Original Contribution

Maternal Fatty Acid Status During Pregnancy and Child Autistic Traits

The Generation R Study

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ω-3 and ω-6 polyunsaturated fatty acids are important for brain function and development. We examined whether maternal polyunsaturated fatty acid status during pregnancy affects risk of autistic traits in childhood. Within the Generation R cohort, we measured maternal plasma polyunsaturated fatty acid concentrations and the ω-3:ω-6 ratio in midpregnancy (Rotterdam, the Netherlands, 2001–2005). Child autistic traits at 6 years were assessed by using the Social Responsiveness Scale short form in 4,624 children. A lower maternal ω-3:ω-6 ratio during pregnancy was associated with more autistic traits (β = −0.008, 95% confidence interval: −0.016, −0.001). In particular, a higher total ω-6 and linoleic acid status were associated with more autistic traits (all P’s < 0.05). Associations were independent of child intelligence, suggesting that the fatty acid distribution specifically affects the development of autistic traits in addition to general neurodevelopment. Maternal plasma ω-3 status was not associated with child autistic traits and, consistently, neither was prenatal dietary fish intake. Our study shows that a lower prenatal ω-3:ω-6 ratio is associated with more child autistic traits, which is largely accounted for by higher ω-6 instead of lower ω-3 status. These results suggest a biological pathway between maternal fatty acid intake during pregnancy and autistic traits in the offspring.

Autism spectrum disorder (ASD) is characterized by impairments in social communication and restricted repetitive behaviors (1). Understanding the pathogenesis of ASD has proven to be challenging. Next to insights from behavioral genetic epidemiologic studies (2), research on environmental risk factors for autism risk suggested associations between various environmental factors and autism (3).

Because of their role in brain development and function, the ω-3 and ω-6 polyunsaturated fatty acids (PUFAs) are among these potential environmental risk factors. ω-3 and ω-6 PUFAs affect numerous processes, including membrane fluidity, neurotransmission, and gene expression (4). Both types of PUFAs exert different and sometimes opposing effects, for example, on the production and activity of various components of the immune system that modulate brain functions (5). During the last century, the Western diet has provided a lower ω-3 and higher ω-6 intake than in previous generations, diminishing the ω-3:ω-6 ratio (6). Given the purported rise in ASD prevalence over the past decades (7), it has been suggested that disturbances in the ω-3:ω-6 ratio may contribute to the emergence of ASD (8). In this respect, maternal PUFAs status during pregnancy may be of particular interest, as the prenatal phase is a time of both rapid neurodevelopment and increased fetal demand of PUFAs (9), for which the fetus depends on maternal supply (10). Prenatal PUFAs status has repeatedly been associated with general child neurodevelopment, expressed by the

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; IQ, intelligence quotient; PUFA, polyunsaturated fatty acid; SRS, Social Responsiveness Scale.
intelligence quotient (IQ) and other scores of global cognitive ability (for an overview, refer to the report by Steer et al. (11)). Whether PUFAs additionally affect the development of ASD is less clear.

Lyall et al. (12) investigated the potential role of maternal PUFAs in the development of ASD in the offspring, using dietary fatty acid intake as a measure of prenatal PUFA status. High intake of maternal linoleic acid (an ω-6 PUFA) was associated with reduced risk, and a very low total ω-3 intake was associated with a higher risk of child ASD. Other, less accurate proxies of PUFA status, such as intake of fish (a major source of ω-3 PUFAs) or fish oil supplementation during pregnancy, were not associated with child ASD (12, 13). Studies of dietary intake, however, have not investigated the ω-3 and ω-6 PUFAs ratio and children’s risk of ASD. More importantly, nutritional biomarkers, which could support a biological relationship between fatty acid intake and child ASD, were not incorporated.

In this prospective population-based study, we explored whether maternal plasma PUFA concentrations in pregnancy predicted autistic traits in the offspring at 6 years of age. Given the association of ASD with child IQ (14), we further examined whether any associations were independent of child IQ. We hypothesized that a lower maternal ω-3:ω-6 ratio during pregnancy is associated with more autistic traits in children.

METHODS

Study population

The present analysis was embedded in the Generation R Study (15). The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki and was approved by the local medical ethics committee. Written, informed consent was obtained from all adult participants and caregivers of participating children.

Out of 8,663 pregnant women who were enrolled before their third trimester of pregnancy (<25 weeks’ gestation), valid fatty acid profiles in plasma were available for 6,999 mothers (80.8%). Overall, 6,611 mothers and their single liveborn infants were eligible at study baseline. A measure of child autistic traits at the age of 6 years was available for 4,624 children (69.9%) (Figure 1). IQ scores were available for 3,838 of these children.

Fatty acid analyses

Between 2001 and 2005, venous samples were drawn in midpregnancy (median, 20.6 weeks; 90% range, 18.9–22.8) and processed as described earlier (16). Briefly, samples were stored at room temperature before being transported to the regional laboratory for processing and storage for future studies. The samples were centrifuged and thereafter stored at −80°C. After being thawed, plasma glycerophospholipid fatty acid was analyzed and the composition determined by a sensitive and precise high-throughput method (17).

We investigated the available ω-3 PUFAs in the PUFA synthesis pathway (α-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid) and the corresponding ω-6 PUFAs (linoleic acid, arachidonic acid, adrenic acid, osbond acid). The ω-3:ω-6 ratio was calculated by summing the ω-3 PUFAs and dividing this by the sum of the ω-6 PUFAs, as previously defined (16) (refer to Web Table 1 available at http://aje.oxfordjournals.org/ for an overview). Fatty acids are expressed as the percentage by weight of all glycerophospholipid fatty acids detected with a chain length between 14 and 22 carbon atoms.

Figure 1. Flowchart of study population in the Generation R Study, Rotterdam, 2001–2012. A total of 3,838 eligible children had data on the intelligence quotient score.

Child autistic traits

The Social Responsiveness Scale (SRS) (Western Psychological Services (WPS), Torrance, California) was administered as a measure of autistic traits in children within a population-based setting (18). The SRS provides a valid quantitative measure of autistic traits as reported by parents (19). At a mean child age of 6.2 (standard deviation, 0.5) years, mothers filled out the 18-item short form of the scale (20). Each item on the SRS is rated from 0 (never true) to 3 (almost always true), covering social, language, and repetitive behaviors; higher scores indicate more problems.

Additionally, at the mean child age of 6 (standard deviation, 0.4) years, mothers filled out the Child Behavior Checklist, which includes a subscale on Pervasive Developmental Problems (21). The Pervasive Developmental Problems subscale is a useful screening instrument to identify children with ASD (22) with good predictive validity identifying preschoolers at risk of ASD (23). Because the SRS and Pervasive Developmental Problems scores correlated well (r = 0.6; P < 0.001; n = 3,567), scores on the Pervasive Developmental Problems subscale were included in the imputation model that was used to impute missing values (number of missing SRS scores = 870; 18.8%).

Child IQ

At the age of 6 (mean = 6.1 (standard deviation, 0.4) years), the children were invited to visit the Generation R research center. During this visit, the children’s nonverbal IQ
was assessed by using 2 subtests of the validated Dutch test battery, “Snijders-Oomen Niet-verbale Intelligenietest–Revisie” (SON-R 2½-7) (24). These subtests were Mosaics (assesses spatial visualizability abilities) and Categories (assesses abstract reasoning abilities). Raw test scores were converted into nonverbal IQ scores by using norms tailored to exact age.

Maternal fish intake

Nutritional intake, including fish consumption, in the past 3 months was assessed in early pregnancy (median, 13.8 weeks; 90% range, 10.8–21.4) by using a modified version of a validated semiquantitative food frequency questionnaire (25). Assessment of fish intake has been described in detail previously (26). Total fish intake (in g/day) includes all types of fish consumed and was adjusted for daily total energy intake by using the residual method (27).

Fish intake data were available for 3,802 mothers of the current study population.

Covariates

Several parental and child characteristics were included as confounding variables, based on previous studies of the associations of perinatal PUFA status with child autistic traits (11–13). These were maternal IQ, age, educational level, national origin, psychopathology in midpregnancy, prepregnancy body mass index, pregnancy planning, parity, marital status, smoking, alcohol consumption, and folic acid supplement use during pregnancy, as well as family income. We also included the age, educational level, national origin, psychopathology, and body mass index of the father (28). Child characteristics comprised gestational age and weight at birth, sex, breastfeeding status at 6 months, day-care attendance during early childhood, and age of the child at assessment. As no information on child PUFA levels was available, we used dietary ω-3 and ω-6 intakes during infancy, as assessed by a 211-item food frequency questionnaire when children were 14 months of age (29), to adjust for child fatty acid status (30). ω-3 and ω-6 intakes were adjusted for total energy intake by the residual method (27) and were available for 2,436 of the children in the current study sample.

Statistical analyses

We used multivariable linear regression to test the association of overall measures of maternal PUFA status with child autistic traits in 2 separate analyses: 1) using the ω-3:ω-6 ratio as predictor in the model (per standard deviation), and 2) using both total ω-3 and ω-6 status as predictors in the model (percentage by weight, per standard deviation). Autistic trait scores were square root transformed to approximate a normal distribution. To further illustrate results, we subsequently divided the maternal ω-3:ω-6 ratio by quintiles in the regression analysis.

In the event of a significant association for 1 or more of the overall measures of maternal PUFA status with child autistic traits, further association analyses of individual maternal PUFAs (percentage by weight, per standard deviation) with child autistic traits were performed in 1 separate analysis. Additionally, as the prenatal nutritional ω-3 deficiency has been found to affect offspring behavior in human (12) and rodent (31, 32) studies, the lowest 10% and 5% of the distribution of total ω-3 status were compared with the remaining 90% and 95% of these distributions, respectively, to examine the associations of very low maternal ω-3 status with child autistic traits in secondary analyses.

We added quadratic terms of the fatty acid variables to test for nonlinear associations and performed sensitivity analyses, in which we excluded subjects with the highest load of autistic traits. Further, we explored potential effect modification by maternal ethnicity (Dutch/non-Dutch) in the fully adjusted models by including the interaction term(s) between maternal fatty acids and ethnicity. Finally, to test whether associations of maternal PUFA status with child autistic traits were explained by child PUFA status, analyses were repeated adjusting for child dietary ω-3 and ω-6 intakes during infancy in a subsample with these data available (n = 2,436).

Similarly, we explored associations of the maternal ω-3:ω-6 ratio and total ω-3 and ω-6 concentrations with child IQ.

Finally, we examined the associations of fish intake during pregnancy with offspring autistic traits and IQ in a subsample of mothers with prenatal dietary intake data available (n = 3,802). The average daily total fish intake (g/day, per standard deviation) was used as both a continuous and dichotomized (yes vs. no) exposure variable.

Analyses of maternal PUFAs and child outcome were controlled for gestational age at venipuncture, as well as for sex and age of the child. In the analysis of total ω-3 and ω-6, as well as the analysis on individual fatty acids, all fatty acids were mutually adjusted for each other in order to test the independent association of total ω-3, total ω-6, and those of the individual fatty acids with child outcome. The other covariates were included in the adjusted models if they changed the effect estimates meaningfully (defined as more than 5%). Following this criterion, parity, marital status, pregnancy planning, child gestational age and weight at birth, breastfeeding status at 6 months, and paternal age and body mass index were excluded from the analyses. Analyses with child autistic traits as an outcome were additionally adjusted for child IQ. Analyses of maternal fish intake were additionally adjusted for daily total energy intake. Investigation of the covariate correlation matrix and the tolerance statistic for detection of collinearity showed that there were no bivariate correlations above 0.80 and no tolerance statistics below 0.20 (33).

Missing values on covariates (0.1%–30.4% missing data; average, 11.4%), child IQ (17.0%), and autistic traits (18.8%, imputed only if a Pervasive Developmental Problems score was available) were imputed by using the Markov Chain Monte Carlo multiple imputation technique with predictive mean matching for continuous variables and generating 5 data sets. All statistical analyses were carried out with PASW Statistics, version 21.0 for Windows, software (IBM Corp., Armonk, New York).

RESULTS

In the nonresponse analysis, we compared child and parental characteristics of the included participants (n = 4,624; 69.9%) and those without child behavioral data (n = 1,987)
Table 1. Associations of Maternal Fatty Acids During Pregnancy With Child Autistic Traits at 6 Years in the Generation R Study (n = 4,624), Rotterdam, the Netherlands, 2001–2012

<table>
<thead>
<tr>
<th>Maternal Fatty Acid</th>
<th>Basic&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Adjusted for Covariates&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Adjusted for Child IQ&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>95% CI</td>
<td>P Value</td>
</tr>
<tr>
<td>ω-3:ω-6 ratio&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-0.033</td>
<td>-0.040, -0.025</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total ω-3&lt;sup&gt;f&lt;/sup&gt;</td>
<td>-0.011</td>
<td>-0.020, -0.003</td>
<td>0.009</td>
</tr>
<tr>
<td>Total ω-6&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.032</td>
<td>0.022, 0.041</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ω-3&lt;sup&gt;h&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Linolenic acid</td>
<td>-0.005</td>
<td>-0.013, 0.003</td>
<td>0.19</td>
</tr>
<tr>
<td>Eicosapentaenoic acid</td>
<td>-0.002</td>
<td>-0.013, 0.008</td>
<td>0.65</td>
</tr>
<tr>
<td>Docosapentaenoic acid</td>
<td>-0.003</td>
<td>-0.012, 0.007</td>
<td>0.58</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>-0.005</td>
<td>-0.017, 0.008</td>
<td>0.44</td>
</tr>
<tr>
<td>ω-6&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>0.033</td>
<td>0.023, 0.044</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>0.022</td>
<td>0.013, 0.030</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adrenic acid</td>
<td>0.020</td>
<td>0.006, 0.033</td>
<td>0.006</td>
</tr>
<tr>
<td>Osbond acid</td>
<td>-0.004</td>
<td>-0.017, 0.008</td>
<td>0.50</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviations: CI, confidence interval; IQ, intelligence quotient; SD, standard deviation.
<sup>b</sup> Model 1: adjusted for gestational age at venipuncture, sex, and age of the child at assessment.
<sup>c</sup> Model 2: model 1, additionally adjusted for maternal IQ, prepregnancy body mass index, educational level, national origin, age at enrollment, psychopathology score in midpregnancy, smoking, alcohol consumption, and folic acid supplement use during pregnancy, as well as family income, child day-care attendance, and paternal educational level, national origin, and psychopathology score.
<sup>d</sup> Model 3: model 2, additionally adjusted for child IQ.
<sup>e</sup> Per SD: 1-unit increase equals an increase of 0.1 in the ω-3:ω-6 ratio.
<sup>f</sup> Expressed as percentage by weight, per SD: 1-unit increase equals an increase of 1.5% of total fatty acid composition.
<sup>g</sup> Expressed as percentage by weight, per SD: 1-unit increase equals an increase of 2.7% of total fatty acid composition.
<sup>h</sup> Expressed as percentage by weight, per SD: 1-unit increase in α-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid equals an increase of 0.1%, 0.3%, 0.2%, and 1.1% of total fatty acid composition, respectively.
<sup>i</sup> Expressed as percentage by weight, per SD: 1-unit increase in linoleic acid, arachidonic acid, adrenic acid, and osbond acid equals an increase of 2.8%, 1.6%, 0.1%, and 0.2% of total fatty acid composition, respectively.

Maternal PUFAs and child autistic traits

Table 1 shows the unadjusted and adjusted associations of maternal PUFA status with child autistic traits. A lower maternal ω-3:ω-6 ratio was associated with more child autistic traits (βω-3:ω-6 ratio = -0.009, 95% confidence interval (CI): -0.017, -0.001). In quintile analyses of the ω-3:ω-6 ratio, the lowest quintile was used as the reference category (quintile 1). Compared with this reference, a maternal ω-3:ω-6 ratio in the second highest quintile (quintile 4) was associated with less autistic traits in the offspring (β = -0.024, 95% CI: -0.047, -0.000; Figure 2). In sensitivity analyses, boys with weighted SRS scores above 1.078 and girls with scores over 1.000 were excluded (34), resulting in a sample of 4,535 children. The results show an effect size similar to that in the full sample (β = -0.008, 95% CI: -0.015, -0.000; P = 0.045).

In addition, higher total ω-6 levels were associated with more child autistic traits (βtotal ω-6 = 0.011, 95% CI: 0.002, 0.020). Of the individual ω-6 PUFAs, only linoleic acid...
remained associated with child autistic traits in fully adjusted analyses ($\beta = 0.012$, 95% CI: 0.001, 0.023). Including quadratic terms of the fatty acids in the model did not suggest nonlinear associations. No association was found between maternal total ω-3 and child autistic traits (Table 1). In secondary analyses, the lowest 10% or 5% of the ω-3 distribution compared with the remaining 90% or 95% of the distribution, respectively, was also not associated with autistic traits (fully adjusted models: $\hat{\beta}_{\text{lowest 10\%}} = 0.007$, 95% CI: −0.022, 0.035; $\hat{\beta}_{\text{lowest 5\%}} = -0.001$, 95% CI: −0.039, 0.036). Consistently, there were no associations of individual ω-3 PUFAs with child autistic traits (Table 1).

We did not find any indication that the observed association between maternal fatty acids and child autistic traits was moderated by maternal ethnicity (all interaction $P$’s > 0.10). Adjustment for child ω-3 and ω-6 intakes in the subsample with these data ($n = 2,436$) did not change the association of the ω-3:ω-6 ratio, total ω-6, or linoleic acid with child autistic traits meaningfully (Web Table 4).

### Maternal PUFAs and child IQ

The associations between maternal PUFA status and child IQ are presented in Table 2. Whereas the maternal ω-3:ω-6 ratio was associated with child IQ ($\beta = 0.52$, 95% CI: 0.03, 1.00), total ω-3 and total ω-6 were not. Associations of the maternal ω-3:ω-6 ratio, total ω-6, and linoleic acid status with child autistic traits did not change after adjustment for child IQ (Table 1).

To further investigate potential bias due to attrition, we additionally created a data set in which all missing data on both covariates and outcome (i.e., child SRS score and IQ score) were imputed for those mother-child pairs that were eligible at baseline ($n = 6,611$) but did not have valid outcome data available ($n = 1,987$). Results show very similar effect estimates with somewhat larger confidence intervals (Web Tables 5 and 6).

### Maternal fish intake and child autistic traits/IQ

In a subsample with prenatal fish intake data available, we tested the association of maternal fish intake during pregnancy with child autistic traits ($n = 3,802$) and IQ ($n = 3,162$) (Web Table 7). Fish intake was not associated with child autistic traits or IQ after adjustment for confounders.

## DISCUSSION

In this population-based study, a lower maternal ω-3:ω-6 ratio during pregnancy was associated with more autistic traits in the offspring at age 6 years. This association was largely accounted for by relatively higher ω-6, in particular, higher maternal linoleic acid. Maternal fish intake, a major source of ω-3, during pregnancy was not associated with child autistic traits.

A low ω-3:ω-6 ratio and high intake of ω-6 PUFAs in adults have been hypothesized to promote pathogenesis of various diseases, such as cardiovascular disease, cancer, and inflammatory and autoimmune diseases (35). With regard to ASD in children, little is known about the potential effects of maternal PUFA intake and even less so for maternal PUFA blood status. We found a lower maternal ω-3:ω-6 ratio and higher total ω-6 PUFA status in midpregnancy to be associated with more offspring autistic traits. This is in line with findings by Strain et al. (36), who reported that a lower ω-3:ω-6 ratio in maternal blood at 28 weeks of gestation was associated with poor communicative development in children at 20 months.

Blood concentrations of PUFAs reflect dietary intake and biological processes such as the endogenous synthesis of α-linolenic acid and linoleic acid to their long chain end products, docosahexaenoic acid and arachidonic acid, respectively. During this synthesis, the ω-3 and ω-6 PUFAs compete for the same enzyme systems (6). As a result, higher intake of ω-6 PUFAs will decrease the synthesis of ω-3 PUFAs, and vice versa. Additionally, fatty acid desaturase genotypes are involved.

### Table 2. Associations of Maternal Fatty Acid Status During Pregnancy With Child Intelligence Quotient at 6 Years in the Generation R Study ($n = 3,838$), Rotterdam, the Netherlands, 2001–2012

<table>
<thead>
<tr>
<th>Maternal Fatty Acid</th>
<th>IQ Score</th>
<th>Basic*</th>
<th>Adjusted for Covariatesb</th>
<th>P Value</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\omega$-3:$\omega$-6 ratio&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.65</td>
<td>2.19, 3.11</td>
<td>&lt;0.001</td>
<td>0.52</td>
<td>0.03, 1.00</td>
<td>0.038</td>
</tr>
<tr>
<td>Total $\omega$-3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.21</td>
<td>0.67, 1.74</td>
<td>&lt;0.001</td>
<td>0.26</td>
<td>−0.28, 0.79</td>
<td>0.34</td>
</tr>
<tr>
<td>Total $\omega$-6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>−2.18</td>
<td>−2.71, −1.65</td>
<td>&lt;0.001</td>
<td>−0.44</td>
<td>−0.99, 0.12</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; IQ, intelligence quotient; SD, standard deviation.

* Model 1: adjusted for gestational age at venipuncture and sex of the child.

b Model 2: model 1, additionally adjusted for maternal IQ, prepregnancy body mass index, educational level, national origin, age at enrollment, psychopathology score in midpregnancy, smoking, alcohol consumption, and folic acid supplement use during pregnancy, as well as family income, child day-care attendance, and paternal educational level, national origin, and psychopathology score.

c Per SD: 1-unit increase equals an increase of 0.1 in the $\omega$-3:$\omega$-6 ratio.

d Expressed as percentage by weight, per SD: 1-unit increase equals an increase of 1.5% of total fatty acid composition.

e Expressed as percentage by weight, per SD: 1-unit increase equals an increase of 2.7% of total fatty acid composition.

in this synthesis pathway and modulate the amounts of PUFA in the blood (37). The association of the maternal ω-3:ω-6 ratio with child autistic traits was accounted for by the ω-6 rather than the ω-3 PUFA status. In particular, we found higher maternal linoleic acid status to be associated with more autistic traits. It should be kept in mind, however, that linoleic acid is the largest contributor to total ω-6 status and, as such, can be measured with more precision than the other ω-6 PUFAs. Additionally, the limited range of total ω-3 status in our study sample may make a potential association between low ω-3 status and child autistic traits hard to detect.

An earlier study reported that higher dietary ω-6 and linoleic acid intake were associated with a lower risk of autism (12). Yet, although correlated, dietary intake and biomarker concentrations cannot be compared straightforwardly. First, as mentioned earlier, biomarkers also reflect biological metabolism and are a more genetically determined characteristic. Second, dietary intake data are prone to information bias due to over- and underreporting on food frequency questionnaires (38). When the same informant (mother) additionally reports on the study outcome, shared method variance bias is introduced (39). A potential solution is the use of nutritional biomarkers that are able to objectively assess dietary status without the bias of self-reported dietary intake errors (40).

ω-3 and ω-6 PUFAs play an important role in various neurodevelopmental processes. The mechanism underlying the association of the maternal ω-3:ω-6 ratio with child autistic traits could be explained by insights from animal studies (8). Studies in mice showed that both ω-3 and ω-6 PUFAs are required for the maturation of nerve growth cones and subsequent synapse formation (41). Rat studies have shown that maternal dietary fatty acids can alter the fetal brain growth cone ω-3 and ω-6 levels (42). Impaired synapse formation has been indicated as a core mechanism in ASD pathology (43). Moreover, ω-3 and ω-6 PUFAs are endogenous ligands for peroxisome proliferator-activated receptors, which are involved in myelination (44). Indeed, expression of proliferator-activated receptors in young mice was positively related to the dietary ω-3:ω-6 ratio during maternal pregnancy and lactation (45), a pathway promoting early brain development.

Finally, this type of nutritional associations in observational studies is sensitive to the effects of confounding factors. A substantial attenuation in the association of maternal PUFA status with the offspring’s autistic traits was noticeable after adjustment for confounders. Although we accounted for many sociodemographic factors, further (residual) confounding cannot be excluded.

Other limitations include selective attrition and possible information bias. Mothers of children not included generally had less favorable PUFA status and socioeconomic circumstances. Whether this selective attrition leads to bias cannot be inferred from our results. However, we considered multiple imputation for the outcome and found very similar effect estimates with somewhat larger confidence intervals. Next, our study was limited by the fact that clinical diagnoses of autism were not at our disposal. Autistic traits are defined as subclinical deficits in socialization, communication, and restricted/stereotypic behaviors that do not meet the formal criteria for an ASD diagnosis (18). However, although not meeting strict diagnostic criteria, comorbid psychiatric traits and psychosocial difficulties have been reported in individuals with increased levels of autistic traits (46). Further, our assessment of PUFA status was based on a single measure of blood plasma in midpregnancy. One measurement, although indicative, is not a reliable reflection of a mother’s long-term nutrient status (47). However, repeated measurements were not feasible in this large study.

In conclusion, we found the maternal plasma ω-3:ω-6 ratio and ω-6 PUFA status in midpregnancy to be associated with child autistic traits at age 6 years. The associations were independent of child intelligence, suggesting that the fatty acid distribution specifically affects the development of autistic traits in addition to general neurodevelopment. Our study suggests that this biological pathway possibly explains the association of maternal dietary PUFA intake during pregnancy and development of ASD in the offspring. Maternal plasma ω-3 status and fish intake during pregnancy were not associated with child autistic traits. Possibly, the focus of dietary interventions should not only be on increasing ω-3 intake, but also on lowering the intake of food products with high ω-6 content. More research is needed to confirm our findings. Additionally, future research on optimal ω-3 and ω-6 PUFA intake should also include blood PUFA concentrations to account for endogenous biological processes that determine the actual bioavailability of ω-3 and ω-6 PUFAs in the human body.

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