Maternal Long-Chain Polyunsaturated Fatty Acid Status during Early Pregnancy and Children's Risk of Problem Behavior at Age 5-6 Years

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Objective To prospectively investigate the association between maternal long-chain polyunsaturated fatty acid (LCPUFA) status and ratio during pregnancy and children's risk of problem behavior at 5 years of age.

Study design Maternal LCPUFA status in plasma phospholipids during pregnancy (M = 13.3, SD = 3 weeks) was available for 4336 women. Children's behavior was rated by their mother (n = 2502) and teacher (n = 2061).

Results When using multivariate logistic regression analyses, we found that greater concentrations of omega-3 fatty acid docosahexaenoic acid (OR 0.75; 95% CI 0.56-0.99; *P* = .05) decreased children's risk for emotional symptoms. Although lower eicosapentaenoic acid and a greater omega-6:omega-3 LCPUFA (ie, arachidonic acid/[docosahexaenoic acid + eicosapentaenoic acid]) tended to increase the risk for emotional symptoms and the risk of hyperactivity/inattention problems for the omega-6:omega-3 LCPUFA, the results were nonsignificant (*P* = .07). No evidence was found for mediation by preterm birth and being small for gestational age. The child's sex and infant feeding pattern did not modify the associations.

Conclusion Our results suggest long-term developmental programming influences of maternal LCPUFA status during pregnancy and stress the importance of an adequate and balanced supply of fatty acids in pregnant women for optimal fetal brain development and subsequent long-term behavioral outcomes. *(J Pediatr 2014;164:762-8)*.

Essential fatty acids and particularly their long-chain polyunsaturated derivatives eicosapentaenoic acid (EPA; omega-3), docosahexaenoic acid (DHA; omega-3), and arachidonic acid (AA; omega-6) are important to neurodevelopmental processes such as neurogenesis, cell proliferation, membrane functioning, and, potentially, myelination.[1] To enable optimal fetal brain development, both a sufficient and balanced supply of omega-3 and omega-6 long-chain polyunsaturated fatty acids (LCPUFAs) from the maternal circulation (ie, placental transfer) are crucial.[2,3] Hence, deficiencies in maternal LCPUFA or an imbalance in the omega-6:omega-3 LCPUFA (ie, AA/[DHA + EPA]) during gestation might affect fetal brain development and influence subsequent long-term behavioral outcomes.

Although a number of studies suggest beneficial effects of maternal omega-3 and omega-6 LCPUFA during pregnancy on long-term neurodevelopmental outcomes of the offspring, they are compromised by methodologic shortcomings, and the results remain inconclusive. First, in 2 studies maternal omega-3 LCPUFA status was represented by maternal fish consumption; therefore, findings could be the result of a nutritious diet or a healthy lifestyle in general.[4,5] To capture maternal LCPUFA status in late gestation, previous studies have assessed maternal venous[6] or umbilical cord blood at birth,[7,8] leaving the potential influence of maternal LCPUFA status during early gestation unstudied. However, there is evidence that LCPUFAs are involved in early neurodevelopmental processes.[1,9] Second, although a balanced supply of omega-3 and omega-6 LCPUFA is known to be important,[10] studies in humans in which researchers have investigated the long-term influence of the omega-6:omega-3 LCPUFA on neurodevelopmental outcomes are lacking. Cheruku et al[6] showed that a greater ratio of total omega-6:omega-3 fatty acids in maternal venous blood at delivery was related to altered sleep patterns in newborns, which is suggestive of central nervous system vulnerability. This might be the result of the LCPUFA precursors linoleic acid (omega-6) and alpha-linolenic acid (omega-3) that compete for the same enzymes for conversion into their long-chain polyunsaturated derivatives. Hence, a greater linoleic acid (omega-6) status, which is typical in Western diets, results in a lower omega-3 (DHA and EPA) status, which potentially influences early neurodevelopmental processes.[3] Third, in previous studies childhood behavior was solely rated by their mothers or

AA	Arachidonic acid
ABCD	Amsterdam Born Children and their Development
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
LCPUFA	Long-chain polyunsaturated fatty acid
SDQ	Strengths and Difficulties Questionnaire
SGA	Small for gestational age

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fathers, and setting-specific behavior problems (eg, home vs school) and cross-informant discrepancies between parents and teachers were not taken into account.[11] Fourth, the potential role of infant feeding (breast milk vs formula) in the association between maternal LCPUFA status and children's neurodevelopmental outcome remains unclear.[12]

Therefore, we prospectively investigated the association between maternal LCPUFA status (EPA; omega-3, DHA; omega-3 and AA; omega-6) and the omega-6:omega-3 LCPUFA during early pregnancy and risk of problem behavior in children at ages 5-6 years in a large multiethnic, community-based birth cohort.

Methods

The current study is part of the Amsterdam Born Children and their Development (ABCD) study. Extensive information about the cohort and procedures regarding data collection is provided elsewhere.[13] In short, pregnant women living in Amsterdam were approached for their participation between January 2003 and March 2004 during their first visit with an obstetric care provider. All women (12 373) received a questionnaire covering sociodemographic, obstetric, lifestyle, and psychosocial conditions, which was filled out by 8266 (67%). Of those respondents, 53% (n = 4389) participated voluntarily in the biomarker study, in which an additional blood sample was taken during routine blood collection for prenatal screening purposes. To be included in the current study, complete data on both maternal fatty acid status and the childhood behavioral assessment had to be available. Additional information about inclusion criteria is provided in the **Figure**. All participating mothers provided their written informed consent. Approval of the study was obtained from the Central Committee on Research involving Human Subjects in The Netherlands, the Medical Ethical Committees of participating hospitals, and from the Registration Committee of the Municipality of Amsterdam.

Maternal LCPUFA concentrations in plasma phospholipids were determined. The absolute amounts of omega-6 AA, omega-3 DHA, and omega-3 EPA (in mg/L plasma) were quantified on the basis of the recovery of an internal standard and expressed as a relative value (percentage of total amount of phospholipids-associated fatty acids).

Problem behavior was reported by mothers and primary school teachers via the Strengths and Difficulties Questionnaire (SDQ), a short behavioral screening questionnaire suitable for 4- to 16-year-old subjects.[14] The SDQ consists of 25 items, which are divided in 5 subscales: emotional symptoms, conduct problems, hyperactivity/inattention problems, peer relationship problems, and prosocial behavior. All items (without prosocial behavior items) added together form a total difficulties score that represents overall problem behavior. The reliability and validity of the SDQ have been established in a Dutch population with satisfactory psychometric characteristics.[15] Interrater reliability between mothers and teachers was calculated with Cohen kappa coefficients for hyperactivity/inattention problems (K = 0.28), conduct problems (K = 0.21), emotional symptoms (K = 0.15), peer relation problems (K = 0.24), prosocial behavior (K = 0.09), and overall problem behavior (K = 0.28) (all $P < .001$).

On the basis of these coefficients, mother and teacher agreement on problem behavior was considered to be slight to fair.[16] Therefore, we chose to identify children to be at risk for problem behavior only when both mother and teacher ratings were consistent. Because no valid norm scores for a Dutch population of young children are available[17] and in accordance with previous work,[18] behavioral outcomes were dichotomized ("no behavior problems" or "at risk for behavior problems") using the 83rd percentile as a cut-off (optimal sensitivity and specificity).[19] Children with SDQ (subscale) scores by both mother and teacher less than the 83rd percentile were not considered to be at risk for problem behavior. In accordance, children with a score greater than the 83rd percentile reported by either mother or teacher also were not considered to be at risk for problem behavior. Only children with a mean (subscale) score greater than the 83rd percentile reported both by their mother and their teacher were considered to be at risk for behavior problems. For prosocial behavior, children with SDQ (subscale) scores by both mother and teacher greater than the 17th percentile were not considered to show suboptimal prosocial behavior. Children with a score less than the 17th percentile reported by either mother or teacher also were not considered to be at risk for suboptimal prosocial behavior. Only children with a score less than the 17th percentile reported both by their mother and their teacher were considered to be at risk for suboptimal prosocial behavior.

Covariates, Mediators, and Moderators

Theoretically based a priori selected potential covariates were: self reported maternal ethnicity defined by country of birth (the Netherlands, other Western country, other non-Western country),[20] maternal age (years), parity (0, >1), prepregnancy body mass index (kg/m^2) based on self-reported height and weight, smoking (no, stopped since pregnant, yes) and alcohol consumption (no, stopped since pregnant, yes), maternal state-anxiety,[21] maternal education (years after primary school), and child's sex and age (years). Birth weight (grams) and gestational age (weeks) were available from Youth Health Care Registration and the Dutch Perinatal Registration (www.perinatreg.nl). Information on infant feeding (formula fed, 1-3 months of exclusive breastfeeding, >3 months of exclusive breast feeding) was from 2 questionnaires (during infancy and when child was 5 years of age) and information available from the Youth Health Care Registration.

Statistical Analyses

We first examined the association between maternal LCPUFA status and children's risk of problem behavior by using a logistic regression model (crude model) that was adjusted for gestational age at blood sampling to account for changes in LCPUFA status during pregnancy.[22] Second,