Breastfeeding, Polyunsaturated Fatty Acid Levels in Colostrum and Child Intelligence Quotient at Age 5-6 Years

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Objective To examine the relationship of polyunsaturated fatty acid (PUFA) in breast milk with children's IQ.

Study design In the French Etude des Déterminants pré- et postnatals précoces du développement et de la santé de l'Enfant (EDEN) mother-child cohort, colostrum samples were collected at the maternity unit. Colostrum omega-6 and omega-3 PUFA were analyzed by gas chromatography. At age 5-6 years, the IQs of 1080 children were assessed using the Wechsler Preschool and Primary Scale of Intelligence-III. The relationships of breastfeeding duration and PUFA levels with children's IQs were examined by linear regression.

Results Full scale IQ of ever breastfed children was 4.5 (95% CI: 2.7, 6.2) higher than never breastfed children in the unadjusted model, but this was not statistically significant in the adjusted model (1.3 points higher [-0.4, 3.0]). Any breastfeeding duration was associated with full scale (0.20 [0.00, 0.41] points/month) and verbal (0.31 [0.09, 0.52]) IQ. Colostrum linoleic acid (LA) levels were negatively associated with Verbal IQ (-0.6 [-1.1, 0.0] points per 1% level increase). Children exposed to colostrum high in LA and low in docosahexaenoic acid (DHA) had lower IQs than those exposed to colostrum high in DHA (3.0 [0.5, 5.5] points) and those exposed to colostrum low in LA and DHA (4.4 [1.6, 7.3] points). Finally, the association between breastfeeding duration and child IQ was stronger when LA levels were high.

Conclusions Duration of breastfeeding and colostrum PUFA levels were associated with children's IQs in the EDEN cohort. These data support breastfeeding and add evidence for the role of early PUFA exposure on childhood cognition. *(J Pediatr 2017;183:43-50).*

O bservational studies have shown that breastfed children score higher on cognitive tests, such as IQ, than formula-fed children.[1] In our Etude des Déterminants pré- et postnatals précoces du développement et de la santé de l'Enfant (EDEN) cohort, we have highlighted a positive and linear association between breastfeeding duration and cognitive development assessed at ages 2 and 3 years with parent-reported questionnaires.[2] This finding since has been replicated by other cohorts with cognitive tests assessed by psychologists.[3-5] Whether this relationship is causal remains controversial because other studies have found no association after adjustment for socioeconomic status and maternal IQ.[6,7] In addition, if truly causal, the underlying mechanism remains unclear.

Human milk provides a nutritional advantage over infant formulas, particularly for lipid contents.[8] The 2 series of polyunsaturated fatty acids (PUFAs), the omega-6 (n-6) and the omega-3 (n-3), specifically their long-chain forms (long chain PUFA [LC-PUFA]), are naturally found in human milk and are needed for the developing brain of the fetus and the infant.[9] Positive effects on child cognition have been shown in some, but not all randomized controlled trials of formulas enriched in docosahexaenoic acid (DHA, 22:6 n-3) or arachidonic acid (AA, 20:4 n-6) (the most important n-3 and n-6 LC-PUFA, respectively). As the evidence has not been consistent, systematic reviews have been inconclusive.[10]

The published observational studies are limited. In sixty-seven 11-month-old Inuit infants exposed to relatively high levels of breast milk PUFA, Jacobson et al[11]

AA	Arachidonic acid
ALA	α-linolenic acid
DHA	Docosahexaenoic acid
EDEN	Etude des Déterminants pré- et postnatals précoces du développement et de la santé de l'Enfant
FAMEs	Fatty acid methyl esters
LA	Linoleic acid
LC-PUFA	Long-chain PUFA
Omega-3	n-3
Omega-6	n-6
PUFAs	Polyunsaturated fatty acids

found no association between the LC-PUFA levels and cognitive and motor outcomes. In a Spanish cohort, 14-month-old infants who were breastfed for a long duration and consumed colostrum containing a low n-6:n-3 had higher cognitive development than those breastfed for a shorter duration (regardless of colostrum content) and those fed with colostrum containing a higher n-6:n-3 (regardless of breastfeeding duration).[12] In their follow-up at 4 years of age, they found no association, suggesting that the effect decreases with age.[13] In our EDEN cohort, we previously found a negative association between the levels of linoleic acid (LA, 18:2 n-6), the precursor of the n-6 series, and cognitive development assessed by parents at 2 and 3 years of age.[14] In this follow-up evaluation, we examined the relationships of breastfeeding duration and breast milk PUFA levels with children's IQ at 5-6 years of age.

Methods

The EDEN study is a French mother-child cohort which started in 2003 and aimed at examining the role of pre- and postnatal determinants of child development and health.[15] Pregnant women (less than 24 weeks gestational age) attending their first antenatal visit in the maternity units of Nancy or Poitiers University Hospitals, France, were invited to participate.

Exclusion criteria were twin pregnancies, known diabetes before pregnancy, illiteracy, and intention to move outside the region within the next 3 years. A total of 2002 women were enrolled.[15] The study was approved by the ethics research committee (Comité Consultatif de protection des personnes dans la recherche biomédicale) of the Bicêtre Hospital, and by the National Data Protection Authority.

From a questionnaire self-administered by the women during pregnancy, we obtained information on the number of siblings, smoking status, alcohol consumption (0, 1-9, ≥10 g/week), and depression symptoms using the French scale of the Center for Epidemiologic Studies–Depression.[16] Prepregnancy body mass index (in kg/m^2) was obtained from the self-reported weight before pregnancy. Height was measured during the first pregnancy visit. Parental education level was indicated by the highest diploma declared by both parents.

Average household income over the study was calculated from parents' declarations during pregnancy and every year from 1 to 5 years. Offspring's sex, gestational age, and birth weight were collected from obstetric and pediatric records. Birth weight z score was calculated according to the French fetal growth reference.[17] During the clinical visit at 5-6 years of age, our research assistants administered 21 items from 3 subscales (language stimulation, learning stimulation, and variety in experience) of the French Home Observation for the Measurement of the Environment Inventory-Short Form.[18]

Feeding modes during the maternity stay and at discharge were obtained from medical records. In questionnaires at 4 months, 8 months, and 1 and 2 years, mothers reported their infants' feeding modes. Mothers also reported the date of when they stopped breastfeeding. Two variables of breastfeeding duration were calculated according to their intensity level: any

breastfeeding duration (in exact days and converted into months) and exclusive breastfeeding duration (in exact months). Any breastfeeding was defined as receiving any breast milk (whether exclusively or partially), and exclusive breastfeeding referred to infants receiving neither formula nor milk other than human milk. Additional details are available from prior publications.[2,19]

About 5 mL of colostrum was collected from lactating new mothers (mean ± SD: 3.9 ± 1.1 days after birth) and stored at -80°C until analysis. Fatty acid methyl esters (FAMEs) were obtained by direct transmethylation of 100 μL of colostrum at 100°C during 1 hour,[20] and analyzed by gas chromatography with a fast BPX-70 column (Clarus 600 GC; Perkin Elmer, Waltham, Massachusetts), as previously reported.[14] An external standard containing exact amounts of several different FAMEs (GLC 674; Nu-Chek Prep, Waterville, Minnesota) was used to calibrate the peak area of each FAME provided by a flame ionization detector such that it was proportional to its quantity. Twelve PUFAs, expressed as percentages of total milk fatty acids, were identified. These include LA, AA, α-linolenic acid (ALA, 18:3 n-3), eicosapentaenoic acid (20:5 n-3), and DHA. Total levels of n-6 LC-PUFA and of n-3 LC-PUFA (≥20 carbons), as well as several ratios (LA/ALA, AA/DHA, total n-6/n-3 PUFA) were calculated.

Between 5 and 6 years of age, 1 of 2 trained psychologists (1 in each study center) administered the French version of the Wechsler Preschool and Primary Scale of Intelligence-Third Edition.[21] They were blinded to the children's infant feeding history. The core subtests of the battery were assessed (information, vocabulary, word reasoning, block design, matrix reasoning, picture concepts, and coding) to obtain age-adjusted composite scores for verbal, performance, and full scale IQ.

Statistical Analyses

The characteristics of mother-child dyads with available IQ data at 5-6 years were described as percentages and means (SD), and compared with the population not analyzed. Linear regression was used to examine the associations between breastfeeding and children's IQs in unadjusted and adjusted models. Adjusted models included the following covariates: study center, maternal age, prepregnancy body mass index, depression during pregnancy, tobacco and alcohol consumption during pregnancy, parity, child's sex, birth weight z score, gestational age, parental education, household income, and family stimulation score. IQs were compared according to breastfeeding status (as a binary variable; ever vs never breastfed). Among ever breastfed children, associations between continuous variables of breastfeeding duration and children's IQs were examined. The same analysis was performed in our population of interest (ie, those with available data on colostrum PUFA. Deviation from linearity was tested by adding the squared term of breastfeeding duration into the models. Model diagnostics were performed to identify potential influential observations (outliers and high leverage).

Associations between PUFA levels (and ratios) and children's IQs were assessed by linear regression. The adjusted models accounted for the delay between birth and colostrum