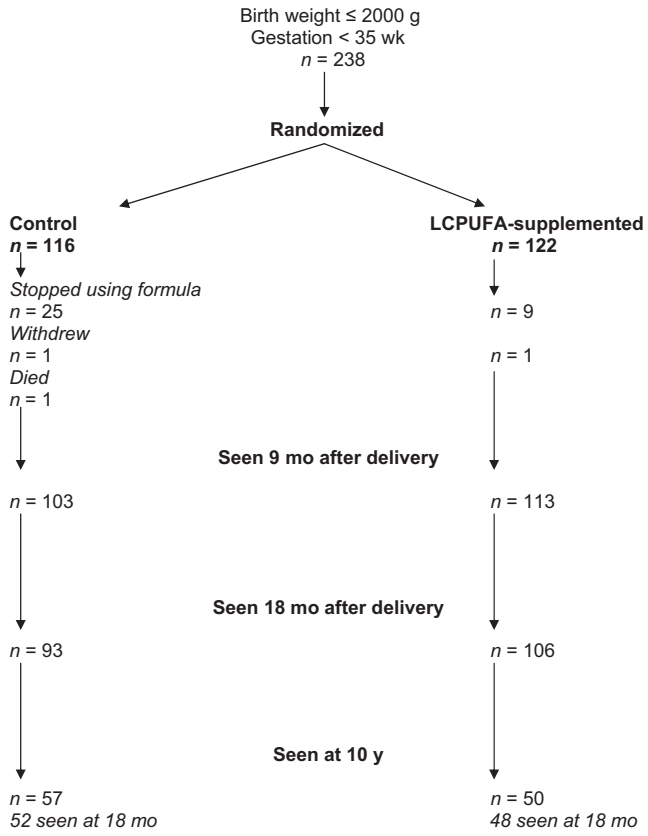

FIGURE 1
Flow chart of progress through the study.

from the Children's Memory Scale (CMS).[20]

Academic Attainments

The Wechsler Individual Achievement Test, Second UK Edition,[21] measured attainments in numeracy (numerical operations subtest), literacy (word reading and pseudoword decoding), and spelling.

Attention

The Test of Everyday Attention for Children[22] measures aspects of visual and auditory attention. We used Sky Search (selective/focused attention), Score! (sustained attention), Sky Search DT (sustained/divided attention), Creature Counting, and Opposite Worlds (attentional control/switching).

Executive Function

We used the Behavioural Assessment of the Dysexecutive Syndrome for Children[23] because of a reported association between LCPUFA status and executive function.[24,25]

Statistical Analyses

Primary Analyses

Group comparisons for baseline characteristics were performed for (1) those seen versus those not seen at the follow-up evaluation and (2) randomly assigned diet groups. The 2 randomly assigned diet groups then were compared with respect to the main outcome measures by using *t* tests.

Preplanned Supplementary Analyses

Comparisons were performed (1) for each feeding group (formula as the sole diet or as a supplement to maternal breast milk) and (2) within each gender. We used analysis of variance to test for dietary interactions within each comparison, to provide additional evidence of any causal subgroup effect.

TABLE 3 Baseline Characteristics for Children Seen or not Seen at 10-Year Follow-up Evaluation

	Seen ($n = 107$)	Not Seen ($n = 131$)	*P*
Birth weight, mean ± SD, g	1485 ± 352	1509 ± 318	.58
Gestational age, mean ± SD, wk	30.8 ± 2.2	31.3 ± 1.9	.03
Duration of ventilation, mean ± SD, d	5.1 ± 5.4	6.4 ± 7.9	.39
Social class 1 or 2, *n* (%)[a]	25 (23)	29 (22)	.48
Mother has higher vocational qualification/degree, *n* (%)	4 (4)	7 (5)	.40
Received maternal breast milk, *n* (%)	68 (64)	62 (47)	.01
Proportion of enteral intake as maternal breast milk, mean ± SD, %	28.8 ± 31.1	21.9 ± 23.6	.15

[a] Highest 2 categories (of 7).

RESULTS

General Characteristics

Table 3 presents baseline data for children who were seen versus not seen at the 10-year follow-up evaluation. Children who were seen had significantly lower gestational ages and were more likely to have received maternal breast milk, but there were no significant differences in socioeconomic factors between these groups. Table 4 presents characteristics of the 2 randomly assigned diet groups at 10 years of age and shows a significant difference in social code values.

Primary Analyses of Whole Group

Cognitive measures for the randomly assigned groups were compared by using *t* tests; data are presented in Table 5. Differences between the control and supplemented formula groups with respect to general and specific cognitive outcomes were small (rarely exceeding 0.3 SD), and none reached a significance level of $P = .05$. Because of social code differences reported above, all analyses were repeated with the use of that factor as a covariate, but the pattern remained the same.

Planned Supplementary Analyses According to Feeding Group

The diet for 39 children consisted solely of the trial formulas (control formula: 23 children; supplemented formula: 16 children). Another 68 children received some breast milk during their hospital stays, in addition to the randomly assigned formula used to meet volume requirements (control formula: 34 children; supplemented formula: 34 children); the mean proportion of breast milk in the diet during hospitalization was 29% (control formula: 28%; supplemented formula: 29%). Table 6 presents characteristics according to diet group; there was a significant difference in social codes between diet groups among those who received formula only. We conducted a nonrandomized comparison between the diet groups within each feeding group and then analyses controlling for social code, because of this group difference, and for maternal education in case of residual confounding.

TABLE 4 Comparison of Randomized Diet Groups at 10-Year Follow-up Evaluation With Respect to Perinatal and Social Variables

	Control ($n = 57$)	Supplemented ($n = 50$)	*P*
Age, mean ± SD, y	10.8 ± 0.7	10.8 ± 0.6	.73
Birth weight, mean ± SD, g	1512 ± 338	1454 ± 369	.60
Gestational age, mean ± SD, wk	30.9 ± 2.0	30.6 ± 2.3	.60
Duration of ventilation, mean ± SD, d	1.7 ± 3.5	3.1 ± 5.3	.90
Social class 1 or 2, *n* (%)[a]	7 (12)	18 (36)	.004
Mother has higher vocational qualification/degree, *n* (%)	9 (1)	3 (6)	.25
Received maternal breast milk, *n* (%)	1 (2)	3 (6)	.09

[a] Highest 2 categories (of 7).

TABLE 5 Results of Primary Analyses Comparing Randomized Groups in Cognitive Outcome Measures

	Score, Mean ± SD		*P*
	Control (*n* = 57)	Supplemented (*n* = 50)	
Wechsler Abbreviated Scale of Intelligence			
VIQ	92.6 ± 12.6	96.7 ± 13.2	.11
Performance IQ	94.5 ± 14.1	94.2 ± 12.7	.93
FSIQ	92.7 ± 12.3	95.1 ± 13.2	.34
Neuropsychological Test for Children			
Memory domain score	97.9 ± 16.6	99.3 ± 16.4	.68
Language domain score	97.6 ± 13.1	97.0 ± 15.4	.85
Tower scaled score	10.0 ± 2.4	10.4 ± 2.5	.37
Inspection time, total accuracy score (maximum: 150)	107.2 ± 12.7	108.0 ± 15.7	.79
Test of Everyday Attention for Children			
Attention scaled score	8.3 ± 2.6	8.2 ± 2.5	.99
Score! scaled score	7.8 ± 3.4	7.7 ± 3.4	.85
Creature counting scaled score	9.6 ± 2.1	10.0 ± 2.7	.42
Dual-task decrement scaled score	7.3 ± 2.8	7.6 ± 2.5	.58
Opposite Worlds different scaled score	8.4 ± 2.8	8.9 ± 3.5	.41
CMS word pairs			
Learning scaled score	11.3 ± 3.5	11.7 ± 3.2	.54
Delayed recall scaled score	10.4 ± 2.9	10.6 ± 3.3	.73
Delayed recognition scaled score	10.6 ± 17.6	11.3 ± 1.9	.13
Behavioural Assessment of Dysexecutive Syndrome for Children, overall score	86.6 ± 17.6	87.8 ± 17.9	
Wechsler Individual Achievement Test			
Word reading standard score	91.9 ± 12.5	94.5 ± 16.7	.73
Pseudoword decoding standard score	95.5 ± 11.1	96.1 ± 11.6	.81
Spelling standard score	90.5 ± 12.8	93.8 ± 14.2	.21
Numerical operations standard score	91.4 ± 13.2	90.6 ± 15.8	.78

For domain scores and standard scores the mean is 100; for scaled scores, the mean is 10.

These analyses were conducted for all cognitive outcome measures; significant results are presented in Table 7. Among the 39 children fed only formula, there were differences between groups in overall VIQ and FSIQ scores. Examination of the Wechsler Abbreviated Scale of Intelligence subtest results showed differences on the 2 verbal subtests. Table 7 also shows that the supplemented formula group had significantly higher CMS word-pair learning scores (significance levels after covariance: VIQ: $P = .005$; FSIQ: $P = .04$; CMS: $P = .07$). No significant differences existed between randomized diet groups within the breastfed group with respect to any cognitive measure.

Before testing for interactions between feeding group and diet for the significant cognitive outcomes, we examined baseline characteristics of the 2 feeding groups and found significant differences in gestational age, birth weight, duration of ventilation, mater-

TABLE 6 Comparison of Subjects Who Received Some Breast Milk With Those Who Were Entirely Formula-Fed, According to Diet Group

	Not Breastfed			Breastfed		
	Control (*n* = 23)	Supplemented (*n* = 16)	*P*	Control (*n* = 34)	Supplemented (*n* = 34)	*P*
Birth weight, mean ± SD, g	1684 ± 238	1545 ± 355	.15	1396 ± 373	1411 ± 373	.86
Gestational age, median (range), wk	31.7 (29–34)	31.3 (27–34)	.47	30.4 (27–34)	30.4 (26–33)	.87
Duration of ventilation, median (range), d	0.5 (0–4)	2.5 (0–7)	.19	3.9 (0–17)	3.9 (0–25)	.28
Proportion of enteral intake as maternal breast milk, %	NA	NA		28	29	
Social class 1 or 2, *n* (%)[a]	1 (0.04)	6 (38)	.01	6 (18)	2 (35)	.17
Mother has higher vocational qualification/degree, *n* (%)	0 (0)	0 (0)		1 (0.03)	3 (9)	.28

NA indicates not applicable.
[a] Highest 2 categories (of 7).

TABLE 7 Comparisons of 2 Formula Groups in Cognitive Measures, Showing Significant Interactions With Diet

	Score, Mean ± SD					
	Not Breastfed			Breastfed		
	Control (*n* = 23)	Supplemented (*n* = 16)	*P*	Control (*n* = 34)	Supplemented (*n* = 34)	*P*
VIQ	86.3 ± 7.8	98.2 ± 13.3	.003 (3.1)[a]	97.6 ± 13.6	96.9 ± 13.2	NS
FSIQ	87.6 ± 9.8	97.0 ± 11.1	.02 (3.3)[a]	97.0 ± 12.7	94.8 ± 13.4	NS
Vocabulary score	37.9 ± 6.4	45.3 ± 9.7	0.007	NA		
Similarities score	43.3 ± 8.8	49.5 ± 7.7	0.03	NA		
Block design score	45.7 ± 9.1	47.1 ± 5.8	0.57	NA		
Matrix reasoning score	44.1 ± 10.8	47.8 ± 8.8	0.27	NA		
Word-pair learning score	10.3 ± 2.2	13.1 ± 3.6	.004 (0.09)[a]	12.2 ± 4.2	11.5 ± 3.1	NS

NS indicates not significant; NA, not applicable.
[a] SE of the difference.

TABLE 8 Scores for 2 Cognitive Outcomes Showing Effects of Gender

	Score, Mean ± SD					
	Boys			Girls		
	Control ($n = 23$)	Supplemented ($n = 16$)	*P*	Control ($n = 34$)	Supplemented ($n = 34$)	*P*
Word reading score	94.2 ± 11.1	91.3 ± 17.2	NS	90.0 ± 13.5	97.6 ± 15.9	.07 (3.9)[a]
Spelling score	92.4 ± 11.4	90.0 ± 14.9	NS	89.0 ± 13.9	97.2 ± 12.8	.02 (3.6)[a]

NS indicates not significant.
[a] SE of the difference.

nal education, and social class. Two-way analyses of variance, with those factors as covariates, showed significant interactions between diet and feeding group for VIQ scores ($P = .002$), FSIQ scores ($P = .04$), and CMS word-pair learning scaled scores ($P = .05$).

Planned Supplementary Analyses According to Gender

In planned analyses according to gender for all cognitive outcomes, significant differences between diet groups were seen only for girls and only for 2 measures of academic attainment from the Wechsler Individual Achievement Test, 1 at a trend level (Table 8). Girls who received supplemented formula had mean scores that were ≥0.5 SD higher than those of the control group; no effects of diet were seen for boys. The gender difference was supported by interactions between gender and diet for both word reading ($P = .07$) and spelling ($P = .02$).

Explanatory Analyses

We compared the 2 groups of girls with respect to a range of possible confounding factors (birth weight, gestational age, social code, maternal age, maternal education, paternal education, Apgar score at 5 minutes, time in the hospital, and duration of ventilation), because this was a nonrandomized comparison; they differed significantly only in the duration of ventilation (median: control formula: 0.00 days; supplemented formula: 2.0 days; $P = .01$). This difference should have minimized the dietary effect, because cognitive scores would be expected to be lower in the group that required more ventilation, and, after adjustment for duration of ventilation, the significance of the dietary effects was increased (word reading: $P = .02$; spelling: $P = .02$).

DISCUSSION

This study is the first, to our knowledge, to investigate the effects of early administration of LCPUFAs on cognition in later childhood, (after 9 years of age), by using an extensive assessment battery to test for specific or subtle effects. As at 18 months,[13] we found no overall difference in cognitive outcomes between randomly assigned groups. Planned subgroup analyses, however, demonstrated significant advantages for girls who received LCPUFA supplementation with respect to 2 indices of literacy (word reading and spelling), as well as providing some evidence that the cognitive benefits of breast milk versus formula feeding are related to the LCPUFA content of breast milk, which has been a matter of debate.[6,7,26]

For 10-year-old girls, supplementation was associated with improved performance in single-word reading accuracy and spelling. No other cognitive measures differentiated the groups, but the fact that these are 2 separate measures of a single cognitive domain adds credence to the finding. The girls who had received LCPUFA-supplemented formula had scores on both tests that were ~0.5 SD higher than the scores for girls who had received control formula; these substantial increases would be meaningful in educational terms. LCPUFAs have been associated with literacy in the past. A LCPUFA intervention trial involving adolescents with dyslexia reported improvement in reading-related tasks after 5 months, but there was no control group for proper evaluation of the changes.[27] More convincing was a study that showed significant correlations between blood levels of ω-3 fatty acids and measures of word reading and spelling for groups of adults with dyslexia and for normal readers.[28] A randomized controlled trial investigating the effects of supplementation for school-aged children with dyspraxia reported that 12 weeks of supplementation with LCPUFAs resulted in significant improvement for the test group in measures of reading and spelling.[29] These studies add weight to the view that the effects on literacy that were found in our study were not attributable to chance but were predictable on the basis of previous literature findings. None of the foregoing studies conducted gender analyses.

The demonstration of gender effects concurs with a growing body of literature describing differences between the genders in brain structure and function,[30–32] the relationship between the brain and cognitive function,[33,34] and the vulnerability of the brain to diverse external influences, such as hypoxic risk,[35] prenatal stress,[36] and several aspects of nutrition.[37,38] There also have been reports of differences in fatty acid metabolism between adult men and women.[39] The positive effects of LCPUFA supplementation on literacy for girls in the present study, however, contrast with the finding of an advantage in BSID-II scores for boys at 18 months.[13] The genders may show differences in susceptibility to the effects

of early LCPUFA supplementation at different time points because the developmental trajectories of their brains differ.[40] Alternatively, because literacy could not be tested at the 18-month follow-up assessment, any intrinsic sensitivity to the intervention in the literacy domain for girls would not have been detectable until the present study. Makrides et al[41] reported that preterm girls, but not boys, born to mothers who had received DHA supplementation during pregnancy showed higher BSID-II Mental Developmental Index scores, compared with those born to mothers who had not received supplementation. It would be interesting to test the literacy skills of those children in later childhood.

Systematic reviews showing the absence of an effect of LCPUFAs on cognition among term or preterm infants might be taken to imply that the cognitive benefits of breast milk versus formula feeding, as shown in numerous studies, could not have been attributable to the presence of LCPUFAs in breast milk. Our study provides preliminary data that challenge this conclusion and illustrate the importance of defining the groups carefully. Sixty-four percent of the participants who took part in the study had received some breast milk in their early diet, which reflects the prevalence of mixed feeding regimens in the United Kingdom. Randomization ensured that our supplement and control groups had received equivalent proportions of breast milk; a comparison of those groups indicated that LCPUFA supplementation had not affected cognition. When we separated the subjects into those who had received formula as the sole diet and those who had received a mixed diet, however, positive effects of LCPUFAs on cognitive outcomes emerged, which indicated that the effects of LCPUFAs were blunted by the inclusion of mixed feeding. There were significant interactions between LCPUFA supplementation and breast milk feeding status for several important cognitive measures. The implication is that some factors promoted cognitive development even in the breastfed control group, whose only source of LCPUFAs was breast milk.

The evidence that a key factor in breast milk promoting cognitive development might be LCPUFAs comes from the nonrandomized analysis, which showed that both diet groups that received breast milk had VIQ scores of ~97 to ~98 (Table 7). Among the 2 nonbreastfed groups, the group fed the control formula had a mean VIQ of only 86, whereas the group fed formula with LCPUFAs had the same VIQ as the breastfed groups (mean: 98), which indicates that it was the addition of LCPUFAs to formula that seemed to correct fully the VIQ deficit of the control formula-fed group, compared with the breastfed groups. The same pattern was found for FSIQ. We emphasize that the sample size was small and that this was an ad hoc statistical exploration. The difference in DHA contents between the supplement formula (0.5%) and breast milk (~0.3%) also must be kept in mind. Nevertheless, our data emphasize the need for further analyses of data at this age, to test this hypothesis.

The significant difference found for the word pairs learning test, which is a memory measure that involves associative learning, is consistent with the observation that LCPUFAs are capable of improving long-term potentiation in the hippocampus.[42] The pattern of scaled scores between the groups and conditions was the same as that for IQ scores. It often is assumed that VIQ scores are particularly vulnerable to the influences of social class and parental education, but these factors were included as covariates with little effect on the results for IQ and word pairs learning. We did not control for maternal intelligence[43] but, in a study examining IQ differences among monozygotic twins,[44] the effects of fetal undernutrition were seen selectively for VIQ with a design in which parents' IQ scores, level of education, and social class were controlled.

Fewtrell et al[45] addressed the issue of cohort attrition at some length, as summarized here. We achieved a follow-up rate of 45%, which increased to 54% among those who were seen at 18 months. Reduced numbers might, of course, affect the power of the study to detect group differences. The projected size of 64 subjects per group would allow the detection of a 0.5-SD difference in outcome measures; the present sample size of 107 subjects allows detection of a 0.6-SD group difference with 80% power at 5% significance. Group differences for all measures rarely exceeded 0.3 SD. Attrition also might introduce bias, especially if the 2 groups at the follow-up assessment differed with respect to some characteristics. We looked for this in each comparison; although few such characteristics were found, all were adjusted for in the relevant analyses. It would seem safe, therefore, to generalize these results to the appropriate population (ie, preterm infants born 10 years ago). We note again, however, that we are aware of the relatively small group numbers, and we urge caution in interpretation.

CONCLUSIONS

This first long-term follow-up study of cognitive function among children who, as preterm infants, took part in a randomized controlled trial of LCPUFA formula supplementation suggests that supplementation does not result in differences in cognition at the age of 10 years. It does indicate, however, that there may be gender-specific

long-term effects, with girls benefiting from enhanced literary skills. It is recommended that all subsequent studies be designed to allow gender analyses. The study also provides some preliminary evidence that cognitive development among children who are partly breastfed is not affected by LCPUA supplementation. Among children who are entirely formula-fed, however, those who do not receive LCPUFA supplementation have IQ and memory scores that are ~0.5 SD lower than the scores of both the supplemented formula-fed group and the breastfed group. These are large meaningful differences, and there is an urgent need to attempt to replicate these results in studies with larger numbers of participants. There also is a need to determine whether these findings extend to term infants.

ACKNOWLEDGMENTS

This study was funded as part of the European Union Early Nutrition Programming Project, as part of the Sixth Framework Program (grant FP6-FOOD-2005-007036). Funding for the original study and trial formulas was provided by Heinz UK.

REFERENCES

1. Carlson SE, Neuringer M. Polyunsaturated fatty acid status and neurodevelopment: a summary and critical analysis of the literature. *Lipids*. 1999;34(2):171–178
2. Hoffman DR, Boettcher JA, Dierson-Schade DA. Toward optimizing vision and cognition in term infants by dietary docosahexaenoic and arachidonic acid supplementation: a review of randomized controlled trials. *Prostaglandins Leukot Essent Fatty Acids*. 2009;81(2–3):151–158
3. Fernstrom JD. Effects of dietary polyunsaturated fatty acids on neuronal function. *Lipids*. 1999;34(2):161–169
4. Eilander A, Hundscheid DC, Osendarp SJ, Transler C, Zock PL. Effects of *n*-3 long chain polyunsaturated fatty acid supplementation on visual and cognitive development throughout childhood: a review of human studies. *Prostaglandins Leukot Essent Fatty Acids*. 2007;76(4):189–203
5. Jacobson SW. Assessment of long-chain polyunsaturated fatty acid nutritional supplementation on infant neurobehavioral development and visual acuity. *Lipids*. 1999; 34(2):151–160
6. Gibson RA, Makrides M. Polyunsaturated fatty acids and infant visual development: a summary of the visual development literature and critical appraisal of randomized clinical trials. *Lipids*. 1999;34(2):179–184
7. Simmer K, Patole SK, Rao SC. Longchain polyunsaturated fatty acid supplementation in infants born at term (Review). *The Cochrane Collaboration*. 2008;(1):John Wiley & Sons
8. Simmer K, Schulzke SM, Patole S. Longchain polyunsaturated fatty acid supplementation in preterm infants. *The Cochrane Collaboration*. 2008;(1):John Wiley & Sons
9. Bayley N. *Bayley Scales of Infant Development: Manual*. 2nd ed. San Antonio, TX: Psychological Corp; 1993
10. Cheatham CL, Colombo J, Carlson SE. *n*-3 fatty acids and cognitive and visual acuity development: methodologic and conceptual considerations. *Am J Clin Nutr*. 2006;(6): 1458S–1466S
11. Willatts P, Forsyth JS, DiMadugno MK, Varma S, Colvin M. Effect of long-chain polyunsaturated fatty acids on infant cognitive function. *Lipids*. 1998;33(10):973–980
12. Hadders-Algra M. Effect of long-chain polyunsaturated fatty acid supplementation on neurodevelopmental outcome in full-term infants. *Nutrients*. 2010;2(8):790–804
13. Fewtrell MS, Abbott RA, Kennedy K, et al. Randomized double-blind trial of long-chain polyunsaturated fatty acid supplementation with fish oil and borage oil in preterm infants. *J Pediatr*. 2004;144(4):471–479
14. Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher J, Arterburn LAM. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. *Am J Clin Nutr*. 2007;85(6): 1457–1464
15. Kennedy K, Ross S, Isaacs EB, et al. The 10-year follow-up of a randomised trial of long-chain polyunsaturated fatty acid supplementation in preterm infants: effects on growth and blood pressure. *Arch Dis Child*. 2010;95(8):588–595
16. Wechsler D. *Wechsler Abbreviated Scale of Intelligence Manual*. San Antonio, TX: Harcourt Brace; 1999
17. Korkman M, Kirk U, Kemp S. *NEPSY*. San Antonio, TX: Psychological Corp; 1998
18. Ryan AS, Astwood JD, Gautier S, Kuratko CN, Nelson EB, Salem N Jr. Effects of long-chain polyunsaturated fatty acid supplementation on neurodevelopment in childhood: a review of human studies. *Prostaglandins Leukot Essent Fatty Acids*. 2010;82(4–6): 305–314
19. Shastri L. Episodic memory and cortico-hippocampal interactions. *Trends Cogn Sci*. 2002;6(4):162–168
20. Cohen MJ. *CMS: Children's Memory Scale*. San Antonio, TX: Psychological Corp; 1997
21. Wechsler D. *Wechsler Individual Achievement Test*. 2nd UK ed. London, England: Pearson; 2005
22. Manly T, Anderson V, Robertson I, Nimmo-Smith I. *The Test of Everyday Attention for Children*. London, England: Thames Valley Test Co; 1999
23. Emslie H, Wilson FC, Burden V, Nimmo-Smith I, Wilson BA. *Behavioural Assessment of the Dysexecutive Syndrome for Children (BADS-C)*. Bury St Edmunds, England: Thames Valley Test Co; 2003
24. Tanaka K, Kon N, Ohkawa N, Yoshikawa N, Shimizu T. Does breastfeeding in the neonatal period influence the cognitive function of very-low-birth-weight infants at 5 years of age? *Brain Dev*. 2009;31(4):288–293
25. Birberg-Thornberg U, Karlsson T, Gustafsson PA, Duchén K. Nutrition and theory of mind: the role of polyunsaturated fatty acids (PUFA) in the development of theory of mind. *Prostaglandins Leukot Essent Fatty Acids*. 2006;75(1):33–41
26. Innis SM, Gilley J, Werker J. Are human milk long-chain polyunsaturated fatty acids related to visual and neural development in breast-fed term infants? *J Pediatr*. 2001; 139(4):532–538
27. Lindmark L, Clough P. A 5-month open study with long-chain polyunsaturated fatty acids in dyslexia. *J Med Food*. 2007;10(4):662–666
28. Cyhlarova E, Bell JG, Dick JR, Mackinlay EE, Stein JF, Richardson AJ. Membrane fatty acids, reading and spelling in dyslexic and non-dyslexic adults. *Eur Neuropsychopharmacol*. 2007;17(2):116–121
29. Richardson AJ, Montgomery P. The Oxford-Durham study: a randomized, controlled trial of dietary supplementation with fatty acids in children with developmental coordination disorder. *Pediatrics*. 2005;115(5): 1360–1366
30. Reiss AL, Kesler SR, Vohr B, et al. Sex differences in cerebral volumes of 8-year-olds born preterm. *J Pediatr*. 2004;145(2): 242–249
31. Luders E, Narr KL, Thompson PM, et al. Gen-

der differences in cortical complexity. *Nat Neurosci*. 2004;7(8):799–800

32. Blanton RE, Levitt JG, Peterson JR, et al. Gender differences in the left inferior frontal gyrus in normal children. *Neuroimage*. 2004;22(2):626–636
33. Gur RC, Turetsky BJ, Matsui M, et al. Sex differences in brain gray and white matter in healthy young adults: correlations with cognitive performance. *J Neurosci*. 1999; 19(10):4065–4072
34. Schmithorst VJ, Holland SK. Functional MRI evidence for disparate developmental processes underlying intelligence in boys and girls. *Neuroimage*. 2006;31(3):1366–1379
35. Lauterbach MD, Raz S, Sander CJ. Neonatal hypoxic risk in preterm birth infants: the influence of sex and severity of respiratory distress on cognitive recovery. *Neuropsychology*. 2001;15(3):411–420
36. Weinstock M. Gender differences in the effects of prenatal stress on brain development and behaviour. *Neurochem Res*. 2007; 32(10):1730–1740
37. Isaacs EB, Gadian DG, Sabatini S, et al. The effect of early human diet on caudate volumes and IQ. *Pediatr Res*. 2008;63(3):308–314
38. Isaacs EB, Fischl BR, Quinn BT, Chong WK, Gadian DG, Lucas A. Impact of breast milk on intelligence quotient, brain size, and white matter development. *Pediatr Res*. 2010; 67(4):357–362
39. Burdge GC, Calder PC. α-Linolenic acid metabolism in adult humans: the effects of gender and age on conversion to longer-chain polyunsaturated fatty acids. *Eur J Lipid Sci Technol*. 2005;107(6):426–439
40. Asato MR, Terwilliger R, Woo J, et al. White matter development in adolescence: a DTI study. *Cereb Cortex*. 2010;20(9):2122–2131
41. Makrides M, Gibson RA, McPhee A, et al. Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid. *JAMA*. 2009;301(2):175–182
42. Yamashima T. A putative link of PUFA, GPR40 and adult-born hippocampal neurons for memory. *Prog Neurobiol*. 2008;84(2): 105–115
43. Der G, Batty GD, Deary IJ. Effect of breast feeding on intelligence in children: prospective study, sibling pairs analysis, and meta-analysis. *BMJ*. 2006;333(7575):945–950
44. Edmonds CJ, Isaacs EB, Cole TJ, et al. The effect of intrauterine growth on verbal IQ scores in childhood: a study of monozygotic twins. *Pediatrics*. 2010;126(5). Available at: www.pediatrics.org/cgi/content/full/126/5/e1095
45. Fewtrell MS, Kennedy K, Singhal A, et al. How much loss to follow-up is acceptable in long-term randomised trials and prospective studies? *Arch Dis Child*. 2008; 93(6):458–461

10-year Cognition in Preterms After Random Assignment to Fatty Acid Supplementation in Infancy

Elizabeth B. Isaacs, Sarah Ross, Kathy Kennedy, Lawrence T. Weaver, Alan Lucas and Mary S. Fewtrell

Pediatrics 2011;128;e890; originally published online September 19, 2011; DOI: 10.1542/peds.2010-3153