Maternal LC-PUFA status during pregnancy and child problem behavior: the Generation R Study

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BACKGROUND: Omega 3 (n-3) and 6 (n-6) long-chain polyunsaturated fatty acids (LC-PUFAs) and the n-3:n-6 ratio are important for brain development. Whether maternal LC-PUFA status during pregnancy affects risk of problem behavior in later childhood is unclear.

METHODS: Within a population-based cohort, we measured maternal plasma docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA) concentrations and n-3:n-6-ratio in mid-pregnancy. Child emotional and behavioral problems at 6 y of age were assessed by parents (child behavior checklist), teachers (teacher report form), and combined parent/teacher report.

RESULTS: Higher maternal DHA and n-3:n-6 ratio were associated with fewer child emotional problems using parent (odds ratio $(OR)_{DHA}$ = 0.82; 95% confidence interval (CI): 0.70, 0.96; P = 0.02 and $OR_{n-3:n-6}$ = 0.83; 95% CI: 0.71, 0.96; P = 0.01; n = 5,307) and combined parent/teacher scores $(OR_{DHA}$ = 0.79; 95% CI: 0.66, 0.95; P = 0.01 and $OR_{n-3:n-6}$ = 0.77; 95% CI: 0.65, 0.92; P < 0.01; n = 2,828). Higher AA was associated with more child behavioral problems using teacher (OR = 1.10; 95% CI: 1.00, 1.20; P = 0.04; n = 3,365) and combined parent/teacher scores (OR = 1.12; 95% CI: 1.02, 1.22; P = 0.02; n = 2,827). Maternal EPA was not associated with child problem behavior.

CONCLUSION: Indications of associations of maternal LC-PUFA status with child emotional and behavioral problems were found. Future research is needed to identify LC-PUFA–sensitive periods of fetal brain development by including multiple assessments of prenatal LC-PUFA status.

T wo families of polyunsaturated fatty acids (PUFAs), the omega 3 (n-3) and 6 (n-6) fatty acids (FAs), are nutrients that play a major role in brain development and functioning. They affect numerous processes, including membrane fluidity, neurotransmission, and gene expression (1). The long-chain PUFAs (LC-PUFAs), eicosapentaenoic acid (EPA; C20:5n-3), docosahexaenoic acid (DHA; C22:6n-3), and arachidonic acid (AA; C20:4n-6) are the main FAs that are metabolically active.

In addition, the ratio between n-3 and n-6 FAs is important for optimal brain functioning. During the last century, however, the Western diet has provided a lower n-3 and higher n-6 intake than that in previous generations, diminishing the n-3:n-6 ratio (2).

Because of their role in brain function and development, maternal LC-PUFAs during fetal development have been hypothesized to affect child problem behavior. Approximately half of the dry weight of the brain is lipid, of which 35% are PUFA, most of which is LC-PUFA (1). Increase in AA and DHA content of brain tissue starts during the third trimester of pregnancy (3), indicating that the fetal period possibly is a sensitive period. Moreover, the fetus mostly depends on maternal LC-PUFA supply for its development, because fetal LC-PUFA synthesis is limited (4).

Research in this field is however scarce, and results are conflicting. Two studies on maternal fish intake, a major source of DHA, during pregnancy and offspring problem behavior were undertaken. They found low maternal fish intake to be associated with suboptimal prosocial behavior and increased risk of hyperactivity in childhood (5,6). Another study on dietary intake of n-3 LC-PUFAs did not find any association with child behavior (7). Animal studies have shown that prenatal and not postnatal DHA deficiency was associated with increased depression and anxiety later in life (8). In humans, there is some indication that perinatal LC-PUFA status (either measured in cord blood or maternal blood during gestation) is associated with child behavior. However, results are inconsistent, ranging from null findings to associations with both emotional and behavioral problems (9–12). Most studies were small and probably underpowered to detect subtle effects of nutrition, as expected in a multicausal phenomenon such as development of the brain and behavior. Moreover, multiple informants—the gold standard in child psychiatric research to measure a child's core characteristics from different perspectives and in different contexts—were generally not used.

A recent, relatively large study in another birth cohort from the Netherlands that did use multiple informants

reported a modest association between maternal DHA level and child emotional symptoms (12). In a study twice as large, with extended behavioral assessment by multiple informants and the availability of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) diagnoses of problem behavior, we aimed to further study the effects of maternal LC-PUFAs during pregnancy on child problem behavior. To this end, we tested the hypotheses that maternal LC-PUFA status is associated with child emotional and behavioral problems at 6 y of age. Because of their metabolic role in brain development, we focused on AA, EPA, and DHA, as well as the n-3:n-6 ratio.

RESULTS

Descriptive characteristics of study participants by determinant and outcome are presented in **Tables 1** and **2** respectively.

Maternal LC-PUFAs and Child Emotional Problems

The fully adjusted associations between maternal LC-PUFA status and child emotional problems, as reported by different informants and assessed with different instruments, are presented in **Table 3**. No associations of maternal AA or EPA with child emotional problems were found. Increased levels of maternal DHA were associated with fewer child emotional problems as reported by parents (child behavior checklist

Table 1. Subject characteristics by maternal n-3:n-6 ratio[a] in mid-pregnancy[b]

	Lowest quintile of n-3:n-6 ratio (n = 1,058)	Other n-3:n-6 quintiles (n = 4,249)	P value[c]
Child characteristics			
Gender (% girl)	49.7	50.0	0.83
Gestational age at birth (wk)[d]	40.0 (35.3–42.4)	40.1 (35.6–42.4)	0.03
Weight at birth (g)[e]	3,381.1 ± 565.2	3,441.3 ± 566.8	<0.01
Breastfeeding (partial) until 6 mo (%)	36.5	33.4	0.58
Daycare attendance (number of hours/wk)	10.4 (0.0–36.3)	20.0 (0.0–39.4)	<0.001
Age at assessment (y)	6.0 (5.6–7.5)	5.9 (5.6–7.1)	<0.001
Child dietary intake	n = 386	n = 2,399	
N-3 intake at 14 mo (g/d)[f]	0.64 ± 0.35	0.63 ± 0.34	0.44
N-6 intake at 14 mo (g/d)[f]	5.3 ± 2.9	4.7 ± 2.4	<0.001
Maternal characteristics	n = 1,050	n = 4,204	
Age at enrollment (y)	28.8 ± 5.2	31.0 ± 4.7	<0.001
Ethnicity (% European)	39.7	70.8	<0.001
Education (%)			
Higher	27.1	53.5	
Secondary	53.9	40.2	<0.001
Primary	19.0	6.3	
Family income (%)			
>2,000 €/mo	38.5	69.8	
1,200–2,000 €/mo	28.3	16.0	<0.001
<1,200 €/mo	33.2	14.2	
General psychiatric symptoms in mid-pregnancy (GSI score)	0.24 (0.00–1.55)	0.13 (0.00–1.22)	<0.001
BMI before pregnancy (kg/m²)	23.3 (17.9–35.7)	22.5 (18.2–34.5)	<0.001
Smoking during pregnancy (%)			
Never	72.3	78.0	
Until pregnancy was known	6.1	8.4	<0.001
Continued throughout pregnancy	21.6	13.6	
Alcohol consumption during pregnancy (%)			
Never	66.1	37.2	
Until pregnancy was known	9.6	14.7	<0.001
Continued throughout pregnancy	24.3	48.1	
Parity (% primipara)	46.9	60.3	<0.001
Marital status (% cohabitation)	89.3	89.1	0.89

GSI score, Global Severity Index score.

[a]Participants in the lowest quintile of n-3:n-6 ratio (1:13.7–1:6.5) vs. those in the other quintiles (1:6.4–1:1.9). [b]Descriptives on imputed data. [c]Derived from linear regression analysis for continuous variables or logistic regression analysis for categorical variables; on imputed data. [d]Median and 95% range in parentheses (all such values). [e]Mean ± SD (all such values). [f]Adjusted for energy intake.

(CBCL); odds ratio $(OR)_{parent\ score}$ = 0.82; 95% confidence interval (CI): 0.70, 0.96; P = 0.02) and using the combined scores of both parents (CBCL) and teachers (teacher report form (TRF); $OR_{combined\ score}$ = 0.79; 95% CI: 0.66, 0.95; P = 0.01). Similarly, a higher n-3:n-6 ratio was associated with a lower risk of emotional problems ($OR_{parent\ score}$ = 0.83; 95% CI: 0.71, 0.96; P = 0.01 and $OR_{combined\ score}$ = 0.77; 95% CI: 0.65, 0.92; P < 0.01). In contrast, no associations of maternal DHA or n-3:n-6 ratio with child emotional problems based on teacher report only were found (OR_{DHA} = 1.04; 95% CI: 0.86, 1.25; P = 0.70 and $OR_{n-3:n-6}$ = 0.98; 95% CI: 0.82, 1.17; P = 0.84). Also, no associations of maternal fatty acids with offspring emotional problems were found using the parent-administered Diagnostic Interview Schedule for Children-Young Child version (DISC-YC; OR_{DHA} = 0.82; 95% CI: 0.57, 1.18; P = 0.28 and $OR_{n-3:n-6}$ = 0.94; 95% CI: 0.65, 1.38; P = 0.76).

Including quadratic terms of the FAs in the model did not suggest nonlinear associations, and results did not change

Table 2. Subject characteristics by child total emotional and behavioral problems[a] at 6 y[b]

	Emotional and behavioral problems (n = 208)	No emotional and behavioral problems (n = 5,099)	P value[c]
Child characteristics			
Gender (% girl)	38.7	50.5	<0.01
Gestational age at birth (wk)[d]	39.9 (33.9–42.2)	40.1 (35.6–42.4)	0.05
Weight at birth (g)[e]	3,353.9 ± 607.8	3,432.3 ± 565.0	0.14
Breastfeeding (partial) until 6 mo (%)	31.5	34.1	0.62
Daycare attendance (number of hours/wk)	14.5 (0.0–39.2)	18.0 (0.0–38.7)	0.01
Age at assessment (y)	6.0 (5.4–7.2)	5.9 (5.6–7.2)	0.14
Child dietary intake	n = 81	n = 2704	
N-3 intake at 14 mo (g/d)[f]	0.67 ± 0.38	0.63 ± 0.34	0.32
N-6 intake at 14 months (g/d)[f]	5.1 ± 2.7	4.8 ± 2.5	0.27
Maternal characteristics	n = 208	n = 5,046	
Age at enrollment (y)	28.9 ± 5.6	30.6 ± 4.8	<0.001
Ethnicity (% European)	41.2	65.6	<0.001
Education (%)			
Higher	29.9	49.0	
Secondary	52.1	42.5	<0.001
Primary	18.0	8.5	
Family income (%)			
>2,000 €/mo	37.6	64.6	
1,200–2,000 €/mo	25.3	18.2	<0.001
<1,200 €/mo	37.1	17.2	
General psychiatric symptoms in mid-pregnancy (GSI score)	0.42 (0.01–2.00)	0.15 (0.00–1.24)	<0.001
BMI before pregnancy (kg/m²)	22.6 (17.9–35.6)	22.7 (18.1–34.6)	0.68
Smoking during pregnancy (%)			
Never	67.2	77.2	
Until pregnancy was known	6.8	8.0	<0.001
Continued throughout pregnancy	26.0	14.8	
Alcohol consumption during pregnancy (%)			
Never	54.4	42.5	
Until pregnancy was known	13.1	13.7	0.01
Continued throughout pregnancy	32.5	43.8	
Parity (% primipara)	62.0	57.5	0.22
Marital status (% cohabitation)	78.1	89.6	<0.001

GSI-score, Global Severity Index score.

[a]Children with problem behavior (based on clinical cutoff scores for CBCL Total Problems scale from a Dutch norm population) vs. children without problem behavior. [b]Descriptives on imputed data. [c]Derived from linear regression analysis for continuous variables or logistic regression analysis for categorical variables; on imputed data. [d]Median, 95% range in parentheses (all such values). [e]Mean ± SD (all such values). [f]Adjusted for energy intake.

Table 3. Associations of maternal prenatal fatty acids with child emotional and behavioral problems at 6 y[a]

	Emotional problems							
	Parent report (n = 5,307)[b]		Teacher report (n = 3,366)[c]		Combined parent and teacher report (n = 2,828)[d]		Parent interview, DSM-IV–based diagnosis (n = 490)[e]	
	OR[g] (95% CI)	P	OR[g] (95% CI)	P	OR[g] (95% CI)	P	OR[g] (95% CI)	P
Maternal fatty acid (wt%)[f]								
AA	1.03 (0.95, 1.13)	0.44	1.03 (0.94, 1.14)	0.52	1.05 (0.96, 1.16)	0.27	1.08 (0.90, 1.29)	0.42
EPA	1.09 (0.65, 1.86)	0.74	0.60 (0.28, 1.28)	0.19	1.00 (0.53, 1.89)	0.99	1.83 (0.64, 5.23)	0.26
DHA	0.82 (0.70;0.96)	**0.02**	1.04 (0.86, 1.25)	0.70	0.79 (0.66, 0.95)	**0.01**	0.82 (0.57, 1.18)	0.28
N-3:N-6 ratio (per SD)	0.83 (0.71, 0.96)	**0.01**	0.98 (0.82, 1.17)	0.84	0.77 (0.65, 0.92)	**<0.01**	0.94 (0.65, 1.38)	0.76
	Behavioral problems							
	Parent report (n = 5,307)[b]		Teacher report (n = 3,365)[c]		Combined parent and teacher report (n = 2,827)[d]		Parent interview, DSM-IV–based diagnosis (n = 1,105)[e]	
	OR[g] (95% CI)	P	OR[g] (95% CI)	P	OR[g] (95% CI)	P	OR[g] (95% CI)	P
Maternal fatty acid (wt%)[f]								
AA	1.04 (0.91, 1.18)	0.60	1.10 (1.00, 1.20)	**0.04**	1.12 (1.02, 1.22)	**0.02**	1.07 (0.95, 1.22)	0.25
EPA	1.58 (0.84, 2.95)	0.15	0.85 (0.45, 1.58)	0.60	1.22 (0.70, 2.10)	0.48	1.09 (0.59, 2.02)	0.79
DHA	0.93 (0.75, 1.16)	0.53	1.23 (1.03, 1.46)	**0.02**	1.07 (0.90, 1.26)	0.47	0.89 (0.72, 1.11)	0.30
N-3:N-6 ratio (per SD)	1.09 (0.88, 1.34)	0.44	1.23 (1.05, 1.44)	**0.01**	1.16 (0.99, 1.36)	0.06	0.89 (0.73, 1.08)	0.24

AA, arachidonic acid; CI, confidence interval; DHA, docosahexaenoic acid; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders; EPA, eicosapentaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acid; n-3, omega 3; n-6, omega 6; OR, odds ratio.
P values <0.05 are indicated in bold.
[a]Values are odds ratios from logistic regression analyses, except for the analyses based on parent interview, which are odds ratios from a case–control approach using conditional logistic regression analyses. [b]Cases based on clinical cutoff scores from a Dutch norm population; cases emotional problems: n = 305; cases behavioral problems: n = 129. [c]Cases based on clinical cutoff scores for the middle norm group ("medium scoring societies") from multicultural norms; cases emotional problems: n = 194; cases behavioral problems: n = 223. [d]Cases based on cutoff scores at 91st percentile in study population; cases emotional problems: n = 255; cases behavioral problems: n = 254. [e]Cases based on DSM-IV–based diagnosis of emotional/behavioral problems with mild impairment; cases emotional problems: n = 98, controls: n = 392; cases behavioral problems: n = 221, controls: n = 884. [f]1 unit increase equals an increase of 1% of total fatty acid composition for the respective LC-PUFA. [g] Analyses are adjusted for the other fatty acids (except in case of the n-3:n-6 ratio), gestational age at venipuncture, sex, age of child at assessment, family income, maternal educational level, European origin, general psychiatric symptoms in mid-pregnancy, smoking and alcohol consumption during pregnancy, age at enrollment, parity, marital status, and child daycare attendance.

after exclusion of preterm children and children with low birth weight.

The analyses were repeated while adjusting for dietary n-3 and n-6 intake at 14 mo of age in a subsample with these data available ($n_{parent\ score}$ = 2,785). Dietary n-3 and n-6 data did not change the effect size of DHA nor the n-3:n-6 ratio on child emotional problems (in this subsample, before and after adjustment for child diet: OR_{DHA} = 0.75; 95% CI: 0.59, 0.96; *P* = 0.02 and $OR_{n\text{-}3:n\text{-}6}$ = 0.74; 95% CI: 0.59, 0.93; *P* = 0.01).

With regard to potential effect modification, the interaction term of maternal n-3:n-6 ratio with child sex, but not maternal educational level, was statistically significant (*P* < 0.05). Analyses stratified by sex showed that the effect of maternal n-3:n-6 ratio on child emotional problems could be attributed to an effect in girls (OR_{girls} = 0.71; 95% CI: 0.56, 0.91; *P* < 0.01 and OR_{boys} = 0.91; 95% CI: 0.74, 1.12; *P* = 0.36).

In **Table 4**, we show the association of maternal n-3:n-6 ratio divided by quintiles with child emotional problems. The lowest quintile was used as the reference category (quintile 1). Compared with this reference, a maternal n-3:n-6 ratio in the highest two quintiles was associated with fewer emotional problems in the offspring (OR_{Q4vsQ1} = 0.62; 95% CI: 0.40, 0.95; *P* = 0.03 and OR_{Q5vsQ1} = 0.61; 95% CI: 0.39, 0.96; *P* = 0.03).

Maternal LC-PUFAs and Child Behavioral Problems
The fully adjusted associations between maternal LC-PUFA status and child behavioral problems are presented in **Table 3**. No associations were found between maternal EPA and child behavioral problems. Higher maternal DHA level and n-3:n-6 ratio were only associated with more child behavioral problems using the teacher report (OR_{DHA} = 1.23; 95% CI: 1.03, 1.46; *P* = 0.02; $OR_{n\text{-}3:n\text{-}6}$ = 1.23; 95% CI: 1.05, 1.44; *P* = 0.01).

However, for both the teacher score and the combined parent/teacher score, increased levels of maternal AA were associated with more child behavioral problems ($OR_{teacher\ score}$ = 1.10; 95% CI: 1.00, 1.20; *P* = 0.04 and $OR_{combined\ score}$ = 1.12; 95% CI: 1.02, 1.22; *P* = 0.02). These positive associations were not confirmed in analyses using parent report only (either CBCL or DISC-YC interview).

Again, including quadratic terms of the FAs in the model did not suggest nonlinear associations, and results did not change

Table 4. Associations of maternal prenatal n-3:n-6 ratio with child emotional and behavioral problems at 6 y[a]

Maternal n-3:n-6 ratio	Emotional problems (n = 5,307)[b]			
	Basic[c]		Adjusted for covariates[d]	
	OR (95% CI)	P	OR (95% CI)	P
Quintiles				
1 (1:13.7–1:6.5)	Reference		Reference	
2 (1:6.4–1:5.6)	0.81 (0.58, 1.13)	0.22	1.02 (0.72, 1.44)	0.91
3 (1:5.5–1:4.9)	0.58 (0.40, 0.86)	**<0.01**	0.82 (0.55, 1.22)	0.33
4 (1:4.8–1:4.2)	0.39 (0.25, 0.59)	**<0.001**	0.62 (0.40, 0.95)	**0.03**
5 (1:4.1–1:1.9)	0.35 (0.23, 0.53)	**<0.001**	0.61 (0.39, 0.96)	**0.03**
Maternal n-3:n-6 ratio	Behavioral problems (n = 5,307)[b]			
	OR (95% CI)	P	OR (95% CI)	P
Quintiles				
1 (1:13.7–1:6.5)	Reference		Reference	
2 (1:6.4–1:5.6)	1.24 (0.64, 2.42)	0.52	1.35 (0.70, 2.60)	0.37
3 (1:5.5–1:4.9)	1.12 (0.60, 2.10)	0.71	1.31 (0.70, 2.43)	0.40
4 (1:4.8–1:4.2)	0.77 (0.39, 1.53)	0.45	1.02 (0.51, 2.06)	0.95
5 (1:4.1–1:1.9)	0.86 (0.45, 1.65)	0.66	1.28 (0.65, 2.54)	0.48

CI, confidence interval; n-3, omega 3; n-6, omega 6; OR, odds ratio.
P values <0.05 are indicated in bold.
[a]Values are odds ratios from logistic regression analyses. [b]Parent report, cases based on clinical cutoff scores from a Dutch norm population; cases emotional problems: n = 305; cases behavioral problems: n = 129. [c]Model 1: adjusted for gestational age at venipuncture, sex, and age of child at assessment. [d]Model 2: model 1, additionally adjusted for family income, maternal educational level, European origin, general psychiatric symptoms in mid-pregnancy, smoking and alcohol consumption during pregnancy, age at enrollment, parity, marital status, and child daycare attendance.

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Adjustment for child n-3 and n-6 intake in a subsample ($n_{teacher score}$ = 1,582 and $n_{combined score}$ = 1,553) did not change the association of AA with child behavioral problems using the teacher report (in this subsample, before and after adjustment for child diet: OR_{AA} = 1.22; 95% CI: 1.04, 1.43; P = 0.02). The association between AA and child behavior using the combined parent/teacher report in this subsample did not reach level of statistical significance, either before or after adjustment for child dietary intake (OR_{AA} = 1.13; 95% CI: 0.99, 1.30; P = 0.07).

DISCUSSION

In this population-based study, we found higher maternal DHA levels and n-3:n-6 ratio to be associated with fewer offspring emotional problems using both a parent and combined parent/teacher report of child problem behavior. This association could not be confirmed using teacher report only or using the DISC-YC interview. If anything, we interpret these findings as an indication of a negative association of maternal DHA levels and n-3:n-6 ratio with offspring emotional problems.

Similarly, we found higher maternal AA levels to be associated with more child behavioral problems using both teacher and combined parent/teacher report. The associations using

parent report and DISC-YC data were statistically null findings. These findings indicate a positive association of maternal AA levels with offspring behavioral problems.

No associations between maternal EPA levels and child emotional or behavioral problems were found.

Interpretation

Our findings closely resemble those from Loomans *et al.* (12), who also found higher maternal DHA—but not AA or EPA—to be associated with fewer child emotional symptoms at the age of 5–6 y based on combined report of mother and teacher using the Strength and Difficulties Questionnaire. We additionally found indications of higher maternal n-3:n-6 ratio and AA levels to be associated with, respectively, fewer emotional and more behavioral problems in the offspring, whereas these associations disappeared after adjustment for confounders in the study by Loomans *et al.* Our study, however, has more power. Additionally, our measures of behavioral problems also comprise aggressive and rule-breaking behaviors, whereas the Strength and Difficulties Questionnaire only measures hyperactivity and inattention.

Our results are also compatible with findings from Krabbendam *et al.* (10), who found higher DHA but not AA cord blood levels to be associated with fewer emotional problems in children at the age of 7 y based on CBCL parent report. Moreover, like Krabbendam *et al.*, who found no association between current child LC-PUFA status and child problem behavior, we did not find infant n-3 and n-6 intake to affect the associations between maternal LC-PUFAs and child emotional or behavioral problems at the age of 6 y. This stresses the potential independent effect of prenatal LC-PUFA status on child emotional and behavioral development.

Our results are generally not in line with those from Kohlboeck *et al.* (11), who found higher levels of AA in cord blood to be associated with fewer emotional problems at the age of 10 y based on parent report using the Strength and Difficulties Questionnaire. Whether statistically significant or not, all associations of maternal AA with child problem behavior in our study suggest effect estimates in the opposite direction, as do results from other studies (9,10,12). Kohlboeck *et al.* also found higher levels of DHA to be associated with less hyperactivity/inattention, whereas we found an indication of higher maternal AA levels to be associated with more behavioral problems. Differences in findings may be attributable to a combination of differences in fatty acid assessment (cord blood serum vs. mid-pregnancy plasma sample), instrument and informant of child problem behavior (parent Strength and Difficulties Questionnaire vs. multiinformant assessment by CBCL, TRF, and DISC-YC interview), and child age at assessment (10 vs. 6 y of age).

Finally, our results are not consistent with those from Dunstan *et al.* (9), who reported no significant associations between cord blood LC-PUFAs and problem behavior at the age of 2.5 y using CBCL parent report. Our study, however, has a much larger sample size and thus increased power to detect associations. Additionally, our study has a longer follow-up

time, during which parents may become better in recognizing and thus reporting problem behavior of their child and during which other informants of child problem behavior, such as teachers, become available.

In contrast to the studies mentioned above, we provide an overview of the associations of maternal LC-PUFAs with child problem behavior as reported by different informants. There is a large body of literature indicating the important variability in behavior across different contexts, such as home vs. school (13). Our approach increases insight in the effect that the difference in multi-informant agreement on children's problem behavior may have on associations under investigation. As a consequence, however, this approach makes it harder to make strong statements when results do not all point toward same conclusion. Therefore, not only findings from studies using one informant/instrument but also findings from the current study should be interpreted with caution.

Mechanism of Risk

Animal studies have shown that maternal dietary LC-PUFA composition may durably alter brain monoamine, dopamine, and serotonin concentrations in the offspring (14,15). Moreover, such modifications in 2-mo-old rats were only reversed by supply of a LC-PUFA–balanced diet that started before weaning (15), when rat brain development is similar to that of a full-term human newborn (16). It is known in humans that maternal LC-PUFA status declines during pregnancy (17), and LC-PUFAs accumulate in the fetal brain, with a spurt in the last trimester of pregnancy (1). However, maternal LC-PUFA status may also affect brain development earlier in pregnancy. Gale *et al.* (6) found oily fish intake during early but not late pregnancy to be associated with less hyperactivity in children. Lower maternal LC-PUFA concentrations directly limit the amount of LC-PUFA available to the fetus (4), and animal studies showed that already early in pregnancy LC-PUFAs promote neurogenesis and then continue to influence synaptogenesis and myelination (18–20). The developmental stage in which inadequate LC-PUFA status occurs during pregnancy may thus additionally account for different effects of LC-PUFA status.

Alternatively, maternal depression underlies the observed association of maternal LC-PUFAs with child problem behavior. Antenatal depression is an established cause of child emotional and behavioral problems (21). Low LC-PUFA status might place women at risk of depression or can result from dietary changes due to depression (22). However, we adjusted for maternal depressive symptoms during pregnancy to control for such an indirect effect.

Finally, this type of nutritional associations in observational studies is sensitive to effects of confounding and effect-modifying factors. A decline in the effect of maternal LC-PUFA status on the offspring's problem behavior was noticeable after adjustment for confounders. Admitting we were not able to adjust for child LC-PUFA concentrations, we found no evidence that child dietary intake of n-3 and n-6 FAs influenced the associations. Effect-modification by

sex further points to a complex causal chain, in which many factors exaggerate effects on child emotional and behavioral development. Although we accounted for many sociodemographic factors, further (residual) confounding cannot be excluded.

Strengths and Limitations

The strengths of our study were its large sample size, the availability of plasma LC-PUFA concentrations, the multi-informant assessment of child behavior, and the ability to adjust for several potential confounders.

The study also has its limitations. Selective attrition may have influenced our results. Mothers of children not included generally had less favorable LC-PUFA status and socioeconomic circumstances. This selective attrition can only lead to bias if associations of maternal fatty acids and child emotional/behavioral problems in those mother–child pairs not included in the study differ from the associations found in those that were included in the study. With regard to the hypothesized biological mechanism underlying the association, we do not consider this likely. It can however not be inferred from our results. Next, our assessment of LC-PUFA status was based on a single measure in blood plasma in mid-pregnancy. One measurement, although indicative, is not a reliable reflection of a mother's long-term FA status. Furthermore, other nutrients that are associated with brain development and that simultaneously affect FA status, such as iron, were not included in this study (18).

Conclusion

We found indications of higher maternal DHA levels and n-3:n-6 ratio during second trimester of pregnancy to be associated with fewer emotional problems and higher AA levels with more behavioral problems in the offspring at the age of 6 y. These findings are a step forward for the growing body of literature in this field and underline the importance of the use of multiple informants of child problem behavior in association studies like these. Further research is needed to identify LC-PUFA–sensitive periods of fetal brain development, optimal intake of LC-PUFAs during pregnancy, and the specific effects of inadequate prenatal LC-PUFA status on emotional and behavioral development in the offspring. These studies should include multiple assessments of FAs during pregnancy, as well as in-depth assessments of child problem behavior by multiple informants and instruments.

METHODS

Study Population

The present study was conducted within the Generation R Study, a population-based cohort from early fetal life onwards in Rotterdam, The Netherlands (23). The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki and was approved by the Medical Ethical Committee of the Erasmus Medical Center. Written consent was obtained from all participants.

Out of 8,663 mothers who enrolled in the study before their third trimester of pregnancy (<25 wk of gestation), valid FA profiles in plasma were available for 6,999 mothers (80.8%). Overall, 6,916

mother–child pairs were eligible at study baseline. In total, at least one measure of child behavior was available for 5,307 children (76.7%) and their mothers (**Figure 1**). Some mothers participated with two ($n = 309$) or three ($n = 6$) children. Since results of all analyses using parent report did not differ after exclusion of twins ($n = 110$) or random exclusion of one or two of the siblings, they were kept in the analyses.

Fatty Acid Analyses

Between 2001 and 2005, venous samples were drawn in mid-pregnancy (median: 20.5 wk of gestation; 90% range: 18.8–22.9 wk) and thereafter stored at room temperature before being transported to the regional laboratory for processing and storage for future studies. Processing was aimed to finish within a maximum of 3 h after venipuncture. The samples were centrifuged and thereafter stored at −80 °C (without antioxidant). To analyze FA concentrations, EDTA plasma samples were picked and transported to the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich Medical Center, in 2010. After being thawed, the analysis of plasma glycerophospholipid FA (hereafter referred to as "plasma FA") composition was performed by a sensitive and precise high-throughput method described recently (24). The average coefficient of variation was 15.7%.

Plasma samples had previously undergone one thaw–freeze cycle. In order to explore the impact of this extra thaw–freeze cycle, 105 new plasma samples from the same series of aliquot tubes were randomly selected for reanalysis, and intraclass correlation coefficients for all 25 FA concentrations were calculated using a two-way mixed-effects model for single measures with absolute agreement. Substantial to almost perfect agreement between the two assessments was found (intraclass correlation coefficients: 0.61–0.96; intraclass correlation coefficient for sum of FAs: 0.87), with only one exception for C22:5n-3 (intraclass correlation coefficient: 0.59).

In the current study, we used AA, EPA, and DHA. The n-3:n-6 ratio was calculated by summing the available n-3 PUFAs and dividing this by the sum of the corresponding n-6 PUFAs in the pathway (see

Figure 1. Flow chart of study population. CBCL, child behavior checklist; [a]Due to twin pregnancies. [b]Analytic sample, missing imputed by multiple imputation.

Supplementary Table S1 online for an overview). FAs are expressed as percentage by weight (wt%) of all glycerophospholipid FAs detected with a chain length between 14 and 22 carbon atoms.

Child Emotional and Behavioral Problems

Mothers were asked to fill out the CBCL when their child was about 6 y old. Since the majority of the children were younger than 6 (58.7%), we used the CBCL 1½–5 for all children. Additionally, children 8 y or older were excluded from the analyses ($n = 61$). In our sample, for all scales, Cronbach's alphas were the same in 5-y-old children and in children older than 5, indicating that problems were also reliably measured in children older than 5.

The CBCL is a self-administered parents-report questionnaire to measure the degree of children's problem behavior (25). It contains 99 problem items rated on a 3-point scale (0 = not true, 1 = somewhat or sometimes true, and 2 = very true or often true), based on the preceding 2 mo. A broad-band emotional problems (i.e., being anxious, withdrawn, and emotionally reactive) and behavioral problems (i.e., inattention and aggression) scale can be computed. Clinical cutoff scores were used to classify children as having emotional or behavioral problems (91st percentile of a Dutch norm group (26)).

The TRF 6–18 is the teacher version of the CBCL 6–18. Good reliability and validity have been reported for the CBCL and TRF (25,27). In total, TRF data was available in 3,366 children whose mother had a fatty acid profile. Clinical cutoff scores (91st percentile of the middle norm group from multicultural samples (27,28) were used to classify children as having emotional or behavioral problems.

Correlations between parent and teacher report were modest ($r_{\text{CBCL-TRF}} = 0.19$ for emotional problems; $r_{\text{CBCL-TRF}} = 0.22$ for behavioral problems), which is in line with literature on cross-informant agreement (13) and reflects important variability in behavior across different contexts.

Parent and teacher scores were also combined by averaging the individual age-standardized sum scores. Since no clinical cutoffs were available from norm populations, we used the 91st percentile in our study sample as a cutoff to define child emotional and behavioral problems in the clinical range.

DSM-IV–based diagnosis of problem behavior was obtained by the DISC-YC in a subsample of the cohort when children were about 6 y old. The DISC-YC is a highly structured DSM-IV–based parent-administered interview that generates DSM-IV diagnoses in four modules: anxiety disorders, mood disorders (emotional), behavioral disorders, and miscellaneous (elimination and tic disorders). Good overall reliability of symptom scales has been published (29). DISC-YC administration in the total Generation R cohort was aimed at children enriched for problem behavior (i.e., scoring above the 85th percentile on CBCL total problems or above the 98th percentile of CBCL syndrome scales; $n = 1,080$) plus a random comparison group ($n = 330$), resulting in oversampling of children at risk for diagnosis. In the current study, interview data were available for 839 children whose mother had a FA profile. Of these, 98 children were classified with a diagnosis "emotional problems with mild impairment" and 221 with "behavioral problems with mild impairment." For each of the cases, four matched (on sex and European origin) controls were selected from the sample with CBCL data available (children scoring below the 50th percentile on both CBCL emotional and behavioral problems). Controls were selected from a total eligible control group of 2,134 children, comprising of 117 children who were not diagnosed with emotional or behavioral problems based on the DISC-YC and an additional group of 2,017 children without interview data.

Covariates

Several maternal and child characteristics were considered as possible confounding variables, based on previous studies of perinatal LC-PUFA status and child emotional and behavioral development (9–12). These were: family income, maternal educational level, pre-pregnancy BMI, age at enrollment, smoking and alcohol consumption during pregnancy, national origin, general psychiatric symptoms in mid-pregnancy (highest prevalence for anxious and depressive symptoms), parity, and marital status. Child characteristics that were considered comprised gestational age and weight at birth, sex,
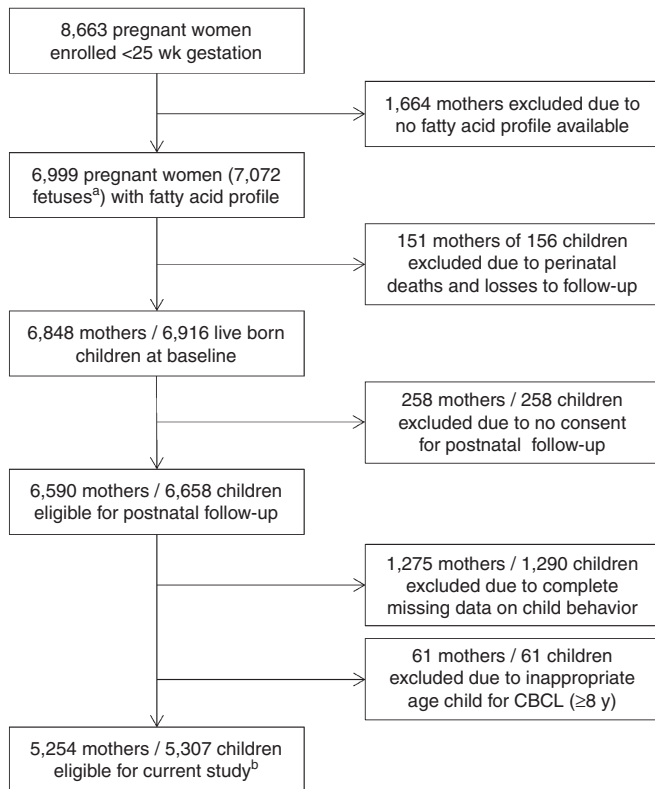
breastfeeding status at 6 mo, daycare attendance during early childhood, and age of the child at the time of assessment.

All analyses of maternal LC-PUFA status and child problem behavior were controlled for gestational age at venipuncture, sex of the child, and the age of the child at assessment, as well as the other FAs (to test the independent effect of each LC-PUFA). Other covariates were included in the adjusted models if they changed the effect estimates meaningfully (defined as more than 5%). Following this criterion, maternal pre-pregnancy BMI and child gestational age and weight at birth and breastfeeding status at 6 mo were excluded from the analyses.

We did not have information on child LC-PUFA levels. Instead, we used dietary n-3 and n-6 intake during infancy, as assessed by a 211-item Food Frequency Questionnaire when children were 14 mo old (30). N-3 and n-6 intake were adjusted for total energy intake using the residual method (31) and were available for 2,785 of the children in the current study sample.

Statistical Analyses

To minimize bias resulting from rater perspectives, we used the parent (CBCL, DISC-YC), teacher (TRF), and combined parent/teacher scores of child emotional and behavioral problems as outcome variables.

First, multivariable logistic regression analyses were used to test for associations of maternal AA, EPA, and DHA levels (wt%) as well as the maternal n-3:n-6 ratio (per SD) with child emotional and behavioral problems using the CBCL, TRF, and the combined CBCL/TRF scores. For the DISC-YC data, we used a case–control approach with conditional logistic regression analysis.

We added quadratic terms of the LC-PUFA variables to test for nonlinear associations. Analyses were repeated while excluding preterm children (<37 wk of gestation) and children with low birth weight (<2,500 g) (n = 396) and adjusting for child dietary n-3 and n-6 intake at the age of 14 mo in a subsample with these data available.

Next, we explored potential effect modification by child sex and maternal educational level in the fully adjusted models using parent reports. If the interaction term between maternal n-3:n-6 ratio and the effect modifier was statistically significant ($P < 0.05$), we performed additional stratified analyses.

Finally, for ease of interpretation, we divided the n-3:n-6 ratio by quintiles in analyses using parent report of child problem behavior.

Missing values on covariates and child outcome data (when at least one other CBCL measure at 1.5 or 3 y was available) were imputed using the Markov Chain Monte Carlo multiple imputation technique with Predictive Mean Matching for continuous variables (0.1–19.9% missing data) and generating five datasets. Statistical analyses were carried out using PASW Statistics, version 20.0 for Windows (SPSS, Chicago, IL).

For the nonresponse analysis, we compared child and maternal characteristics of included participants (n = 5,307; 76.7%) with those from whom we did not obtain behavioral data (n = 1,609). Excluded children on average had a 108 g (95% CI: 76, 140; $P < 0.001$) lower birth weight compared with included children. The mothers of children not included on average had a 0.2 wt% (95% CI: 0.17, 0.29; $P < 0.001$) lower DHA level in mid-pregnancy, were on average 3.3 y (95% CI: 3.0, 3.6; $P < 0.001$) younger, and less educated (22.5% primary education compared with 8.1%; χ^2 = 520 (2); $P < 0.001$). They also had more severe psychopathological complaints (median: 0.25 (95% range: 0–1.98) compared with 0.15 (95% range: 0–1.29); $P < 0.001$).

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