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Maternal polyunsaturated fatty acids in association with child autism spectrum disorder in the MARBLES high-risk study

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Abstract

Background: Prior research suggest that maternal polyunsaturated fatty acids (PUFAs) could have protective effects on neurodevelopmental outcomes.

Objective: To examine associations between maternal PUFA intake during pregnancy and risk for autism spectrum disorder (ASD) and other non-typical development (Non-TD) in a prospective cohort.

Design: Eligible women already had a child with ASD and were planning a pregnancy or were pregnant with another child. Children were clinically assessed longitudinally and diagnosed at 36 months. Maternal PUFA intake during pregnancy was estimated using food frequency questionnaires. Maternal third-trimester plasma PUFA concentration was measured by Gas Chromatography.

Results: 258 mother-child pairs were included. Mothers consuming more total omega3 in the 2nd half of pregnancy were 40% less likely to have children with ASD (RR = 0.6, 95% CI: 0.3–0.98). No significant associations were observed between maternal third-trimester plasma PUFA subtype concentrations and risk of ASD. However, higher plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations were associated with lower Non-TD risk (RR ranging from 0.93–0.99).

Conclusions: This study provides suggestive evidence on associations between maternal omega3 intake during pregnancy and risk of ASD in the children but not with third-trimester plasma PUFAs. Further research is needed to evaluate these potential relationships.

Lay Abstract

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Conflict of Interest:
The authors declare that they have no conflict of interest.

Ethical approval:
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent:
Informed consent was obtained from all individual participants included in the study.

Prior studies suggest that maternal polyunsaturated fatty acids (PUFAs) intake during pregnancy may have protective effects on autism spectrum disorder (ASD) in their children. However, they did not examine maternal PUFA intake by detailed timing during pregnancy as well as via evaluating levels in plasma samples. This study investigates whether maternal PUFAs in defined time windows of pregnancy, assessed by both questionnaires and biomarkers, are associated with risk of ASD and other non-typical development (Non-TD) in the children. Food frequency questionnaires were used to estimate maternal PUFA intake during the first and second half of pregnancy. Gas Chromatography measured maternal plasma PUFA concentrations in the third-trimester. 258 mother-child pairs from a prospective cohort were included. Mothers were those who already had a child with ASD and were planning a pregnancy or pregnant with another child. Children were clinically assessed longitudinally and diagnosed at 36 months. For PUFA intake from questionnaires, we only found mothers consuming more omega3 in the second half of pregnancy were 40% less likely to have children with ASD. For PUFA concentrations in the third-trimester plasma, we did not observe any statistical significance in relation to the risk of ASD. However, our study confirmed associations from previous studies between higher maternal docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) plasma concentrations in the late pregnancy and reduced risk for Non-TD. This study markedly advanced understandings of whether and when maternal PUFA intake influence risk for ASD and set the stage for prevention at the behavioral and educational level.

Keywords

omega3; omega6; polyunsaturated fatty acids; dietary fat; autism; pregnancy

Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that affects approximately 1 in 59 children in the United States (Baio et al. 2018). ASD is characterized by impaired social interaction and communication as well as restricted interests and/or stereotyped behaviors, and its prevalence has increased over the past twenty years. This increase can partly be explained by changes in diagnostic criteria and greater awareness (Hertz-Picciotto and Delwiche 2009); however environmental factors could also play a critical role (Hallmayer et al. 2011; Levine et al. 2018).

Polyunsaturated fatty acids (PUFAs), especially omega3, play significant roles in the structural and functional development of human brains (Freeman et al. 2006; Haggarty 2004). Maternal diet strongly influences the fetal PUFA supply (Peet et al. 1996; Richardson et al. 2000a). Dietary and supplemental omega3 consumption during pregnancy have already been associated with improved neurodevelopmental outcomes in children, such as intelligence quotient (IQ) (Richardson et al. 2000b), cognitive and social skills (Richardson and Ross 2000). However, relationships between maternal PUFA intake and children's risk of ASD are not well understood. Although some previous studies (Lyall et al. 2013; Steenweg-de Graaff et al. 2016; Suren et al. 2013) suggest maternal PUFA insufficiencies, including lower omega3 and linoleic acid (LA), are significantly related to either higher ASD risk or more autistic traits in their offspring, these studies were unable to examine

maternal intake by detailed timing during pregnancy. Moreover, they only used self-reported intake and did not access these relationships using maternal PUFA concentrations in plasma during pregnancy. Plasma PUFA concentrations could reflect dietary PUFA status over the past 1 to 4 weeks (Harris and Thomas 2010), and are considered to be more representative of real PUFA status. Understanding these associations is of particular importance given the substantial evidence linking higher maternal intakes of certain nutrients and vitamin supplements to a reduction in the ASD risk (Lyall et al. 2014).

In this study, we used a prospective cohort design to investigate maternal PUFAs during pregnancy in defined time windows, assessed by both questionnaires and biomarkers, in relation to ASD or other non-typical development (non-TD) in the offspring at 3 years of age. Omega3, specifically docosahexaenoic acid (DHA), becomes incorporated into the phospholipid membrane of retina and brain and accumulate quickly during the latter part of pregnancy, primarily in the third trimester (Denomme et al. 2005; Greenberg et al. 2008; Jacobson et al. 2008; Jensen 2006). These accumulations are preferentially transferred across the placenta, suggesting a particular need for PUFAs at this time period (Hornstra 2000; Innis 2000; Innis 2007a; Innis 2007b). On this basis, we hypothesized that higher maternal omega3 during late pregnancy would be associated with reduced ASD risk in the offspring.

Methods

Study Population

*M*arkers of *A*utism *R*isk in *B*abies-*L*earning *E*arly *S*igns (*MARBLES*) study is the first prospective cohort study to recruit mothers of children with ASD in a subsequent pregnancy, who were thus at high risk for delivering another child with ASD. Families were recruited from lists of children receiving autism services obtained via the California Department of Developmental Services Department of Developmental Services (DDS), from other studies at the MIND Institute, or by self-referrals. The inclusion criteria were: a) mother or father had a biological child with ASD, and the mother was b) at least 18 years old; c) pregnant or planning a pregnancy, and biologically able to become pregnant; d) living within 2 hours of the MIND Institute; e) sufficiently fluent in English. Participants in this study enrolled into the *MARBLES* study between 2006 and 2019, and included only one child per family. Both the Institutional Review Board at the University of California, Davis and the State of California Committee Committee approved this study.

Outcome

At the 36-month visit, children were classified into 1 of 3 algorithmically-defined neurodevelopmental outcome groups: ASD, typical development (TD), and non-TD (and not ASD). The outcome algorithm (Schmidt R.J., et.al. 2019) was defined following previously published methods from the Baby Siblings Research Consortium (Chawarska et al. 2014; Ozonoff et al. 2015). It was derived from 4 subscale score (fine motor, expressive language, receptive language, and visual reception) on the Mullen Scales of Early Learning (MSEL) (EM 1995) as well as the scores on Autism Diagnostic Observation Schedule (ADOS) (Lord et al. 2000). Children with an ASD diagnosis scored over the ADOS cutoff as well

as met Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria for ASD (American Psychiatric Association (2013). Children with TD outcomes had all MSEL T-scores within 2 standard deviations (SD) and no more than 1 MSEL subscale score that was 1.5 SD below the normative mean, while their ADOS scores were at least 3 or more points below the ADOS cutoff. All the rest of children were categorized into the Non-TD group (e.g., those with low MSEL or high ADOS scores, who did meet criteria for ASD).

Maternal PUFA measurements

a. Questionnaire: maternal dietary PUFA intake—We used the Block 2005 Food Frequency Questionnaire (FFQ) (Johnson et al. 2007) to assess the comprehensive history of maternal dietary intake in the 1st and 2nd half of pregnancy. It has 114 food items, and each item has 9 frequency options, ranging from never to daily, and several quantity options, such as 1/4 cup per day and 2 cups per day. Individual portion size was also asked for each food, and pictures were provided to enhance the accuracy of quantification. Block FFQs were sent to NutritionQuest (Berkeley, California) to calculate nutrients as previously described (Johnson et al. 2007). The frequency of each food item was defined as the decimal fraction from 0 (=Never) to 1 (=Every day), and the proportion of each item was converted into grams. The formula used in the calculation of average nutrients per day was: ((frequency * proportion)/100) * (nutrient per 100g). Nutrient per 100g were derived from Food and Nutrient Database for Dietary Survey (FNDDS), United States Department of Agriculture (USDA) data files, published sources, imputation and manufactures or label data. At last, the average daily nutrients for all foods were added up to obtain the full average dietary intake per day, including omega3, omega6, DHA, eicosapentaenoic acid (EPA), alpha-linolenic acid (ALA), arachidonic acid (AA), and LA.

b. Biomarker: maternal PUFA concentrations—Third trimester maternal plasma samples were collected in sodium citrate vacutainers, processed the same day, and immediately placed into −80 °C freezers for storage. Staff working with these samples received annual biosafety training, took precautions such as personal protective equipment, and followed laboratory safety standards. The sample aliquots of 100 ul were then shipped to the analytical lab OmegaQuant (Sioux Falls, South Dakota), which is CLIA-certified (Number: 43D1105229). As previously described (Harris et al. 2013; Jimenez et al. 2015), internal standard (C17:0 or C 23: 0 in chloroform) was added to heparinized plasma samples, and vortex-mixing methods were used to extract fatty acids twice. After centrifugation, the chloroform extract was combined, dried and then used BF3 in methanol to hydrolyze and methylate to fatty acid methyl esters. Samples were extracted twice with n-hexane and quantitatively measured by a capillary GC2010 Gas Chromatograph (Shimadzu). PUFA concentrations were identified by comparison with a standard mixture of fatty acids characteristic of plasma (GLC 727, NuCheck Prep), which were also used to determine individual PUFA response factors. 5 PUFA plasma concentrations (mg/l) were sent back to us and included in this study, including LA, ALA, AA, EPA, DHA.

c. Questionnaire: maternal supplemental PUFA intake—As described in more detail elsewhere (Hertz-Picciotto I 2017), trained interviewers obtained data on omega3 supplementation via telephone calls with the mother. She was asked whether or not each

item had been consumed and, if so, what brand and dose had been consumed, how frequently, and in which months (1st month of pregnancy and continuing throughout each month of gestation) the supplement was taken. Based on this information, we calculated average daily intake for each product and summed all values to a total omega3 supplementation amount per month (100 mg/day, 1st month to 9th month or end of pregnancy) for each woman.

Statistical Analyses

Descriptive and univariable analyses were conducted to summarize maternal PUFA intake, child outcomes, and demographic characteristics. When examining associations between maternal PUFAs and diagnosis of ASD or Non-TD, we first investigated maternal PUFA intake collected from the FFQ questionnaire during the 1st and 2nd half of pregnancy in relation to risk of ASD and Non-TD in children, including total omega3, omega6, DHA, EPA, ALA, AA and LA. In the secondary analyses, we explored relationships between maternal PUFA plasma concentrations in the 3rd trimester, including DHA, EPA, ALA, AA and LA, and risk of ASD and Non-TD in the offspring. Sensitivity analyses were also conducted for PUFA plasma samples in the 3rd trimester with no previous thaws. In addition, we evaluated associations between maternal omega3 supplementation from the 1st to 9th month during pregnancy and risk of both ASD and Non-TD in the offspring.

Potential confounders were those who had been reported to be associated with either ASD or non-TD in previous publications. Demographic variables included: maximum education in the household (categorized as either “some college or less”, “bachelor’s degree”, or “graduate or professional degree (i.e., MD, DDS, and DVM), maternal pre-pregnancy body mass index (BMI) (categorized as either “normal and underweight” (<25 kg/m^2), “overweight” (25–30 kg/m^2), or “obese” (> 30 kg/m^2)), mom’s race (categorized as either “white” or “non-white”), maternal age (years), paternal age (years), gestational age (days) at delivery and at sample collection. We also considered other maternal nutrition intake factors from both dietary intake and supplement during the same pregnancy period as each exposure of interest, including total energy intake (kcals), dietary folate equivalents intake (DFE, mcg), iron intake (mg), calcium intake (mg), magnesium intake (mg), vitamin B1 intake (mg), vitamin B2 intake (mg), vitamin C intake (mg), vitamin E intake (mg), vitamin B6 intake (mg), vitamin A intake (mcg), vitamin B12 intake (mcg), vitamin K intake (mcg), folic acid supplement (yes/no), calcium supplement (yes/no), vitamin C supplement (yes/no), vitamin E supplement (yes/no), vitamin B6 supplement (yes/no), vitamin A supplement (yes/no) and vitamin B12 supplement (yes/no). Moreover, to account for sample quality, we also examined laboratory variables, including sample numbers of thaws (categorized as either “0” or “>1”) and storage time (days).

Multinomial logistic regression was used to examine associations between maternal PUFAs and diagnosis of ASD or Non-TD outcome relative to TD. Since our exposures were from different sources, model building was carried out separately for each exposure of interest to ensure correct confounders in final models. Bivariate analyses examined unadjusted associations of potential confounders with both outcomes (ASD and non-TD) and the exposure of interest separately to identify those that were broadly associated (P<0.3).

Then, multivariable models were built, separately for ASD and non-TD risk, via adding 1 variable at a time to the multinomial logistic model and retaining those that caused at least a 10% change in the exposure parameter estimates. This approach led to a various set of confounders for different exposures. 5 variables met our model selection criteria across all models, including maternal food iron intake (outcome: dietary omega3 intake, omega6 intake, LA intake, ALA intake, LA plasma concentration and ALA plasma concentration), gestational age at delivery (outcome: ALA plasma concentration), sample numbers of thaws (outcome: all plasma concentration), sample storage time (outcome: AA, EPA and DHA plasma concentration), and paternal age (outcome: EPA plasma concentration, supplemental omega3 intake). After selecting final models, multivariable adjusted relative risk (RR) [with 95% confidence intervals (CI)] for ASD and non-TD were calculated directly using SAS macro %RELRISK9 (Wacholder 1986). All analyses were carried out using SAS version 9.4 (SAS Institute Inc., Cary, NC). Tests were two-sided, with $\alpha = 0.05$.

Results

258 (57 ASD, 62 Non-TD, and 139 TD) mother-child pairs were eligible for the study and had maternal supplemental PUFA intake information. Among them, 32 (56 %) ASD, 33 (53 %) Non-TD and 82 (59 %) TD reported the FFQ questionnaire on maternal PUFA dietary intake in the 1st half of pregnancy, while 30 (52%) ASD, 31 (50%) Non-TD and 70 (50%) TD had these information on the 2nd half of pregnancy. Of the 252 participant pairs who have information on maternal plasma PUFA concentrations in the third trimester, 218 (87%) children had 36-month diagnoses, including 50 (88%) ASD, 52 (84%) Non-TD, and 116 (83%) TD.

Characteristics of mother and child pairs are shown in Table 1. Children with ASD were significantly more likely to have longer gestational age ($P = 0.0002$), and their mothers' plasma samples were stored for shorter periods ($P = 0.02$). Mothers with ASD children had borderline significantly lower iron intake from food in the 2nd half of pregnancy than mothers of TD children ($P = 0.06$), but their total energy intake was not significantly different. Mothers of Non-TD children were more likely to be non-white, compared to those of TD children ($P = 0.04$).

In the 2nd half of gestation, maternal mean dietary intake of total omega3 (unit: gms/day) in mothers of children with ASD (1.2, standard deviation (SD) = 0.7) was significantly lower than in those of children with TD (1.5, SD=0.6, $P = 0.04$). After adjusting for confounders, this association was still evident (RR: 0.6, 95% CI: 0.3–0.98) (Table 2). However, no other statistically significant associations were found for all other PUFA subtypes intake during both the 1st and 2nd half of pregnancy (Table 2).

All maternal fatty acid concentrations in the 3rd trimester plasma were similar among mothers of children with ASD or TD in both unadjusted and adjusted multivariable analyses (Table 3). However, after adjusting for confounders, higher maternal EPA (RR = 0.927, CI: 0.868–0.990) and DHA (RR = 0.987, CI: 0.975–0.998) plasma concentrations were significantly associated with lower risk of Non-TD in their children (Table 3). However,

in our sensitivity analyses for PUFA samples with no previous thaws, we did not observe similar significant results (Supplmentary S1).

Additionally, there were no significant associations between average intake of supplemental omega3 from vitamins and supplements (100 mg/day) during pregnancy (1st month to 9th month) and risk of ASD (RR is ranging from 0.91 to 1.00) or Non-TD (RR is ranging from 0.96 to 0.99) after adjusting for confounder, including paternal age.

Discussion

In our study, we found that higher maternal total omega3 intake in the 2nd half of pregnancy was statistically associated with 40% lower risk of ASD in the child. However, intake of PUFA subtypes, including LA and ALA, were not statistically associated with risk of ASD in both 1st and 2nd half of pregnancy, even after adjusting for confounders. These non-significant associations were also observed in plasma measures of PUFA subtypes in the 3rd trimester, which might also suggest the protective effects of total omega3 instead of their subtypes on child risk of ASD. The total amount of omega3 collected by the FFQ contained other fatty acids, such as docosapentaenoic acid (DPA) and stearidonic acid (SDA), which were not measured in the plasma of this study. However, the limited statistical power to detect small effect sizes in this study could also have contributed to the lack of associations observed for omega3 in plasma, given the effect sizes of omega3 subtypes in the late pregnancy were in the same direction as that of the total omega3. In addition, our study confirmed relationships from general population studies between higher maternal DHA and EPA concentrations in the late pregnancy and reduced risk for Non-TD in a high-risk ASD younger sibling population.

Taking purified fish oil supplements during pregnancy is highly recommended by physicians (Genuis and Schwalfenberg 2006). Our findings suggest that maternal omega3 intake in the 2nd half of gestation has a protective association with the child's risk of ASD. Similar findings have been documented in a sub-cohort of the Nurses' Health Study (NHS) II with 317 mother-child pairs, where researchers showed that children whose mothers had very low omega3 consumption during pregnancy were associated with increased risk of ASD (Lyall et al. 2013) in the offspring. This study used the Willett FFQ to measure PUFA intake, which is different with our Block FFQ in several aspects, including food items and portion size; However, these two questionnaires have been demonstrated to have the similar capacity to predict diet-disease risk (Caan et al. 1998; Subar et al. 2001). We also observed that the 2nd half of pregnancy appeared to be a critical time window for this association, which is consistent with previous evidence about the significant influence of PUFAs in the late pregnancy upon neurodevelopmental outcomes. For example, studies reported that the third trimester is a critical time for maternal DHA intake levels in relation to child cognitive development (Rees et al. 2014). Previous studies have also reported that children whose mothers consumed cod liver oil (rich in omega3) in late pregnancy had improved IQ and mental processing scores at 4 years old (Helland et al. 2003; Willatts 2002). Nevertheless, in our study, all other subtypes of maternal PUFA dietary and supplemental intake, such as DHA and EPA, were similar among mothers of children with ASD or TD within all-time windows. Same findings were also observed in maternal plasma samples in the 3rd trimester.

These results are consistent with a study in the Norwegian Mother and Child cohort with 85,176 participants (Suren et al. 2013). Fish oil supplements (mainly EPA+DHA) consumed from 4 weeks before pregnancy to 8 weeks after pregnancy were reported to be not associated with risk of ASD in the children. Similarly, a randomized clinical trial with 726 mother-child pairs also observed that autism diagnoses did not differ in groups with or without prenatal DHA supplementation (Makrides et al. 2014). This could be evidence for residual confounding, given intake may be related to other healthy behaviors.

The finding that there is an association between maternal omega3 intake in late pregnancy and the likelihood of offspring's risk of ASD is noteworthy. This relationship might be due to biological effects of omega3 fatty acids on brain development. Omega3 fatty acids occupy 20% of the brain's dry weight (Bourre et al. 1991; Freeman et al. 2006), and previous animal studies indicate that it has potential therapeutic effects on ASD symptoms as well as other cognitive and behavioral capacities (Davis-Bruno and Tassinari 2011; Neuringer et al. 1986). Since omega3 fatty acids cannot be synthesized by the fetus and must be provided by placental transfer from the mother, prenatal intake is critical for brain development in later stages of gestation (Genuis and Schwalfenberg 2006) and plays important roles in gene expression, signal transduction and as components of cell membranes (Casper 2004; Richard J Deckelbaum 2006). Animal studies have also shown that omega3 fatty acid deprivation during pregnancy was associated with behavioral deficits, which could not be reversed with postnatal supplementation (Nesheim MC 2007). Moreover, omega3 fatty acids are anti-inflammatory precursors (Green et al. 2008) and may be able to counter damage from neuroinflammation, which has been demonstrated in some individuals with ASD (Careaga et al. 2010; Onore et al. 2012).

Several limitations of the FFQ dietary data should be considered when interpreting our findings. First, since FFQ is a self-administrated questionnaire with a fixed list of foods, the reported amount may be underestimated if the individual consumed food items that were not on the list. For example, although salmon is a primary contributor to the omega3 fatty acid intake, salmon consumption was not specifically asked in this FFQ version. However, this information might have been captured by the "Other fish. Not fried" question in the FFQ. Second, although the FFQ asked participants to report portion sizes, individuals may not be able to describe and conceptualize food sizes accurately, and substantial within-person variation may exist (Haraldsdottir et al. 1994). Under these circumstances, non-differential misclassification might have occurred, which could have weakened the observed associations, especially for specific subtypes of PUFAs (WC 1998).

Our study is also the first of which we are aware to investigate maternal PUFA plasma concentrations in the third trimester and risk of ASD or Non-TD in high-risk children. Even though findings on statistical associations between subtypes and risk of ASD are consistent with results from our questionnaire, these results should also be interpreted cautiously due to the differences between these two methods. First, the plasma PUFA concentrations may not be highly correlated with the amount of the FFQ dietary intake. Plasma is a sensitive marker of short-term changes in fatty acid intake (Harris and Thomas 2010) and easily affected by food intake changes across seasons or even throughout the courses of a single day. FFQs, on the other hand, are designed to measure long-term habits. Moreover, the FFQ

data were completed for the entire 2nd half of pregnancy in our study, thus representing a longer period than the plasma samples which were specifically collected in the third trimester and represented episodic status. Moreover, because the 2–4 weeks within the trimester represented by our plasma measurement differed across individuals, this could have averaged out and thus muddied our exposure measurement and comparisons within the trimester, which would attenuate our ability to find differences by adding non-differential bias towards the null. Additionally, total omega 3 plasma concentrations were lacking, and future studies with this information should be conducted to replicate our questionnaire findings.

In this prospective cohort study, detailed information on demographic factors, medical conditions, maternal nutrient intake during pregnancy and laboratory information were systematically collected and examined as potential confounders. However, limitations of the study design still should be noted while interpreting our results. First, this study was conducted in a high-risk population of families affected by ASD, therefore, our findings might not be generalizable to the general U.S. population. Second, we only measured the third trimester plasma PUFA concentrations, because the late pregnancy was hypothesized to be the critical window for these relationships based on results from previous studies of other neurodevelopmental outcomes (Denomme et al. 2005; Greenberg et al. 2008; Jacobson et al. 2008; Jensen 2006). However, we acknowledge that critical windows could differ across neurodevelopmental disorders with unique etiologies. We did not assess PUFA biomarkers in early pregnancy, which could be a critical period for ASD development (Levine et al. 2018; Lyall et al. 2014). Additionally, we did not have information on concentrations of fish contaminants including mercury. While mercury is a common contaminant of fish, one of the main sources of PUFAs, Kern and colleagues (Kern et al. 2016) recently reviewed and summarized that the majority (74%) of studies suggest that mercury is a risk factor for ASD development, revealing both direct and indirect effects. Thus, it is possible that mercury and other fish contaminants might be a potential and uncontrolled confounder in our analyses.

Conclusion

In summary, this prospective study of high-risk younger sibling pregnancies provides evidence for an association between higher maternal total omega3 fatty acid intake in the 2nd half of pregnancy and reduced risk of ASD in the offspring. However, no statistical significances are observed for all other PUFA subtypes from both self-reported questionnaires and third-trimester plasma. Future studies with larger sample size, mercury measurement, and measures at different time during pregnancy should seek to replicate these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Demographic and Clinical Characteristics of Children and Their Mothers

	TD (*n*=139)	ASD (*n*=57)		Non-TD (*n*=62)	
		Value	*P*	Value	*P*
Maximum education in the household[1], ***n* (%)**					
Some college or less	52 (37.4)	25 (46.3)	0.46	27 (43.6)	0.70
Bachelor's degree	49 (35.3)	18 (33.3)		19 (30.7)	
Master's, professional or doctoral degree	38 (27.3)	11 (20.4)		16 (25.8)	
Maternal pre-pregnancy BMI[2], ***n* (%)**					
Underweight and normal	66 (48.5)	23 (40.4)	0.58	29 (47.5)	0.61
Overweight	38 (27.9)	19 (33.3)		14 (23.0)	
Obese	32 (23.5)	15 (26.3)		18 (36.0)	
Maternal race[3], ***n* (%)**					
White	84 (60.4)	29 (51.8)	0.27	28 (45.2)	0.04
Non-white	55 (39.6)	27 (48.2)		34 (54.8)	
Number of thaws for samples[4], ***n (%)***					
0	60 (81.1)	29 (93.6)	0.10	28 (82.3)	0.87
≥1	14 (18.9)	2 (6.5)		6 (17.7)	
Maternal age, *mean* (*SD*), *y*	33.9 (5.0)	34.3 (5.3)	0.63	34.5 (4.3)	0.37
Paternal age[5], ***mean* (*SD*), *y***	36.1 (6.2)	37.7 (5.9)	0.10	37.1 (4.6)	0.27
Gestational age at delivery[6], ***mean* (*SD*), *d***	270.4 (11.3)	278.2 (6.4)	0.0002	272.2 (9.3)	0.36
Gestational age at sample collection[6], ***mean* (*SD*), *d***	235.1 (21.6)	230.2 (20.2)	0.40	225.3 (20.4)	0.10
Plasma sample storage time[7], ***mean* (*SD*), *d***	373.6 (76.3)	332.7 (77.7)	0.02	368.5 (91.6)	0.76
Dietary folate equivalents[8], ***mean* (*SD*), *mcg***					
1st half of pregnancy	457.8 (219.5)	475.4 (196.7)	0.70	442.5 (147.2)	0.71
2nd half of pregnancy	481.8 (192.0)	436.4 (257.4)	0.33	476.6 (191.3)	0.90
Maternal food iron intake[8], ***mean* (*SD*), *mg***					

	TD (*n*=139)	ASD (*n*=57)		Non-TD (*n*=62)	
		Value	*P*	Value	*P*
1st half of pregnancy	11.2 (4.8)	11.0 (3.8)	0.89	11.6 (4.1)	0.67
2nd half of pregnancy	12.3 (4.2)	10.4 (5.4)	0.06	12.3 (4.6)	0.99
Maternal total energy intake[8]**, *mean* (*SD*), *kcals***					
1st half of pregnancy	1545.2 (611.3)	1466.6 (504.4)	0.53	1582.1 (433.0)	0.74
2nd half of pregnancy	1641.4 (527.4)	1466.7 (798.2)	0.20	1622.8 (448.4)	0.86

Abbreviations: TD, typical developing; ASD, autism spectrum disorders; Non-TD, nontypical development; BMI: body mass index; SD: standard deviation.

P values for categorical variables were derived from chi-square tests comparing the ASD or Non-TD (separately) to the TD group; *P* values for continuous variables were derived from two sample t- tests comparing ASD or Non-TD (separately) to the TD group;

[1]Professional degree is a degree that prepares someone to work in a particular profession, often meeting the academic requirements for licensure or accreditation. Data missing for 3 children in ASD group;

[2]Calculated by NIH standard categories for obesity; Underweight and normal: 18–25, Overweight: 25–30, Obese:>30; Data missing for 3 children in TD group and 1 in non-TD group;

[3]Data missing for 1 child in ASD group;

[4]Data missing for 65 children in TD group, 26 in ASD and 28 in Non-TD;

[5]Data missing for 4 children in TD group, 2 in ASD and 3 in Non-TD;

[6]Data missing for 52 children in TD group, 20 in ASD and 20 in Non-TD;

[7]Data missing for 65 children in TD group, 26 in ASD and 28 in Non-TD;

[8]Data missing for 57 children in TD group, 27 in ASD and 29 in Non-TD in the 1st half of pregnancy; Data missing for 69 children in TD group, 27 in ASD and 31 in Non-TD in the 2nd half of pregnancy;

Table 2.

Unadjusted and Adjusted Associations of Maternal PUFA Dietary Intake (gms/day) during Pregnancy with Child Outcome

	36-mo Outcome, mean (SD)			Un-adjusted RR (95% CI)		Adjusted RR (95% CI)	
	TD	ASD	Non-TD	ASD vs. TD	Non-TD v.s. TD	ASD vs. TD	Non-TD v.s. TD
Omega3 [1]							
1st half of pregnancy	1.4 (0.7)	1.2 (0.5)	1.6 (0.9)	0.8 (0.5, 1.3)	1.2 (0.9, 1.7)	0.7 (0.4, 1.3)	1.3 (0.9, 1.8)
2nd half of pregnancy	1.5 (0.6)	1.2 (0.7)	1.5 (0.6)	0.4 (0.3, 0.8)	0.97 (0.6, 1.5)	0.6 (0.3, 0.98)	0.96 (0.6, 1.5)
Omega6 [1]							
1st half of pregnancy	11.2 (5.4)	10.6 (3.9)	12.2 (5.3)	0.98 (0.9, 1.1)	1.0 (1.0, 1.1)	0.97 (0.9, 1.1)	1.0 (1.0, 1.1)
2nd half of pregnancy	12.3 (4.8)	10.4 (5.9)	11.9 (4.9)	0.9 (0.9, 1.0)	0.99 (0.93, 1.05)	0.97 (0.9, 1.1)	0.98 (0.9, 1.1)
LA [1]							
1st half of pregnancy	11.0 (5.4)	10.5 (3.9)	12.1 (5.3)	0.98 (0.9, 1.1)	1.0 (1.0, 1.1)	0.97 (0.9, 1.1)	1.0 (0.96, 1.1)
2nd half of pregnancy	12.2 (4.8)	10.3 (5.8)	11.8 (4.9)	0.9 (0.9, 1.0)	0.99 (0.9, 1.1)	0.97 (0.9, 1.1)	0.98 (0.9, 1.1)
ALA [1]							
1st half of pregnancy	1.2 (0.7)	1.1 (0.4)	1.4 (1.0)	0.7 (0.4, 1.3)	1.2 (0.9, 1.7)	0.6 (0.3, 1.3)	1.2 (0.8, 1.8)
2nd half of pregnancy	1.3 (0.5)	1.1 (0.6)	1.3 (0.6)	0.6 (0.3, 1.0)	1.0 (0.6, 1.7)	0.8 (0.4, 1.6)	1.0 (0.5, 1.8)

Abbreviations: TD, typical developing; ASD, autism spectrum disorders; Non-TD, nontypical development; SD: standard deviation; RR: relative risk; CI: confidence interval; LA: linoleic acid; ALA: alpha-linolenic acid; EPA: eicosapentaenoic acid; PUFA: polyunsaturated fatty acids;

[1]RRs were adjusted for maternal food iron intake in the same period of pregnancy; models for EPA, DHA and AA were not converged; For the 1st half of pregnancy, we have 82 TD, 30 ASD and 33 Non-TD; For the 2nd half of pregnancy, we have 70 TD, 30 ASD and 31 Non-TD;

Table 3.

Unadjusted and Adjusted Associations of Maternal PUFA Plasma Concentration (*mg/l*) in the 3rd trimester of Pregnancy with Child Outcome

	36-mo Outcome, mean (SD)			Un-adjusted RR (95% CI)		Adjusted RR (95% CI)	
	TD (*n* = 116)	ASD (*n* = 50)	Non-TD (*n* = 52)	ASD vs. TD	Non-TD vs. TD	ASD vs. TD	Non-TD vs. TD
LA [1]	1100.7	1084.3	1076.2	0.999	0.999	0.999	0.999
	(226.8)	(195.7)	(237.6)	(0.998, 1.001)	(0.998, 1.001)	(0.998, 1.001)	(0.998, 1.001)
ALA [2]	28.2	28.0	24.6	0.999	0.975	0.982	0.969
	(10.7)	(11.4)	(10.4)	(0.977, 1.021)	(0.952, 0.999)	(0.954, 1.012)	(0.942, 1.000)
AA [3]	214.3	209.6	201.3	0.999	0.996	0.998	0.996
	(49.3)	(49.9)	(52.3)	(0.994, 1.003)	(0.991, 1.000)	(0.992, 1.005)	(0.990, 1.002)
EPA [4]	11.3	8.6	8.3	0.960	0.953	0.948	0.927
	(9.1)	(5.2)	(5.7)	(0.920, 1.002)	(0.913, 0.997)	(0.898, 1.002)	(0.868, 0.990)
DHA [3]	77.0	72.4	67.2	0.995	0.990	0.997	0.987
	(30.5)	(26.8)	(25.9)	(0.987, 1.005)	(0.981, 0.999)	(0.985, 1.008)	(0.975, 0.998)

Abbreviations: TD, typical developing; ASD, autism spectrum disorders; Non-TD, nontypical development; SD: standard deviation; RR: relative risk; CI: confidence interval; PUFA, polyunsaturated fatty acids; LA: linoleic acid; ALA: alpha-linolenic acid; EPA: eicosapentaenoic acid; AA, arachidonic acid; DHA, docosahexaenoic acid.

[1] RRs were adjusted for maternal food iron intake and sample numbers of thaws;

[2] RRs were adjusted for maternal food iron intake, gestational age at sample collection and sample numbers of thaws;

[3] RRs were adjusted for sample numbers of thaws and sample storage time;

[4] RRs were adjusted for paternal age, sample numbers of thaws and sample storage time.