



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

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ABSTRACT

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 edn), 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 breast milk 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 to 10.2) and 7.02 (1.56 to 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.

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}

What is already known about 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.

Much of the evidence for the importance of LCPUFAs has come from observational studies comparing cognition in children who were breastfed with those fed an infant milk formula or those breastfed 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 heritable,¹⁰ and women of higher intelligence are more likely to initiate breastfeeding and continue it for longer.¹¹

Randomised 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 4-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 20–34 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 publicised 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 18 mg/100 ml of formula. For each baby, we calculated the total number of days to age 6 months that he/she was fed breast milk, 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 three 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 100 g, 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 edn; 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 five-point difference in IQ between milk-feeding groups in infancy. As children reached their fourth birthday, their mothers were invited to bring them for a dual-energy x-ray absorptiometry (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 3 years for which the study was funded, 396 mothers were contacted. Of these, 268 (68%) participated. Mothers provided written informed consent. Analyses are based on 241 children with complete data.

Compared with 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 a 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 with 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 breast milk 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 breast milk group had been fed breast milk 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. The mean (SD) total estimated DHA intake from milk between birth and age 6 months in the three groups was, respectively, 12.1 (1.88), 13.0 (5.6) and 2.14 (1.98) g ($p<0.001$).

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 breast milk group and lowest in the unfortified-formula group. The mean IQ

and mean age at birth were highest in the breast milk group, lowest in the unfortified-formula group and intermediate in the fortified-formula group. Mothers in the breast milk 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 birth weight 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 breast milk or fortified-formula groups compared with those in the unfortified-formula group.

In unadjusted analysis, full-scale and verbal IQ scores were higher in children in the breast milk and fortified-formula groups compared with 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

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

| Characteristics | Breast milk n=130 | Fortified formula n=65 | Unfortified formula n=46 | p Value |
|---|---------------------|------------------------|--------------------------|---------|
| | Mean (SD) or No (%) | Mean (SD) or No (%) | Mean (SD) or No (%) | |
| Mother | | | | |
| 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 (year) | 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 first birthday (no (%)) | 78 (60.0) | 35 (53.9) | 30 (65.2) | 0.474 |
| Child | | | | |
| 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 |
| Birth weight (kg) | 3.51 (0.48) | 3.37 (0.68) | 3.35 (0.47) | 0.040 |
| Gestational age (weeks) | 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 breast milk or DHA-fortified formula groups compared with the unfortified formula group

| Feeding group | Regression coefficient (95% CI) | | |
|--------------------------|---------------------------------|----------------------|----------------------|
| | Full scale IQ | Verbal IQ | Performance IQ |
| Breast milk | | | |
| Unadjusted | 5.29 (1.16 to 9.43) | 7.61 (2.71 to 12.5) | 2.25 (-2.33 to 6.84) |
| Adjusted for: | | | |
| Maternal IQ | 3.70 (-0.71 to 8.10) | 4.82 (-0.35 to 9.98) | 1.59 (-3.33 to 6.50) |
| Education | 2.59 (-1.72 to 6.91) | 3.95 (-1.12 to 9.02) | 0.82 (-4.05 to 5.69) |
| Social class | 4.15 (0.1 to 8.30) | 6.41 (1.47 to 11.3) | 1.14 (-3.47 to 5.76) |
| On benefits | 4.85 (0.69 to 9.00) | 6.91 (2.00 to 11.8) | 1.82 (-2.80 to 6.43) |
| Age at birth | 5.71 (1.50 to 9.91) | 8.32 (3.35 to 13.3) | 2.23 (-2.44 to 6.90) |
| Birth weight | 4.85 (0.71 to 8.99) | 7.46 (2.52 to 12.4) | 1.37 (-3.15 to 5.89) |
| All | 2.51 (-1.97 to 6.98) | 3.66 (-1.66 to 8.92) | 0.29 (-4.69 to 5.27) |
| Fortified formula | | | |
| Unadjusted | 6.52 (1.88 to 11.1) | 8.57 (3.07 to 14.1) | 3.31 (-1.84 to 8.46) |
| Adjusted for: | | | |
| Maternal IQ | 5.63 (0.92 to 10.3) | 7.00 (1.48 to 12.5) | 2.93 (-2.32 to 8.18) |
| Education | 5.21 (0.62 to 9.81) | 6.80 (1.40 to 12.2) | 2.61 (-2.58 to 7.81) |
| Social class | 6.14 (1.55 to 10.7) | 8.16 (2.71 to 13.6) | 2.93 (-2.17 to 8.03) |
| On benefits | 6.51 (1.87 to 11.1) | 8.54 (3.08 to 14.0) | 3.29 (-1.85 to 8.43) |
| Age at birth | 6.82 (2.12 to 11.5) | 9.09 (3.56 to 14.6) | 3.29 (-1.91 to 8.49) |
| Birth weight | 6.46 (1.83 to 11.1) | 8.55 (3.04 to 14.1) | 3.17 (-1.87 to 8.22) |
| All | 5.62 (0.98 to 10.2) | 7.02 (1.56 to 12.4) | 2.99 (-2.18 to 8.16) |

Estimates are shown unadjusted, adjusted separately for each potential confounder, then fully adjusted.

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 breast milk or fortified-formula groups compared with those in the unfortified-formula group. There were no statistically significant differences in scores on any of these tests.

The total estimated DHA intake in milk up to age 6 months ranged from 0 to 23.8 g. Mothers of children with higher DHA intakes tended to have a higher IQ ($r=0.28$, $p<0.001$), to be better educated ($r_s=0.28$, $p<0.001$) and to be of a higher social class ($r_s=-0.16$, $p=0.013$). Table 4 shows the change in test score for a 1 g 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 to 0.73) of a point for a 1 g 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 with 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 an LCPUFA-fortified formula showed a reduced occurrence of abnormal movements at 3 months in comparison with 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 preterm 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 preterm 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

Table 3 Differences in other neurocognitive test scores at age 4 between the breast milk or DHA-fortified formula groups compared with 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 to 1.54) | 0.17 (−0.26 to 0.60) | 0.77 (−0.25 to 1.79) | 0.67 (−0.16 to 1.50) | −0.13 (−1.08 to 0.82) |
| | Fortified formula | 0.27 (−0.74 to 1.28) | 0.40 (−0.09 to 0.88) | 0.40 (−0.75 to 1.54) | 0.76 (−0.17 to 1.70) | 0.43 (−0.63 to 1.49) |
| Multivariate-adjusted* | Breast milk | 0.20 (−0.82 to 1.21) | 0.31 (−0.18 to 0.79) | 1.06 (−0.07 to 2.18) | −0.19 (−1.09 to 0.71) | −0.53 (−1.59 to 0.52) |
| | Fortified formula | 0.21 (−0.83 to 1.25) | 0.45 (−0.05 to 0.95) | 0.62 (−0.54 to 1.78) | 0.34 (−0.59 to 1.28) | 0.25 (−0.84 to 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 g 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 to 0.48) | 0.32 (−0.03 to 0.68) | −0.01 (−0.34 to 0.32) | 0.06 (−0.01 to 0.12) | 0.03 (−0.01 to 0.06) | 0.01 (−0.06 to 0.09) | 0.02 (−0.04 to 0.08) | −0.01 (−0.07 to 0.06) |
| | 0.06 (−0.24 to 0.37) | 0.14 (−0.21 to 0.50) | −0.07 (−0.41 to 0.27) | 0.05 (−0.02 to 0.12) | 0.03 (−0.01 to 0.06) | 0.02 (−0.06 to 0.10) | −0.01 (−0.07 to 0.05) | −0.02 (−0.09 to 0.05) |

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

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 with 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 breast milk 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 breastfeeding promotion.

The strengths of this study include the detailed milk feeding histories collected during infancy, the comprehensive psychological assessment of the children at age 4 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. First, compared with women who did not take part, the women who agreed to participate in this follow-up study were better educated and of a higher social class, and a higher proportion had breastfed their child. Compared with the entire SWS birth cohort, women from more disadvantaged socio-economic 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. Second, 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 a 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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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.

REFERENCES

1. **Svennerholm L.** Distribution and fatty acid composition of phosphoglycerides in normal human brain. *J Lipid Res* 1968;**9**:570–9.
2. **Martin RE, Bazan NG.** Changing fatty acid content of growth cone lipids prior to synaptogenesis. *J Neurochem* 1992;**59**:318–25.
3. **Martinez M.** Tissue levels of polyunsaturated fatty acids during early human development. *J Pediatr* 1992;**120**:S129–38.
4. **Clandinin MT, Chappell JE, Leong S, et al.** Extruterine fatty acid accretion in infant brain: implications for fatty acid requirements. *Early Hum Dev* 1980;**4**:131–8.
5. **Calderon F, Kim HY.** Docosahexaenoic acid promotes neurite growth in hippocampal neurons. *J Neurochem* 2004;**90**:979–88.
6. **Kitajka K, Sinclair AJ, Weisinger RS, et al.** Effects of dietary omega-3 polyunsaturated fatty acids on brain gene expression. *Proc Natl Acad Sci USA* 2004;**101**:10931–6.
7. **McNamara RK, Carlson SE.** Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins Leukot Essent Fatty Acids* 2006;**75**:329–49.
8. **McCann JC, Ames BN.** Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. *Am J Clin Nutr* 2005;**82**:281–95.
9. **Anderson JW, Johnstone BM, Remley DT.** Breast-feeding and cognitive development: a meta-analysis. *Am J Clin Nutr* 1999;**70**:525–35.
10. **Deary IJ, Spinath FM, Bates TC.** Genetics of intelligence. *Eur J Hum Genet* 2006;**14**:690–700.
11. **Silva PA, Buckfield P, Spears GF.** Some maternal and child developmental characteristics associated with breast feeding: a report from the Dunedin Multidisciplinary Child Development Study. *Aust Paediatr J* 1978;**14**:265–8.
12. **Eilander A, Hundscheid DC, Osendarp SJ, et al.** 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**:189–203.
13. **Simmer K, Patole SK, Rao SC.** Long-chain polyunsaturated fatty acid supplementation in infants born at term. *Cochrane Database Syst Rev* 2007;**3**:CD000376.
14. **Simmer K, Schulzke SM, Patole S.** Long-chain polyunsaturated fatty acid supplementation in preterm infants. *Cochrane Database Syst Rev* 2008;**1**:CD000375.
15. **Inskip HM, Godfrey KM, Robinson SM, et al.** Cohort profile: the Southampton Women's Survey. *Int J Epidemiol* 2006;**35**:42–8.
16. **Everitt BSLM, Landau S.** *Cluster analysis*. London: Arnold, 2001.
17. Department of Health. *Dietary reference values for food, energy and nutrients for the United Kingdom*. London: HMSO, 1991.

18. **Yuhas R**, Pramuk K, Lien EL. Human milk fatty acid composition from nine countries varies most in DHA. *Lipids* 2006;**41**:851–8.
19. **Wechsler D**. *Wechsler pre-school and primary scale of intelligence. Third UK edition (WPPSI-III UK)*. London: Psychological Corporation, 2004.
20. **Korkman M**, Kirk U, Kemp S. *NEPSY: a developmental neuropsychological assessment manual*. San Antonio, Texas: Psychological Corporation, 1997.
21. **Gardner MF**. *Test of visual-perceptual skills (non-motor)*. San Francisco: Psychological and Educational Publications, 1996.
22. Office of Population Censuses and Surveys. *Standard occupational classifications 1990*. London: HMSO, 1990.
23. **Wechsler D**. *Wechsler abbreviated scale of intelligence*. San Antonio: The Psychological Corporation, 1999.
24. **Caldwell BM**, Bradley RH. *Home observation for measurement of the environment*. Little Rock, Arkansas: University of Arkansas, 1984.
25. **Auestad N**, Scott DT, Janowsky JS, *et al*. Visual, cognitive, and language assessments at 39 months: a follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. *Pediatrics* 2003;**112**:e177–83.
26. **Birch EE**, Garfield S, Castañeda Y, *et al*. Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula. *Early Hum Dev* 2007;**83**:279–84.
27. **Bouwstra H**, Dijk-Brouwer DA, Wildeman JA, *et al*. Long-chain polyunsaturated fatty acids have a positive effect on the quality of general movements of healthy term infants. *Am J Clin Nutr* 2003;**78**:313–18.
28. **Bouwstra H**, Dijk-Brouwer DA, Boehm G, *et al*. Long-chain polyunsaturated fatty acids and neurological developmental outcome at 18 months in healthy term infants. *Acta Paediatr* 2005;**94**:26–32.
29. **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**:471–9.
30. **Clandinin MT**, Van Aerde JE, Merkel KL, *et al*. Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. *J Pediatr* 2005;**146**:461–8.
31. **Jensen CL**, Voigt RG, Prager TC, *et al*. Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. *Am J Clin Nutr* 2005;**82**:125–32.
32. **Richards M**, Wadsworth M, Rahimi-Foroushani A, *et al*. Infant nutrition and cognitive development in the first offspring of a national UK birth cohort. *Dev Med Child Neurol* 1998;**40**:163–7.
33. **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**:945.
34. **Angelsen NK**, Vik T, Jacobsen G, *et al*. Breast feeding and cognitive development at age 1 and 5 years. *Arch Dis Child* 2001;**85**:183–8.
35. **Gale CR**, O'Callaghan FJ, Godfrey KM, *et al*. Critical periods of brain growth and cognitive function in children. *Brain* 2004;**127**:321–9.
36. **Kramer MS**, Aboud F, Mironova E, *et al*. Breastfeeding and child cognitive development: new evidence from a large randomized trial. *Arch Gen Psychiatry* 2008;**65**:578–84.