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Breastfeeding, the use of docosahexaenoic acid–fortified formulas in infancy and neuropsychological function in childhood

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Objective: To investigate the relation between breastfeeding, use of docosahexaenoic acid (DHA)-fortified formula and neuropsychological function in children.

Design: Prospective cohort study.

Setting: Southampton, UK.

Subjects: 241 children aged 4 years followed up from birth.

Main outcome measures: IQ measured by the Wechsler Pre-School and Primary Scale of Intelligence (3rd edition), visual attention, visuomotor precision, sentence repetition and verbal fluency measured by the NEPSY, and visual form-constancy measured by the Test of Visual-Perceptual Skills (Non-Motor).

Results: In unadjusted analyses, children for whom breast milk or DHA-fortified formula was the main method of feeding throughout the first 6 months of life had higher mean full-scale and verbal IQ scores at age 4 years than those fed mainly unfortified formula. After adjustment for potential confounding factors, particularly maternal IQ and educational attainment, the differences in IQ between children in the breastmilk and unfortified formula groups were severely attenuated, but children who were fed DHA-fortified formula had full-scale and verbal IQ scores that were respectively 5.62 (0.98, 10.2) and 7.02 (1.56, 12.4) points higher than children fed unfortified formula. However, estimated total intake of DHA in milk up to age 6 months was not associated with subsequent IQ or with score on any other test.

Conclusions: Differences in children's intelligence according to type of milk fed in infancy may be due more to confounding by maternal or family characteristics than to the amount of long-chain polyunsaturated fatty acids they receive in milk.

Keywords: breastfeeding, infant formula, long-chain polyunsaturated fatty acids, infancy, intelligence, neuropsychology

Introduction

There has been considerable interest in the role that long-chain polyunsaturated fatty acids (LCPUFAs) might play in neurodevelopment. LCPUFAs, particularly the n-3 docosahexaenoic acid (DHA) and the n-6 arachidonic acid (AA), are found in high concentrations in the brain and retina,^{1,2} and accumulate during the spurt in brain growth that occurs between the last trimester of pregnancy and the first year of life.^{3,4} LCPUFAs, especially DHA, are involved in cell signalling, regulation of gene expression and neuronal growth.⁵⁻⁷ Animal studies suggest that reductions in DHA accrual may lead to neurocognitive deficits.^{7,8}

Much of the evidence for the importance of LCPUFAs has come from observational studies comparing cognition in children who were breast-fed with those fed an infant milk formula or those breast-fed for different durations. Most studies have found that breastfeeding is associated with better cognition, but few adjusted for the confounding effect of maternal intelligence.⁹ Intelligence is heritable¹⁰ and women of higher intelligence are more likely to initiate breastfeeding and continue it for longer.¹¹

Randomized controlled trials into the effects of LCPUFA-supplemented formula on cognitive or visual function have produced inconsistent findings.^{8,12-14} Most trials have been carried out in babies or very young children. Whether such formulas are associated with longer-term benefits in neurocognitive performance is unclear.

We investigated the relation between breastfeeding, use of LCPUFA-fortified formula in infancy and neuropsychological function in four-year-old children, controlling for the influence of maternal intelligence and other potential confounding factors.

Methods

The Southampton Women's Survey

The Southampton Women's Survey (SWS) is a study of a population sample of non-pregnant women aged 20 to 34 years, in Southampton, UK. Details of recruitment to the study and the representativeness of the sample have been described in detail previously.¹⁵ In brief, between 1998 and 2002 all general practitioners in Southampton were asked to help us recruit their female patients aged 20-34 years to the study. The study was publicized locally and we recruited women not registered with GPs or who were registered with an incorrect address by approaching women at local events and in supermarkets in the city. Of those women contacted about the study, 12 583 (75%) agreed to participate. The initial study and follow-ups were approved by Southampton and South West Hampshire Local Research Ethics Committee. Women gave written informed consent.

Dietary assessment

Children born to SWS women were followed-up at 6 months when a milk feeding history since birth was recorded. Mothers reported type of milk feeding (including brand of formula) and duration. At the time we carried out our study there were 12 infant formulas on the market that contained added DHA, with concentrations ranging

from 6.8 to 18mg/100ml of formula. For each baby we calculated the total number of days to age 6 months that he/she was fed breastmilk, DHA-fortified formula or unfortified formula. We used k-means cluster analysis¹⁶ to identify groups that were homogeneous in the length of time they had received each type of milk. This produced 3 clusters.

To obtain an estimate of DHA exposure up to 6 months, we used average milk volumes¹⁷ for each month of feeding together with the DHA content of the formula milk fed (obtained from the manufacturers); we assumed breast milk contained 9.56 mg of DHA per 100g, based on a recent estimate of the DHA content of breast milk in the UK.¹⁸

Neuropsychological assessment

At age 4 years, children's IQ was assessed at home using the Wechsler Pre-School and Primary Scale of Intelligence (3rd edition; WPPSI-III UK).¹⁹ We assessed attention, sensorimotor ability, memory and language using the subtests visual attention, visuomotor precision, sentence repetition and verbal fluency from the Developmental Neuropsychological Assessment (NEPSY).²⁰ We assessed visual perception abilities separate from motor skills using the visual form-constancy subtest of the Test of Visual-Perceptual Skills (Non-Motor).²¹

Maternal and child characteristics

Details of mother's age, number of children, educational attainment, receipt of means-tested benefits, and her and her partner's current occupation were obtained at the pre-pregnant interview. Occupational social class was defined according to the highest

social class of the mother or her partner.²² At birth, the child was weighed using digital scales. During the 4-year visit, maternal intelligence was assessed with the Wechsler Abbreviated Scale of Intelligence.²³ The quality of the child's environment was assessed using the short form of the Home Observation for Measurement of the Environment scale (HOME-SF).²⁴

Participants

There were 1981 singleton live births to women in the SWS up to the end of 2003. After exclusion of infants with major congenital abnormalities and neonatal deaths, 1973 infants were available for follow-up. Of these, 1645 (83%) were visited at 6 months and 1618 (82%) at 12 months for dietary assessment. Children with complete dietary data were invited to participate in studies at 4 years to assess bone mass, body composition and cognitive function. For the cognitive function study, we aimed to recruit around 400 children. Power calculations – using observations from a previous study that the mean (SD) IQ of breastfed children were 104 (13) and that of formula fed children were 99 (15) - suggested that this would give us >90% power to detect a 5-point difference in IQ between milk-feeding groups in infancy. As children reached their 4th birthday, their mothers were invited to bring them for a DXA scan at the Southampton Osteoporosis Centre. During this visit they were given an information sheet about the cognitive function study. Psychologists from the research team subsequently contacted the mothers by phone and invited them to take part in the study. During the three years for which the study was funded, 396 mothers were contacted. Of these, 268 (68%) participated. Analyses are based on 241 children with complete data.

Compared to the rest of the SWS cohort with children born in 1999-2003 who did not take part in this study, the mothers who participated tended to have a higher level of educational attainment ($p=0.05$), were of higher social class ($p=0.01$) and had breastfed their baby for longer ($p<0.001$).

Statistical analysis

We used *t*-tests, the Mann-Whitney test, analysis of variance, χ^2 tests and correlation coefficients to examine characteristics. We used linear regression to examine differences in scores in the breastfeeding and fortified-formula feeding groups compared to the unfortified-formula group, and differences in test scores according to total estimated dose of DHA up to age 6 months.

Results

There were 130 children in the breastmilk group (53.9%), 65 in the fortified-formula group (27%) and 46 in the unfortified-formula group (19.1%). Up to age 6 months, the breastmilk group had been fed breastmilk for a mean (SD) of 143.4 (30.4) days, the fortified-formula group had been fed fortified formula for 146.5 (34.4) days, and the unfortified-formula group had been fed unfortified formula for 147.3 (30.0) days. Mean (SD) total estimated DHA intake from milk between birth and age 6 months in the 3 groups was, respectively, 12.1 (1.88), 13.0 (5.6) and 2.14 (1.98) grams ($p<0.001$).

Table 1 shows characteristics according to feeding group. The proportions of mothers with A levels or a degree or from social classes I or II were highest in the breastmilk group and lowest in the unfortified-formula group. Mean IQ and mean age at birth were highest in the breastmilk group, lowest in the unfortified-formula group, and intermediate in the fortified-formula group. Mothers in the breastmilk group were less likely to have received means-tested benefits than those in the formula groups. There were no differences between the groups in scores on the HOME cognitive stimulation or emotional support scales. There was a gradient in birthweight across feeding groups, but sex, birth order or gestational age was not associated with feeding group. Only characteristics that were significantly associated with infant feeding group were used as potential confounding factors in subsequent analyses.

Table 2 shows regression coefficients for differences in IQ between children in the breastmilk or fortified-formula groups compared to those in the unfortified-formula group. In unadjusted analysis, full-scale and verbal IQ scores were higher in children in the breastmilk and fortified-formula groups compared to those in the unfortified formula group. There were no differences in performance IQ. Adjustment for maternal education or intelligence attenuated the differences in full-scale and verbal IQ between the breastfed and the unfortified-formula groups such that they ceased to be statistically significant. Control for potential confounding factors had less effect on the differences in full-scale and verbal IQ scores between children in the fortified- and unfortified formula groups. Adjustment for maternal education or intelligence diminished the differences slightly, while adjustment for social class and other covariates had little or no attenuating effect. In fully adjusted models, while the

differences in full-scale and verbal IQ scores between the breastfed and unfortified-formula groups were not significant, the differences between the fortified- and unfortified-formula groups persisted.

Table 3 shows differences in the other test scores between children in the breastmilk or fortified-formula groups compared to those in the unfortified-formula group. There were no statistically significant differences in scores on any of these tests.

Total estimated DHA intake in milk up to age 6 months ranged from 0 to 23.8 grams. Mothers of children with higher DHA intakes tended to have a higher IQ ($r=0.28$, $p<0.001$), be better educated ($r_s=0.28$, $p<0.001$) and of higher social class ($r_s=-0.16$, $p=0.013$). Table 4 shows change in test score for a one-gram increase in total DHA intake. In unadjusted analyses, there were weak positive associations between estimated total DHA intake and scores for visual form constancy and visual attention that remained of borderline significance when adjusted for potential confounders, but in general there were no indications to link increasing total DHA intake with better test performance. We repeated these analyses restricting the sample to children in the two formula-fed groups. In unadjusted analyses there was a weak positive association between DHA intake and verbal IQ, such that verbal IQ rose by 0.37 (95% CI 0.02, 0.73) of a point for a 1-gram increase in DHA intake, but after controlling for maternal IQ or educational attainment, this ceased to be statistically significant and it was attenuated further by additional adjustment for other potential confounders. There were no associations in the formula-fed groups between DHA intake and scores for full-scale or performance IQ or any other test.

Discussion

Several trials have investigated the effect of LCPUFA-fortified formula on neurodevelopment. In 276 term-born children followed up at 39 months, no differences were found in IQ, visual-motor skills or visual acuity between children randomised to LCPUFA-fortified formula in infancy, those randomised to unfortified formula and a breastfed comparison group.²⁵ A trial of 79 term-born children found lower verbal IQ at age 4 years in children randomised to DHA-fortified or unfortified formula in infancy compared to a breastfed reference group, but there was no adjustment for potential confounding factors associated with the decision to breastfeed, and no evidence of a difference in IQ between the fortified and unfortified formula groups.²⁶ Term-born babies randomised to a LCPUFA-fortified formula showed a reduced occurrence of abnormal movements at 3 months in comparison to those receiving a control formula in a trial of 472 infants,²⁷ but there was no evidence of improved neurodevelopment at 18 months according to scores on Bayley Scales.²⁸ In 238 pre-term infants, LCPUFA-fortified formula was associated with higher scores on the Mental Development Index of the Bayley Scales at 18 months, but only in boys; overall there were no differences in neurodevelopment between the formula groups.²⁹ In a similar trial in 179 pre-term infants, LCPUFA-fortified formula was linked with significantly higher Bayley Scales scores at 18 months.³⁰ One trial examined the effect of supplementing breastfeeding women with DHA and found that the children of supplemented women scored significantly higher than those of a control group on the Psychomotor Development Index of the Bayley Scales at age 30 months, though there was no difference in score on the Mental Development Index.³¹

In this study, where neurodevelopment was assessed at a later age than in most of the above trials, we found a difference in mean full-scale and verbal IQ between children who had been fed fortified or unfortified formula that persisted after adjustment. Viewed in isolation, this might suggest that the LCPUFA content of fortified formula had benefited these children's cognitive development. However, the lack of any trend between estimated total intake of DHA up to age 6 months and subsequent IQ makes this unlikely. While this might reflect inaccuracies in our estimation of DHA intake, our results suggest that the apparent IQ advantage associated with fortified formula may be due less to the LCPUFA content of these milks, than to unmeasured factors in the home environment that influence the choice of fortified versus unfortified formula. The fact that fortified formula was linked with differences in verbal but not performance IQ provides further support for this explanation.

Although many observational studies have found that children who were breastfed gain higher scores on tests of intelligence, our findings suggest that this association is largely due to confounding. Adjustment for maternal education or intelligence severely attenuated the association; no other covariate had such an effect. These results are consistent with findings in the children of members of the National Survey of Health and Development, where adjustment for maternal cognition or education removed the positive association between IQ and being breastfed.³² Similarly, in the US National Longitudinal Survey of Youth, breastfeeding had little or no effect on intelligence compared to bottle feeding after controlling for maternal IQ.³³ Longer duration of breastfeeding has been linked with higher intelligence in the offspring in many studies.^{34,35} Our finding that total intake of DHA in milk up to age 6 months

was not linked with subsequent intelligence provides a further indication that the association between breastmilk and cognitive development may not be due to the LCPUFA content of the milk.

Evidence suggesting a role for breastfeeding in cognitive development has recently come from a trial of breastfeeding promotion. Children in the experimental group had higher mean verbal IQ scores at age 6 compared with the control group,³⁶ but other differences in intelligence or in teacher ratings were small and often non-significant. It is possible that women who were more intelligent or better educated, and hence had more verbally competent children, were more receptive to breast feeding promotion.

The strengths of this study include the detailed milk feeding histories collected during infancy, the comprehensive psychological assessment of the children at age four years, and the availability of data on a range of potential confounding factors, including maternal intelligence and quality of the home environment.

The study also has limitations. Firstly, compared to women who did not take part, the women who agreed to participate in this follow-up study were better educated, of higher social class, and a higher proportion had breastfed their child. Compared to the entire SWS birth cohort, women from more disadvantaged socioeconomic groups and those who fed their child primarily on formula during infancy are therefore under-represented in this study. However, it is unlikely that this will have biased our findings unless the relation between type of milk fed and child's neurocognitive function is different in those who did not take part in our study. Secondly, our calculation of total DHA intake in milk is likely to contain inaccuracies: it is

dependent on maternal recall of the brands of formula used, and in the absence of data on the actual DHA content of each mother's breast milk and on the volume of milk drunk, we had to base our calculations on recent estimates of the average DHA content of breast milk in the UK and of the amount of milk drunk in the first few months of life. This might have affected our ability to detect any relation between DHA intake and later performance. Finally, the number on which our study was based was smaller than originally planned, so we may have lacked statistical power to detect differences in scores on some of the tests. A post-hoc power calculation showed that our study had >90% power to detect differences in verbal IQ between milk feeding groups of the size found here in unadjusted analyses, and >70% power in the case of full-scale IQ.

In this study, we found that differences in IQ between children who were breastfed and those fed unfortified formula in infancy were largely explained by maternal educational attainment and intelligence. Children fed DHA-enriched formula did have higher full-scale and verbal IQ than those fed unfortified formula, despite control for a range of potential confounders, but this did not appear to be due to the DHA content of these milks.

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collection, analysis or interpretation of data, in the writing of the report or in the decision to submit the manuscript for publication.

Contributors

SMR, CRG and CNM conceived the idea for the study. CRG and SMR are guarantors. CRG, SMR, CNM, HMI, KMG, CML, CC, and JL planned the study, oversaw data collection and interpreted the results. CW carried out the cognitive and neuropsychological testing. LDM and SMR derived the data on fatty acid intake in milk. CRG carried out the statistical analyses and wrote the first draft of the manuscript. All authors subsequently revised the manuscript.

Competing interests

None declared.

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What is already known on this topic

Studies in animals show that lack of the long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA) during periods of rapid brain growth may lead to impaired neurodevelopment, but trials of the effect of DHA-fortified formula in babies have produced conflicting results.

Whether the use of such formulas is associated with longer-term benefits in children's cognitive or neuropsychological performance is unclear.

What this study adds

Children fed predominantly with DHA-fortified formula in infancy had higher full-scale and verbal IQ scores at age 4 years than those fed with unfortified formula, but these differences were not explained by higher intakes of DHA in infancy.

Differences in children's intelligence according to type of milk fed in infancy may be due more to confounding by maternal or family characteristics than to the amount of long-chain polyunsaturated fatty acids they receive in milk

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Table 1: Characteristics of the study participants according to milk-feeding group

Characteristics	Breastmilk n=130	Fortified formula n=65	Unfortified formula n=46	P value
	Mean (SD) or No (%)	Mean (SD) or No (%)	Mean (SD) or No (%)	
<i>Mother</i>				
Educated to A level or higher, no (%)	97 (74.6)	35 (53.9)	17 (37.0)	<0.001
Social class I or II, no (%)	94 (72.3)	31 (47.7)	20 (43.5)	<0.001
On benefits, no (%)	8 (6.2)	11 (16.9)	8 (17.4)	0.027
Full-scale IQ	111.7 (10.9)	107.0 (9.3)	100.9 (11.0)	<0.001
Age at birth, yr	30.8 (3.3)	30.3 (3.5)	29.2 (3.7)	0.007
HOME cognitive stimulation	9.09 (1.18)	8.97 (1.16)	9.00 (1.07)	0.543
HOME emotional support	2.19 (0.96)	1.92 (0.91)	2.22 (0.92)	0.733
Returned to work before child's 1 st birthday, no (%)	78 (60.0)	35 (53.9)	30 (65.2)	0.474
<i>Child</i>				
Female, no (%)	61 (46.9)	33 (50.8)	17 (37.0)	0.341
First or only child, no (%)	60 (46.2)	39 (60.0)	22 (47.8)	0.178
Birthweight, kg	3.51 (0.48)	3.37 (0.68)	3.35 (0.47)	0.040
Gestational age, wks	39.8 (1.66)	39.5 (2.41)	39.7 (1.70)	0.435

Table 2: Differences in IQ scores at age 4 between the breastmilk or DHA-fortified formula groups compared to the unfortified formula group. Estimates are shown unadjusted, adjusted separately for each potential confounder, then fully adjusted

Feeding group		Regression coefficient (95% CI)		
		Full scale IQ	Verbal IQ	Performance IQ
Breastmilk	Unadjusted	5.29 (1.16, 9.43)	7.61 (2.71, 12.5)	2.25 (-2.33, 6.84)
	<i>Adjusted for:</i>			
	Maternal IQ	3.70 (-0.71, 8.10)	4.82 (-0.35, 9.98)	1.59 (-3.33, 6.50)
	Education	2.59 (-1.72, 6.91)	3.95 (-1.12, 9.02)	0.82 (-4.05, 5.69)
	Social class	4.15 (0.1, 8.30)	6.41 (1.47, 11.3)	1.14 (-3.47, 5.76)
	On benefits	4.85 (0.69, 9.00)	6.91 (2.00, 11.8)	1.82 (-2.80, 6.43)
	Age at birth	5.71 (1.50, 9.91)	8.32 (3.35, 13.3)	2.23 (-2.44, 6.90)
	Birthweight	4.85 (0.71, 8.99)	7.46 (2.52, 12.4)	1.37 (-3.15, 5.89)
	All	2.51 (-1.97, 6.98)	3.66 (-1.66, 8.92)	0.29 (-4.69, 5.27)
	Fortified formula	Unadjusted	6.52 (1.88, 11.1)	8.57 (3.07, 14.1)
<i>Adjusted for:</i>				
Maternal IQ		5.63 (0.92, 10.3)	7.00 (1.48, 12.5)	2.93 (-2.32, 8.18)
Education		5.21 (0.62, 9.81)	6.80 (1.40, 12.2)	2.61 (-2.58, 7.81)
Social class		6.14 (1.55, 10.7)	8.16 (2.71, 13.6)	2.93 (-2.17, 8.03)
On benefits		6.51 (1.87, 11.1)	8.54 (3.08, 14.0)	3.29 (-1.85, 8.43)
Age at birth		6.82 (2.12, 11.5)	9.09 (3.56, 14.6)	3.29 (-1.91, 8.49)
Birthweight		6.46 (1.83, 11.1)	8.55 (3.04, 14.1)	3.17 (-1.87, 8.22)
All		5.62 (0.98, 10.2)	7.02 (1.56, 12.4)	2.99 (-2.18, 8.16)

Table 3: Differences in other neurocognitive test scores at age 4 between the breastmilk or DHA-fortified formula groups compared to the unfortified formula group. Estimates are shown unadjusted, then multivariate-adjusted*

Adjustments	Feeding group	Visual form constancy scaled score	Visual attention scaled score	Visuomotor precision scaled score	Sentence repetition scaled score	Verbal fluency scaled score
Unadjusted	Breast milk	0.44 (-0.46, 1.54)	0.17 (-0.26, 0.60)	0.77 (-0.25, 1.79)	0.67 (-0.16, 1.50)	-0.13 (-1.08, 0.82)
	Fortified formula	0.27 (-0.74, 1.28)	0.40 (-0.09, 0.88)	0.40 (-0.75, 1.54)	0.76 (-0.17, 1.70)	0.43 (-0.63, 1.49)
Multivariate-adjusted*	Breast milk	0.20 (-0.82, 1.21)	0.31 (-0.18, 0.79)	1.06 (-0.07, 2.18)	-0.19 (-1.09, 0.71)	-0.53 (-1.59, 0.52)
	Fortified formula	0.21 (-0.83, 1.25)	0.45 (-0.05, 0.95)	0.62 (-0.54, 1.78)	0.34 (-0.59, 1.28)	0.25 (-0.84, 1.34)

*adjusted for birth weight, maternal age, IQ, educational qualifications, social class, and receipt of benefits .

Table 4: Change in cognitive and neuropsychological test scores at age 4 for a 1-gram increase in estimated DHA intake from milk in the first 6 months of life. Estimates are shown unadjusted, then multivariate-adjusted*

Adjustments	Regression coefficient (95% CI)							
	Full-scale IQ	Verbal IQ	Performance IQ	Visual form constancy scaled score	Visual attention scaled score	Visuomotor precision scaled score	Sentence repetition scaled score	Verbal fluency scaled score
Unadjusted	0.18 (-0.12, 0.48)	0.32 (-0.03, 0.68)	-0.01 (-0.34, 0.32)	0.06 (-0.01, 0.12)	0.03 (-0.01, 0.06)	0.01 (-0.06, 0.09)	0.02 (-0.04, 0.08)	-0.01 (-0.07, 0.06)
Multivariate-adjusted*	0.06 (-0.24, 0.37)	0.14 (-0.21, 0.50)	-0.07 (-0.41, 0.27)	0.05 (-0.02, 0.12)	0.03 (-0.01, 0.06)	0.02 (-0.06, 0.10)	-0.01 (-0.07, 0.05)	-0.02 (-0.09, 0.05)

*adjusted for birth weight, maternal age, IQ, educational qualifications, social class, and receipt of benefits.