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The Dietary n6:n3 Fatty Acid Ratio during Pregnancy Is Inversely Associated with Child Neurodevelopment in the EDEN Mother-Child Cohort[1–3]

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Abstract

Long-chain polyunsaturated fatty acids (LC-PUFAs) of the n6 (ω6) and n3 series are essential for the development of a child's brain. Fetal LC-PUFA exposure as well as infant exposure via breast milk depend on the maternal intake of these LC-PUFAs and of their respective dietary precursors (PUFAs). We aimed to investigate the associations between maternal LC-PUFA and PUFA [(LC)PUFA] dietary intake during pregnancy and child neurodevelopment at ages 2 and 3 y. In 1335 mother-child pairs from the EDEN cohort, we evaluated associations between daily maternal (LC)PUFA intake during the last 3 months of pregnancy with the child's language at age 2 y and with different assessments of development at age 3 y. Associations were investigated separately in breastfed and never-breastfed children. We examined interactions between the ratios of n6 and n3 (LC)PUFA intakes (n6:n3 fatty acid ratio) and duration of breastfeeding. Breastfeeding mothers had a lower n6:n3 fatty acid ratio (8.4 vs. 8.8; $P = 0.02$). Among never-breastfed children ($n = 338$), we found negative associations between maternal dietary n6:n3 fatty acid ratios and neurodevelopment, as reflected by the child's language at age 2 y ($\beta \pm$ SE = -2.1 ± 0.7; $P = 0.001$) and development assessed with the Ages and Stages Questionnaire at age 3 y (-1.5 ± 0.8; $P = 0.05$). Among mothers with a high n6:n3 fatty acid ratio only, breastfeeding duration was positively associated with language at age 2 y (P-interaction < 0.05). This suggests that the ratio between maternal dietary n6 and n3 (LC)PUFA intake possibly influences the child's brain development during fetal life but not during or by breastfeeding. However, breastfeeding might compensate for prenatal imbalance in maternal dietary n6:n3 fatty acid ratio. J. Nutr. 143: 1481–1488, 2013.

Introduction

Long-chain (LC) PUFAs, the longer-chain, more-unsaturated derivatives of the essential PUFA linoleic acid (LA; 18:2n6) and α-linolenic acid (ALA; 18:3n3), are important for brain growth and maturation of the fetus and the infant. EPA (20:5n3), DHA (22:6n3), and arachidonic acid (AA; 20:4n6) are involved in membrane structure and signaling functions, especially in the brain, retina, and neural cells (1–3). Animal studies showed that deficiency in n3 LC-PUFAs during fetal and early postnatal periods could affect cognitive development (4). Fetal exposure to LC-PUFAs is highly correlated with maternal status in both PUFA and LC-PUFA [(LC)PUFA] intakes, and therefore depends on maternal fatty acid intakes, metabolism, and stores (5). The supply of (LC)PUFAs

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[3] Supplemental Table 1 and Supplemental Figure 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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[7] Abbreviations used: AA, arachidonic acid; ALA, α-linolenic acid; ASQ, Ages and Stages Questionnaire; CDI, MacArthur Communicative Development Inventories; EDEN, Étude des Déterminants pré- et postnatals précoces du développement et de la santé de l'ENfant; FADS, fatty acid desaturase; LA, linoleic acid; LC, long-chain; (LC)PUFA, long-chain-PUFA and PUFA; PMT-5, Peg Moving Task 5.

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through maternal diet in pregnancy supports the fetal maturation of the brain and could enhance neurodevelopment. The ratio between dietary n6 and n3 (LC)PUFAs (n6:n3 fatty acid ratio) is also of possible importance because endogenous synthesis of n6 and n3 LC-PUFAs competes for the same elongases and desaturases. During the past few decades, the n6:n3 fatty acid ratio has increased in Western diets, which could have some consequences for child neurodevelopment (6).

Randomized controlled trials investigated the effects of LC-PUFA supplementation during pregnancy on child neurodevelopment (7,8). Systematic reviews concluded that there was no clear benefit of supplementation (9,10). The main food source of n3 LC-PUFAs is fish and other seafood, and their consumption is usually used as a proxy for n3 LC-PUFA intake. In moderate- and high-fish-eating populations, many observational studies reported a positive association between maternal seafood consumption during pregnancy and neurodevelopment (11–14). This association might be difficult to detect in low-fish-eating populations, because n3 LC-PUFA intake also comes from other foods. To our knowledge, no observational study has investigated the overall dietary (LC)PUFA intake during pregnancy and its association with child neurodevelopment.

Many studies, including the EDEN (Étude des Déterminants pré- et postnatals précoces du développement et de la santé de l'ENfant) study, have reported a positive association between breastfeeding and neurodevelopmental assessment scores (15–17). Breast milk contains LC-PUFAs, but the content also depends on fatty acid intakes and stores during pregnancy (18,19). Maternal diet affects the child's exposure during pregnancy by an in utero prenatal pathway and after delivery by a postnatal pathway through breast milk if the child is breastfed. However, little is known about the relative importance of these 2 exposure windows.

Our objective was to study in the EDEN mother-child cohort, a low-fish-eating population, the associations between maternal (LC)PUFA intake during pregnancy and the neurodevelopment of their children at 2 and 3 y of age, assessed by both parental questionnaires and neurodevelopmental tests. We also aimed to explore whether breastfeeding and maternal (LC)PUFA intake had independent effects on child neurodevelopment, or whether there was an interaction.

Participants and Methods

Study design. The EDEN study is a French mother-child cohort that aims to investigate the role of pre- and postnatal determinants of child development and health. Recruitment started in 2003 in the hospitals of Poitiers and Nancy and lasted 27 mo. Pregnant women with <24 wk of amenorrhea were invited to participate in the cohort at a clinic visit during pregnancy. Exclusion criteria were multiple pregnancies, known diabetes before pregnancy, illiteracy, and intention to move outside the region in the next 3 y. A total of 2002 women were enrolled, and the participation rate was 55%. Details of the EDEN study protocol have been previously published (20). Informed written consent for the parents was obtained at enrollment and consent for the child was obtained from both parents after the child's birth. 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 Data Protection Authority (Commission Nationale de l'Informatique et des Libertés).

Data collection. During a visit between 24 and 28 wk of gestation, research midwives measured maternal height and mothers declared weight before pregnancy, highest maternal education, and household income and completed a questionnaire. We determined obesity status (BMI ≥30 kg/m^2), tobacco consumption (yes/no), and alcohol consumption during pregnancy (never or <10 g or ≥10 g of ethanol/wk). Household income in euros per month was categorically ordered (≤800, 800–1500, 1500–2300, 2300–3000, 3000–3800, ≥3800). During the mothers' pregnancy, the fathers reported their highest diploma obtained, and parental education was defined as the average number of years of education for both parents. Data on parity (first born of the siblings: yes/no) and infant's gender and gestational age at delivery were collected from obstetric and pediatric records. Data on breastfeeding practices, reasons for the child's hospitalizations, medical consultations, child care, and preschool entry were collected from the mailed questionnaires at 4 and 8 mo and at 1, 2, and 3 y. In the 2-y questionnaire, mothers reported the main caregiver of the child during daytime: mother, family (father, grandparents), nursery, or other (babysitter, neighbor). An index of the weekly frequency of maternal stimulations was calculated by averaging the frequencies of storytelling, singing, and playing with the child (every day, 3–5 times/wk, 1–2 times/wk, <1 time/wk, never). The duration, in months, of preschool attendance before the age of neurodevelopmental assessment was calculated. Breastfed children were defined as those who had received breast milk at any time since birth. The duration of any breastfeeding (exclusive or mixed breastfeeding) was expressed as a continuous variable, in months, as previously described (17).

Dietary assessment. Maternal diet during the last trimester of pregnancy was evaluated within a few days after delivery by using an FFQ and a portion-size picture booklet. The FFQ used was very similar to the FFQ of the Fleurbaix-Laventie Ville Santé study (21,22), and had some additional items about intake of seafood and foods rich in folates, n3 (LC)PUFAs, and vitamin A. By using a food composition database (23), we estimated maternal nutrient intake of LA, AA, ALA, EPA, DHA, total n6 (LC)PUFAs, total n3 (LC)PUFAs, and total (LC)PUFAs (in g/d) and total energy intake (in kJ/d). To evaluate the balance between n6 and n3 (LC)PUFA intake in the maternal diet, we calculated the n6:n3 fatty acid ratio. Energy and nutrient intakes were not estimated when >3 items in the FFQ were missing. Details of the dietary assessment and fatty acid intakes in the EDEN study have been previously published (20).

Neurodevelopmental assessments. In the 2-y questionnaire, the child's language ability was evaluated with the French short version of the MacArthur Communicative Development Inventory (CDI) (24,25). From a list of 100 words, parents reported those words that the child was able to produce spontaneously (score ranged between 0 and 100). Development was investigated at age 3 y with the second French edition of the Ages and Stages Questionnaire (ASQ) (26). This is a parent-completed assessment including 5 domains of development (communication, gross motor, fine motor, problem solving, and personal-social). Although the ASQ is a screening tool created to diagnose developmental delays, its quantitative use has been considered in epidemiologic studies (27). The total score ranged between 0 and 300 points. In the case of 1 or 2 missing data per domain, the mean value of the nonmissing items was imputed (n = 297); when ≥3 items were missing, the ASQ was considered as not valid (26). To have neurodevelopmental measures other than parental questionnaires, at age 3 y children also had several neuropsychological tests covering different aspects of cognitive and motor development. Among those, we present results for the 2 tests for motor and visuospatial abilities [the Peg Moving Task (PMT) 5 and a test of design copying] and the test among 5 that cover the language domain and that showed the greatest variability in our sample with no ceiling effect (the verbal fluency test). The PMT was initially proposed by Annett (28) for the assessment of hand skill in children and adults. The PMT-5 is a reduced "5 holes" version of Annett's PMT (29) and is particularly adapted to very young children. The child had to move each of the 5 pegs, 1 by 1, to the hole on the opposite row, as fast as possible. The task started with the preferred hand, and 2 trials were performed with each hand (29). For the present investigation, we considered the total time in seconds of trials realized with both hands as a measure of overall hand skill. The design copying task is part of the NEPSY (developmental NEuroPSYchological assessment) battery (30,31). It measures motor and visual-perceptual skills and consisted of reproducing drawings (a vertical line, a horizontal line, and a circle). For the verbal fluency assessment, children had to first recall the names of as many animals as possible during 90 s and then the names of objects present in their home.

The score was the sum of the number of names recalled in the best consecutive 60 s of each trial (32). Higher neurodevelopmental scores and lower PMT-5 times indicated better performance.

Participants. From the 1907 women in the cohort who gave birth, mothers without dietary assessments, very preterm children (gestational age <33 wk), children with known disorders such as deafness and epilepsy, and those with incomplete or untimely neurodevelopmental assessments (before age 22 mo and after age 26 mo for the 2-y assessment; before age 34 and after age 39 mo for the 3-y assessments) were excluded. **Figure 1** shows the flow chart of the EDEN population from enrollment to each neurodevelopmental assessment.

Statistical analysis. Characteristics of the study participants are presented as means ± SDs or percentages (*n*). Frequency plots were used to check the distribution of continuous variables. Student's *t* test was used to compare maternal fatty acid intakes during the last 3 months of pregnancy between breastfeeding and nonbreastfeeding mothers.

Associations between maternal fatty acid intakes during pregnancy and the child's scores in the neurodevelopmental assessments were evaluated by multivariable linear regressions. Models were adjusted for covariates identified in the literature as potentially associated with child neurodevelopment, such as maternal diet (13) or breastfeeding (15), and that were available in the EDEN study. These covariates concerned the child (gender, gestational age, and age at assessment), the mother (age at inclusion, total energy intake during pregnancy, obesity, tobacco and alcohol consumption), and the social environment (parental education, household income, whether child was firstborn, main daytime caregiver, frequency of maternal stimulations). In the models with assessments at 3 y, we additionally adjusted for duration of preschool attendance.

Interactions with breastfeeding were tested by adding into the models an interaction term between maternal fatty acid intake during pregnancy and breastfeeding status (never-breastfed vs. breastfed children). Then, model analyses were performed separately for never-breastfed and breastfed children to dissociate those with a prenatal exposure only from children who were also exposed to PUFAs from maternal diet through breast milk. **Supplemental Figure 1** shows a causal diagram of these potential relations according to breastfeeding status.

Finally, among breastfed children only, we investigated whether breastfeeding duration was associated with child neurodevelopment according to maternal dietary n6:n3 fatty acid ratio during pregnancy. For this purpose, we added, into the models previously described, an interaction term between tertiles of maternal dietary n6:n3 fatty acid ratio and breastfeeding duration.

To evaluate the significance of interactions, Wald's test applied to the β-coefficients of the interaction terms was used. Frequency plots were used to check the distribution of residuals of the regression models. Analyses were performed with SAS 9.3 (SAS Institute).

Results

Characteristics of the mother-child pairs are presented in **Table 1**. Mothers who breastfed had higher lipid ($P = 0.02$) and n3 (LC) PUFA ($P = 0.003$) intakes during pregnancy than those who never breastfed (**Table 2**). In addition, the n6:n3 fatty acid ratio of their dietary lipids was lower ($P < 0.02$), because of higher intakes of n3 (LC)PUFAs ($P \leq 0.01$). Summary statistics of the child neurodevelopmental assessments are shown in **Table 3**.

After adjustments for potential maternal, child, and social confounders, no associations were observed between maternal fatty acid intake and results of the design copying task (results not shown). Among both breastfed and never-breastfed children, PMT-5 time was positively associated with maternal n6:n3 fatty

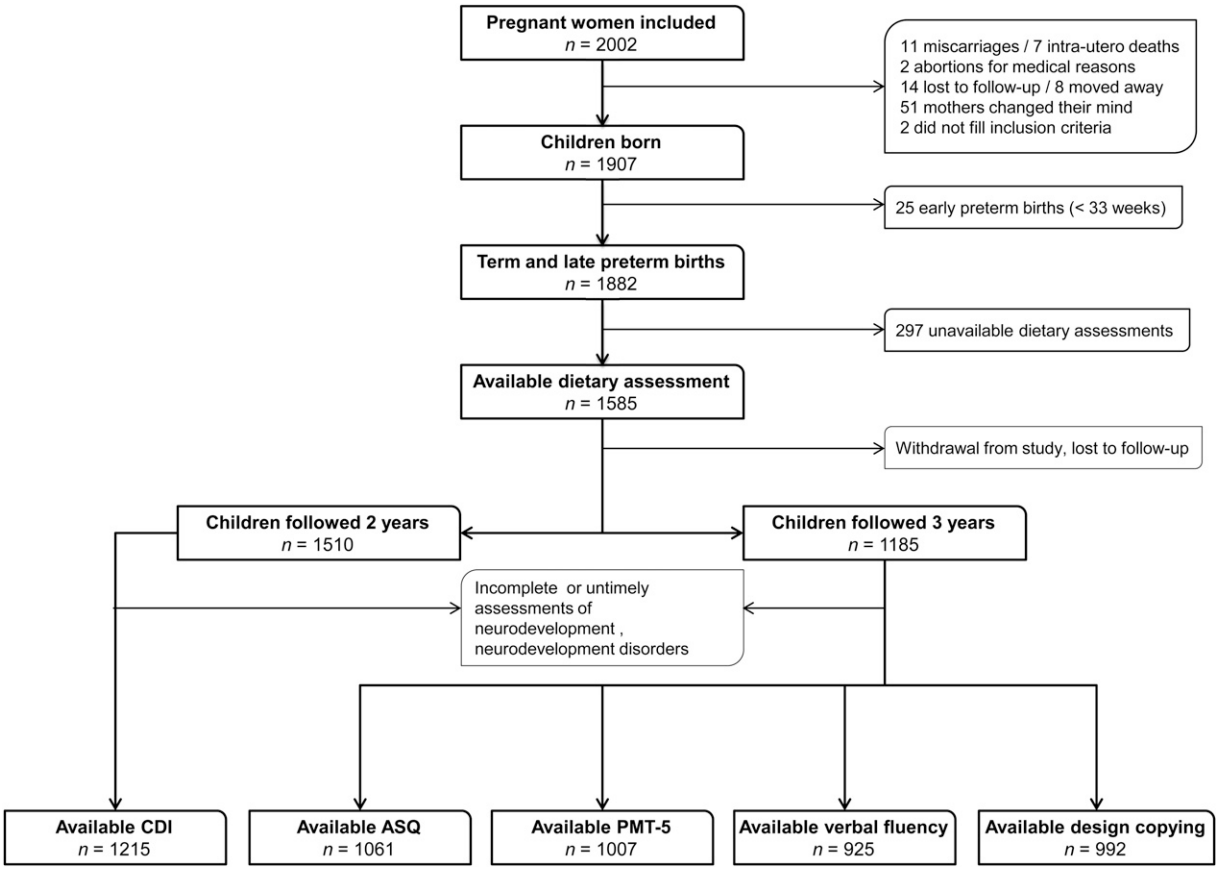

FIGURE 1 Flow diagram of the children with available neurodevelopmental assessments at ages 2 and 3 y in the EDEN (Étude des Déterminants pré- et postnatals précoces du développement et de la santé de l'ENfant) mother-child cohort study. ASQ, Ages and Stages Questionnaire; CDI, Communicative Development Inventory; PMT-5, Peg Moving Task 5.

TABLE 1 Characteristics of participants in the EDEN study[1]

	Value
Maternal characteristics	
Recruitment center, *% (n) from Poitiers*	50.4 (673)
Age at conception, *y*	29.2 ± 4.8
Prepregnancy BMI, *kg/m²*	23.1 ± 4.2
Obese, *% (n)*	7.3 (97)
Smoking during pregnancy, *% (n)*	23.5 (314)
Alcohol consumption, *% (n)*	
Never	55.1 (734)
<10 g/wk	37.0 (493)
≥10 g/wk	7.9 (105)
Total energy intake, *kJ/d*	9130 ± 3020
Child characteristics	
Gender, *% male (n)*	52.2 (697)
Gestational age, *wk*	39.3 ± 1.5
Breastfeeding initiated, *% (n)*	74.7 (997)
Any breastfeeding duration, *mo*	4.7 ± 4.5
Family and social environment characteristics	
Parental education, *y*	13.6 ± 2.3
Household income class, *% (n)*	
≤800 €	2.8 (37)
801–1500 €	9.6 (128)
1501–2300 €	28.2 (377)
2301–3000 €	29.1 (389)
3001–3800 €	17.4 (232)
≥3801 €	12.9 (172)
Firstborn, *% (n)*	46.4 (618)
Frequency of activities with mother, *% (n)*	
<1 time/wk	1.0 (11)
1–2 times/wk	27.3 (296)
3–6 times/wk	33.6 (364)
≥7 times/wk	38.2 (414)
Main daytime caregiver, *% (n)*	
Nursery	20.5 (273)
Other	40.2 (536)
Mother	9.9 (132)
Family	29.5 (394)
Preschool at age 3 y, *% (n)*	66.3 (750)

[1] Values are means ± SDs for continuous variables and percentages (*n*) for categorical variables; *n* = 1335. EDEN, Étude des Déterminants pré- et postnatals précoces du développement et de la santé de l'ENfant.

acid ratio and was negatively associated with ASQ and verbal fluency scores (**Supplemental Table 1**). In the multiple linear regression, we observed an interaction between breastfeeding (yes/no) and maternal n6:n3 fatty acid ratio, with CDI as the outcome (*P*-interaction = 0.004), and with verbal fluency (*P*-interaction = 0.06).

Among breastfed children, no significant associations were found between maternal fatty acid intakes and neurodevelopment (**Table 4**). Among never-breastfed children, maternal n6:n3 fatty acid ratio was inversely associated with CDI, ASQ, and verbal fluency scores ($P < 0.05$), and tended to be inversely associated with the PMT-5 ($P = 0.06$), but was not associated with the other language tests (data not shown). Whatever the feeding mode, the maternal n6:n3 fatty acid ratio tended to be positively associated with PMT-5 time ($P = 0.06$). Maternal n6 (LC)PUFA intake during pregnancy was negatively associated with CDI ($P = 0.03$) and ASQ ($P = 0.04$) but only among never-breastfed children.

As shown in **Figure 2**, among breastfed children, breastfeeding duration was more strongly associated with CDI score in children exposed to a higher maternal n6:n3 fatty acid ratio (third tertile, n6:n3 fatty acid ratio >9.3) during pregnancy than among children exposed to a lower maternal n6:n3 fatty acid ratio (first tertile, n6:n3 fatty acid ratio <7.2) (*P*-interaction = 0.05, *P*-trend across tertiles = 0.02).

Discussion

Our results showed associations in the EDEN mother-child cohort between maternal n6:n3 fatty acid ratio in the diet during pregnancy and some measures of child neurodevelopment at 2 and 3 y of age. We highlighted interactions with breastfeeding status. In never-breastfed but not in breastfed children, maternal dietary n6:n3 fatty acid ratio was negatively related with 3 measures of neurodevelopment: CDI, ASQ, and verbal fluency. We also observed an interaction with breastfeeding duration among breastfed infants, suggesting a greater benefit of breastfeeding on CDI among children initially exposed to a high n6:n3 fatty acid ratio. Finally, in never-breastfed children, CDI and ASQ were negatively associated with maternal n6 (LC)PUFA intake. We did not observe an association with n3 (LC)PUFA intake.

A strength of our study is the prospective data collection of exposure and outcome, which minimizes recall bias. However, the EDEN study was not representative of the general population because mothers had a higher socioeconomic status than average French mothers. This selection bias may lead to a lower variability in our data than in the general population, which is more likely to underestimate than overestimate associations. Even when we adjusted for potential confounders, especially socioeconomic factors, we cannot exclude the possibility that our results were due to residual confounding.

Different domains of child neurodevelopment at ages 2 and 3 y were assessed with both parent-reported questionnaires and neuropsychological evaluations. We found consistent associations with maternal n6:n3 fatty acid ratio among never-breastfed children with neurodevelopmental assessments. Maternal n6 (LC)PUFA intake was negatively associated with the CDI and ASQ only. These 2 assessments are parent-reported questionnaires and could be affected by self-report bias. However, previous studies have confirmed their validity against standardized tests (33,34). In

TABLE 2 Maternal fatty acid intake during pregnancy according to breastfeeding practice in the EDEN study[1]

	BF mothers (*n* = 997)	NBF mothers (*n* = 338)	*P*[2]
Total energy, *kJ/d*	9200 ± 2950	8930 ± 3190	0.15
Total lipids, *g/d*	96.7 ± 38.4	91.3 ± 38.3	0.02
(LC)PUFAs	11.4 ± 4.98	10.9 ± 4.82	0.10
n6 (LC)PUFAs	9.75 ± 4.48	9.35 ± 4.31	0.16
LA	9.60 ± 4.44	9.20 ± 4.26	0.15
AA	0.151 ± 0.079	0.153 ± 0.087	0.64
n3 (LC)PUFAs	1.17 ± 0.454	1.09 ± 0.45	0.003
ALA	0.878 ± 0.344	0.823 ± 0.338	0.01
EPA	0.081 ± 0.057	0.071 ± 0.053	0.004
DHA	0.170 ± 0.111	0.148 ± 0.990	0.002
n6:n3 fatty acid ratio	8.43 ± 2.33	8.78 ± 2.40	0.02

[1] Values are means ± SDs. AA, arachidonic acid; ALA, α-linolenic acid; BF, breastfeeding; EDEN, Étude des Déterminants pré- et postnatals précoces du développement et de la santé de l'ENfant; LA, linoleic acid; (LC)PUFA, long-chain-PUFA and PUFA; NBF, nonbreastfeeding.
[2] Based on Student's *t* test.

TABLE 3 Characteristics of neurodevelopmental scores and ages at assessment of children in the EDEN study[1]

Neurodevelopmental assessment	*n*	Score	Age at assessment
			mo
CDI	1215	60.8 ± 29.4 (1–100)	24.3 ± 0.60 (22.7–27.0)
ASQ	1061	270.2 ± 29.4 (80–300)	37.8 ± 0.7 (34.9–40.0)
PMT-5	1007	44.6 ± 10.2 (23–102)	38.0 ± 0.7 (34.2–40.0)
Verbal fluency	925	6.86 ± 3.86 (0–22)	38.0 ± 0.7 (34.2–40.0)
Design copying	992	9.59 ± 2.30 (0–12)	38.0 ± 0.7 (35.3–40.0)

[1] Values are means ± SDs (ranges). ASQ, Ages and Stages Questionnaire; CDI, Communicative Development Inventory; EDEN, Étude des Déterminants pré- et postnatals précoces du développement et de la santé de l'ENfant; PMT-5, Peg Moving Task 5.

addition, assessments made with the neuropsychological tests partly confirmed these results. Among the language tests, verbal fluency was the only test showing an association, but it was also the test showing the greatest variability in our sample with no ceiling effect.

The accuracy of the evaluation of maternal (LC)PUFA intake during pregnancy is a limitation of our study. The FFQ that we used was adapted from the FFQ from the Fleurbaix-Laventie Ville Santé study and has not been validated in its current version (21,22). The food composition table provides estimates of nutrients but cannot cover all of the variability in the nutrients according to the origin, quality, and cooking processes involved. However, the FFQ remains the instrument of choice in epidemiologic studies because of its practicality. Even if estimates are less accurate than repeated 24-h recalls, intakes of (LC)PUFA derived from FFQs are correlated with biological markers (35). Observed associations may also be due to other nutrients associated with (LC)PUFA consumption. Compared with other studies, which considered only maternal fish intake in relation to the child's neurodevelopment, our assessment of maternal LC-PUFA consumption allowed us to consider LC-PUFAs from sources other than fish. EDEN mothers were low fish consumers: 63% consumed <2 servings/wk, and only 54% of the DHA intake came from fish. Another specificity of our study was to consider the diet during the last 3 months of pregnancy, when the LC-PUFA accretion rate in the fetal brain is maximal (36).

To our knowledge, no observational study has previously investigated the association between maternal dietary intake of (LC)PUFA during pregnancy and child neurodevelopment. Studies have investigated LC-PUFAs in umbilical plasma phospholipids, a proxy for perinatal LC-PUFA exposure, and cognitive and motor development (37–39). Their authors found no association between AA and DHA and cognitive development in 4-y-old (37) and 7-y-old (38) children but found a positive association between DHA and motor development in 7-y-old children (39). In our study, maternal DHA intake was not associated with neurodevelopment and our results with regard to maternal AA intake were conflicting. Some randomized controlled trials showed higher neurodevelopment in children

TABLE 4 Linear regression models examining maternal fatty acid intakes during pregnancy and child neurodevelopment assessed with the CDI, the ASQ, the PMT-5, and the verbal fluency test among breastfed and never-breastfed children of the EDEN study[1]

	CDI			ASQ			PMT-5[2]			Verbal fluency		
	n	β ± SE	*P*	*n*	β ± SE	*P*	*n*	β ± SE	*P*	*n*	β ± SE	*P*
Breastfed children	901			786			746			682		
Total lipids (g/d)		−0.01 ± 0.06	0.83		0.04 ± 0.06	0.47		0.01 ± 0.02	0.59		0.01 ± 0.01	0.51
(LC)PUFAs (g/d)		0.23 ± 0.29	0.43		−0.19 ± 0.29	0.51		0.10 ± 0.11	0.37		0.00 ± 0.04	1.00
n6 (LC)PUFAs (g/d)		0.25 ± 0.30	0.40		−0.22 ± 0.30	0.45		0.12 ± 0.12	0.32		−0.01 ± 0.05	0.88
LA		0.25 ± 0.30	0.40		−0.23 ± 0.30	0.45		0.12 ± 0.12	0.30		−0.01 ± 0.05	0.86
AA		6.29 ± 14.72	0.67		−0.09 ± 15.02	1.00		−7.67 ± 5.94	0.20		3.52 ± 2.35	0.13
n3 (LC)PUFAs (g/d)		−2.23 ± 4.00	0.58		3.61 ± 3.97	0.36		−1.11 ± 1.59	0.49		0.84 ± 0.64	0.19
ALA		−2.77 ± 6.06	0.65		3.29 ± 5.97	0.58		0.29 ± 2.41	0.90		0.57 ± 0.96	0.56
EPA		−8.55 ± 17.46	0.62		15.28 ± 17.30	0.38		−9.80 ± 6.99	0.16		2.57 ± 2.74	0.35
DHA		−3.63 ± 9.23	0.69		7.48 ± 9.18	0.42		−3.26 ± 3.71	0.38		1.73 ± 1.44	0.23
n6:n3		0.38 ± 0.40	0.34		−0.63 ± 0.39	0.11		0.30 ± 0.16	0.06		−0.06 ± 0.06	0.36
Never-breastfed children	309			270			257			241		
Total lipids (g/d)		−0.07 ± 0.10	0.49		−0.28 ± 0.13	0.03		0.05 ± 0.04	0.21		−0.02 ± 0.02	0.17
(LC)PUFAs (g/d)		−1.13 ± 0.55	0.04		−1.33 ± 0.64	0.04		0.17 ± 0.21	0.44		−0.09 ± 0.08	0.28
n6 (LC)PUFAs (g/d)		−1.24 ± 0.57	0.03		−1.37 ± 0.67	0.04		0.17 ± 0.22	0.43		−0.10 ± 0.09	0.25
LA		−1.23 ± 0.58	0.03		−1.34 ± 0.67	0.05		0.17 ± 0.22	0.44		−0.10 ± 0.09	0.26
AA		−33.93 ± 25.36	0.18		−104.25 ± 29.52	0.001		5.07 ± 10.04	0.61		−3.79 ± 3.78	0.32
n3 (LC)PUFAs (g/d)		7.02 ± 8.24	0.39		−2.43 ± 9.74	0.80		−1.72 ± 3.25	0.60		1.41 ± 1.26	0.26
ALA		13.84 ± 12.27	0.26		−2.39 ± 14.71	0.87		−1.30 ± 4.66	0.78		1.56 ± 1.80	0.39
EPA		13.98 ± 36.50	0.70		24.51 ± 43.16	0.57		−10.55 ± 14.88	0.48		5.67 ± 5.81	0.33
DHA		6.14 ± 19.86	0.76		−1.77 ± 24.15	0.94		−4.35 ± 8.21	0.60		2.75 ± 3.21	0.39
n6:n3		−2.13 ± 0.65	0.001		−1.52 ± 0.76	0.05		0.49 ± 0.26	0.06		−0.26 ± 0.01	0.01
P-interaction between n6:n3 fatty acid ratio and breastfeeding practice			0.004			0.2			0.8			0.06

[1] Values are regression coefficients ± SEs or *P* values of the linear regression. Models were adjusted for center, child gender and age, gestational age, maternal age, obesity, energy intake, tobacco and alcohol consumption, parental education and income, firstborn, main daytime caregiver, and frequency of maternal stimulations. ASQ, PMT-5, and verbal fluency models additionally adjusted for preschool attendance. AA, arachidonic acid; ALA, α-linolenic acid; ASQ, Ages and Stages Questionnaire; CDI, Communicative Development Inventory; EDEN, Étude des Déterminants pré- et postnatals précoces du développement et de la santé de l'ENfant; LA, linoleic acid; (LC)PUFA, long-chain-PUFA and PUFA; PMT-5, Peg Moving Task 5.

[2] Lower PMT-5 time corresponds to better neurodevelopment.

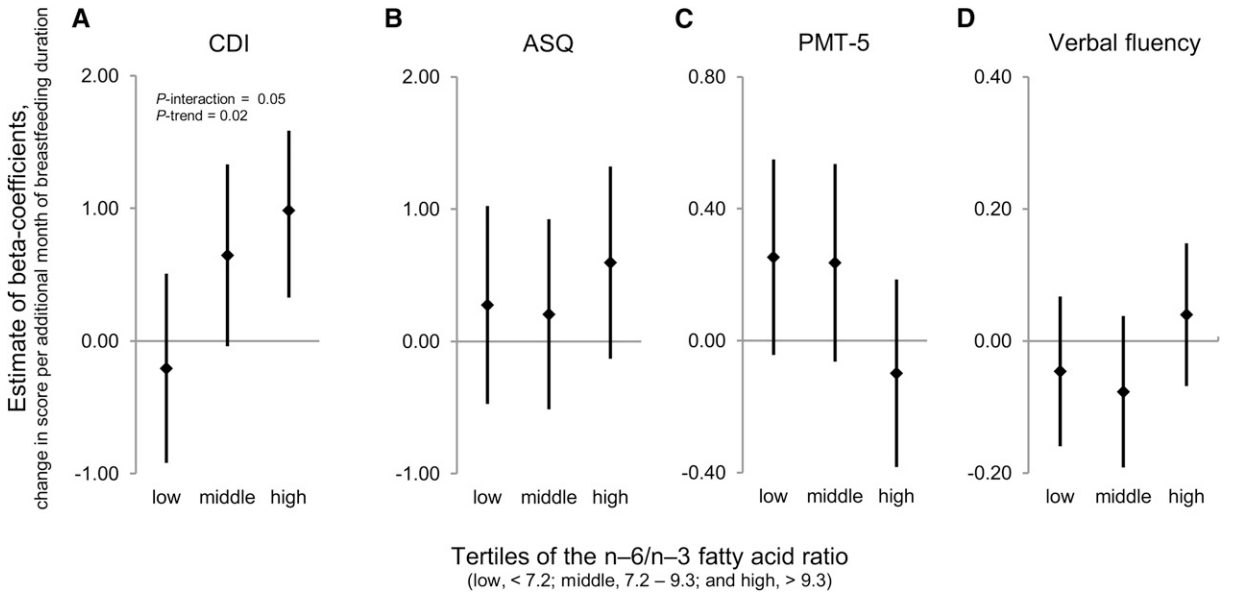

FIGURE 2 Estimates of associations between breastfeeding duration and neurodevelopment of breastfed children, as assessed by CDI (n = 901; *A*), ASQ (n = 786; *B*), PMT-5 (n = 745; *C*), and verbal fluency (n = 682; *D*) scores, according to tertiles of n6:n3 fatty acid ratio in the maternal diet during pregnancy (low, <7.2; middle, 7.2–9.3; and high, >9.3). A lower PMT-5 time corresponds to better neurodevelopment. Results are β-coefficients ± SEs. ASQ, Ages and Stages Questionnaire; CDI, Communicative Development Inventory; PMT-5, Peg Moving Task 5.

from n3 LC-PUFA–supplemented mothers during pregnancy than in children from nonsupplemented mothers, but results are inconsistent across studies (9,10). Observational studies showed consistently that high fish consumption during pregnancy was associated with better child neurodevelopment, even though fish intake is correlated with exposure to methylmercury, a neurotoxicant that is highly concentrated in fish and associated with adverse childhood neurological outcomes (40).

In our study, maternal n6:n3 fatty acid ratio was negatively associated with some aspects of child neurodevelopment, especially among never-breastfed children. It appeared to be mainly driven by a negative association with n6 (LC)PUFAs, with negative associations observed between n6 (LC)PUFAs and the CDI and ASQ assessments. The excess of n6 (LC)PUFAs could in itself not be beneficial for brain development; although AA is needed for neuronal structure and signaling, it is also the precursor of proinflammatory eicosanoids. Even if the proinflammatory effects of n6 (LC)PUFA are debated (41), chronic inflammation might be detrimental for the brain, as suggested for Alzheimer disease (42). A second hypothesis concerns the increase in the balance between n6 and n3 (LC)PUFAs in some modern diets (6). In our sample, 326 of the 1335 (24%) mothers had an n6:n3 fatty acid ratio >10, whereas the recommended ratio is usually 5. An excess of n6 (LC)PUFAs could decrease synthesis of n3 LC-PUFAs because the metabolic pathways of n6 and n3 LC-PUFAs compete for the same enzymes, especially δ-5 and δ-6 desaturases, which are encoded by fatty acid desaturase (FADS) 1 and FADS2, respectively (43). However, the n6:n3 fatty acid ratio is a concept that makes no consensus in nutrition science (44,45), since authors have found a positive correlation between AA and DHA concentrations in maternal plasma phospholipids (46).

To consider prenatal and postnatal exposure periods, we studied separately breastfed and never-breastfed children. Many studies previously reported that breastfeeding is associated with better child neurodevelopment (15), even if some authors have suggested that this was due to residual confounding (47). Breast milk contains LC-PUFAs, especially DHA and AA (48), that can accrete in the brain until the age of 2 y, and postnatal LC-PUFA exposure may potentially contribute to neurodevelopment (49). In our study, we found a statistical interaction between maternal n6:n3 fatty acid ratio and breastfeeding duration: the duration of breastfeeding was also associated with language at 2 y among children exposed to a high maternal n6:n3 fatty acid ratio but not in children exposed to a low maternal n6:n3 fatty acid ratio. The trend was similar for other tests. Future research should investigate this major finding.

In conclusion, our results suggest that a higher n6:n3 fatty acid ratio in the maternal diet is associated with poorer child development at ages 2 and 3 y. In low-fish-eating populations, n6 (LC)PUFAs are important contributors to an imbalance in LC-PUFA status, and future research should consider this pathway. Through the maternal diet during pregnancy, the prenatal period appears to be a critical period for child neurodevelopment. The existence of a postnatal pathway is nevertheless suggested by the protective effects of a long duration of breastfeeding among mothers with a low dietary n6:n3 fatty acid ratio. These data argue in favor of the promotion of breastfeeding and the limitation of intakes of n6 (LC)PUFAs during pregnancy.

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