American Journal of Epidemiology

Vol. 178, No. 2
DOI: 10.1093/aje/kws433
Advance Access publication:
June 27, 2013

Original Contribution

Maternal Dietary Fat Intake in Association With Autism Spectrum Disorders

Kristen Lyall*, Kassandra L. Munger, Éilis J. O'Reilly, Susan L. Santangelo, and Alberto Ascherio

* Correspondence to Dr. Kristen Lyall, Harvard School of Public Health, 655 Huntington Avenue, Building II 3rd Floor, Boston MA 02115 (e-mail: klyall@hsph.harvard.edu).

Initially submitted May 13, 2012; accepted for publication October 24, 2012.

Our goal in this study was to determine whether maternal fat intake before or during pregnancy was associated with risk of autism spectrum disorder (ASD) in the offspring. Our primary analysis included 317 mothers who reported a child with ASD and 17,728 comparison mothers from the Nurses' Health Study II (index births in 1991–2007). Dietary information was collected prospectively through a validated food frequency questionnaire. Binomial regression was used to estimate crude and adjusted risk ratios. Maternal intake of linoleic acid was significantly inversely associated with ASD risk in offspring, corresponding to a 34% reduction in risk in the highest versus lowest quartiles of intake. Mothers in the lowest 5% of ω-3 fatty acid intake had a significant increase in offspring ASD risk as compared with the remaining distribution (risk ratio = 1.53, 95% confidence interval: 1.00, 2.32); this association was also seen in the subgroup of women (86 cases and 5,798 noncases) for whom dietary information during pregnancy was available (risk ratio = 2.42, 95% confidence interval: 1.19, 4.91). Thus, variations in intake of polyunsaturated fats within the range commonly observed among US women could affect fetal brain development and ASD risk. Because the number of women with diet assessed during pregnancy was small, however, these results should be interpreted cautiously.

autism; dietary fat; linoleic acid; ω-3 fatty acids; ω-6 fatty acids; polyunsaturated fatty acids

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; FFQ, food frequency questionnaire; NHS II, Nurses' Health Study II; PUFA, polyunsaturated fatty acid.

Autism spectrum disorders (ASDs) are defined by significant deficits in communication and social functioning and by the presence of repetitive behaviors. Less than 25% of cases can be accounted for by known causes; for the remainder, multifactorial etiology, including both genetic and environmental factors, is likely (1, 2). Research indicates structural and functional brain differences in children with autism and points to the perinatal and neonatal periods as etiologically relevant time frames (3, 4). Several maternal prenatal factors also have been shown to influence risk of ASD (5).

Although maternal nutrition is essential to fetal development, relatively little research has been dedicated to the topic of maternal diet in association with ASD. Maternal intake of fatty acids has been associated with birth weight, gestational age and length, and offspring intelligence quotient (6–8), but no prior report has specifically addressed maternal intake of these fats in association with ASD. Maternal fish intake, a source of ω-3 fatty acids, has been examined in association with broader child developmental outcomes, though results are somewhat conflicting (9–11). Given the known importance of fatty acids in brain development and the correlation between maternal intake and availability to the developing fetus, determination of whether maternal fatty acid intake alters ASD risk is a logical and potentially informative next step for ASD research.

In this study, we examined maternal intake of fats and risk of having a child with ASD. We used data from a large national cohort, the Nurses' Health Study II (NHS II), to address whether women with high and low intakes of fats and fatty acids differ in risk of having a child with ASD. In particular, we hypothesized that high maternal intakes of ω-3 and other polyunsaturated fats might be protective against ASD because of their role in brain development, and conversely, that low intakes would increase risk.

MATERIALS AND METHODS

The study population is drawn from the NHS II, an ongoing, large US cohort of female nurses who were 25–42 years of age when recruited in 1989 to be followed up by mailed biennial questionnaires. Since baseline, 116,430 nurses have reported on their medical conditions and lifestyle factors and, more recently, on conditions in their children. Additional details of the cohort have been reported previously (12), and all NHS II questionnaires are available online at http://www.channing.harvard.edu/nhs/?page_id=246. Only women with index births between 1991 (the year of first collection of dietary information) and 2007 were included in primary analyses. Index pregnancies were defined as the case birth for nurses reporting a child with ASD, and, for multiparous mothers not reporting a child with ASD, 1 child was randomly selected for the purpose of comparing diet before or during pregnancy. After exclusions, 18,045 women were available for analysis in this study (Figure 1).

Case definition

Cases were defined according to maternal report of autism, Asperger syndrome, or pervasive developmental disorder not otherwise specified (PDD-NOS) on the NHS II 2005 and 2009 questionnaires. Information on case year of birth was obtained from the NHS II questionnaires or in a follow-up study. Women reporting a child with ASD in either 2005 or 2009, but not both, were included only if 1) the reason for nonreporting on the other questionnaire was nonparticipation in that questionnaire year; 2) the nurse confirmed the diagnosis in a previous substudy; or 3) for women reporting on the 2009 questionnaire only, the child was born after 2000 (in which case, the child might have been too young for report of diagnosis by the 2005 questionnaire mailing). Noncases were defined as women who did not skip either ASD question and did not report any ASD at any time point. Women reporting competing diagnoses (fragile X syndrome, Rett syndrome, tuberous sclerosis, Down syndrome, trisomy 18; $n = 6$) in a previous substudy were not included.

In a subgroup of 50 cases, the Autism Diagnostic Interview–Revised (13) was conducted over the phone as part of our follow-up study conducted in 2009. Of these, 43 (86%) met full diagnostic criteria for ASD; the remainder narrowly missed domain cutoffs but met the ASD onset criterion and at least 1 other domain (5 cases missed by just 1 point in 1 domain; 2 met 1 or 2 domains). In addition, Social Responsiveness Scales (14) were sent to participants in a nested case-control study in NHS II for further diagnostic validation. Results from Social Responsiveness Scale T-scores also suggested a high degree of validity, with 94% of persons with maternally reported ASD falling within the ASD range according to established cutoffs (14) and 93% of individuals whose mothers reported no ASD falling within the "normal" range.

Exposure information

Information on dietary factors was collected by a 131-item food frequency questionnaire (FFQ), which has been used and described in numerous studies (15–17). Briefly, the FFQ items assess how often, on average, during the previous year a person has consumed foods and beverages. Nine options for frequency of intake, ranging from never or 1 time per month to 6 times per day, are given per item, and on the basis of these values and serving sizes, nutrient contents are calculated with information from the US Department of Agriculture and from food manufacturers. The reproducibility and validity of the FFQ have been demonstrated; in particular, total fat has been validated by changes in blood lipid levels, and FFQ-measured intake of specific fatty acids has been shown to correlate well with both diet records (Pearson correlation coefficients ranging from 0.48 to 0.73) and fatty acid composition of subcutaneous fat aspirates ($r = 0.48$ for ω-3; $r = 0.51$ for *trans*-fat) (17–20).

FFQs were administered every 4 years beginning in 1991. Primary exposures were the major types of fat, including total, saturated, monounsaturated, *trans*, and polyunsaturated fatty acids (PUFA), among which are the following fatty acids: ω-3, ω-6, eicosapentaenoic acid, docosahexaenoic acid, α-linolenic acid, linoleic acid, and arachidonic acid. Primary analyses of ω-3 and -6 total fatty acids did not include intake from supplements. Fish intake, as a main food source contributor of these PUFAs, was also examined. All nutrient intakes were adjusted for total energy intake by the residual method (21).

Statistical analysis

For the primary analysis, only exposure information collected from the FFQ before the birth of the index child was used. In a secondary analysis, we also included individuals who reported breastfeeding and who completed an FFQ within 1–2 years after the index child's year of birth. Dietary intakes of fats and fish were categorized in quartiles. Categories for the highest and lowest 10% of the distribution of intake were created to determine if relative extremes of intake were associated with ASD, as compared with the middle 80% of the intake distribution. Similarly, the highest 5% and lowest 5% of the distribution of intake were compared with the remaining 90% of the distribution for primary fats of interest to examine the effect of very high and low intakes.

In univariate analysis, demographic and lifestyle factors were compared between quartiles of intake of fats. Binomial regression was used to estimate crude and adjusted risk ratios for ASD (22, 23), with the lowest quartile of intake of a particular nutrient as the referent. Tests of trend were conducted with the ordinal score test, with the median value used for each quartile. Potential confounders were selected on the basis of a priori knowledge and associations with exposures and outcome. Multivariate models included, in addition to total calories, maternal race (binary: white or other, given that 96% of the NHS II is white), maternal age at index birth (continuous), child's year of birth (continuous by year), household income level (3 indicators for <75,000, 75–99,000 (referent), and ≥100,000, plus a missing indicator), maternal smoking status before pregnancy (regular smoking: yes or no), and maternal body mass index (weight (kg)/height $(m)^2$; 4 indicators: ≤20, >20–<25 (referent), ≥25–<30, ≥30). Given the exploratory nature of certain analyses and the

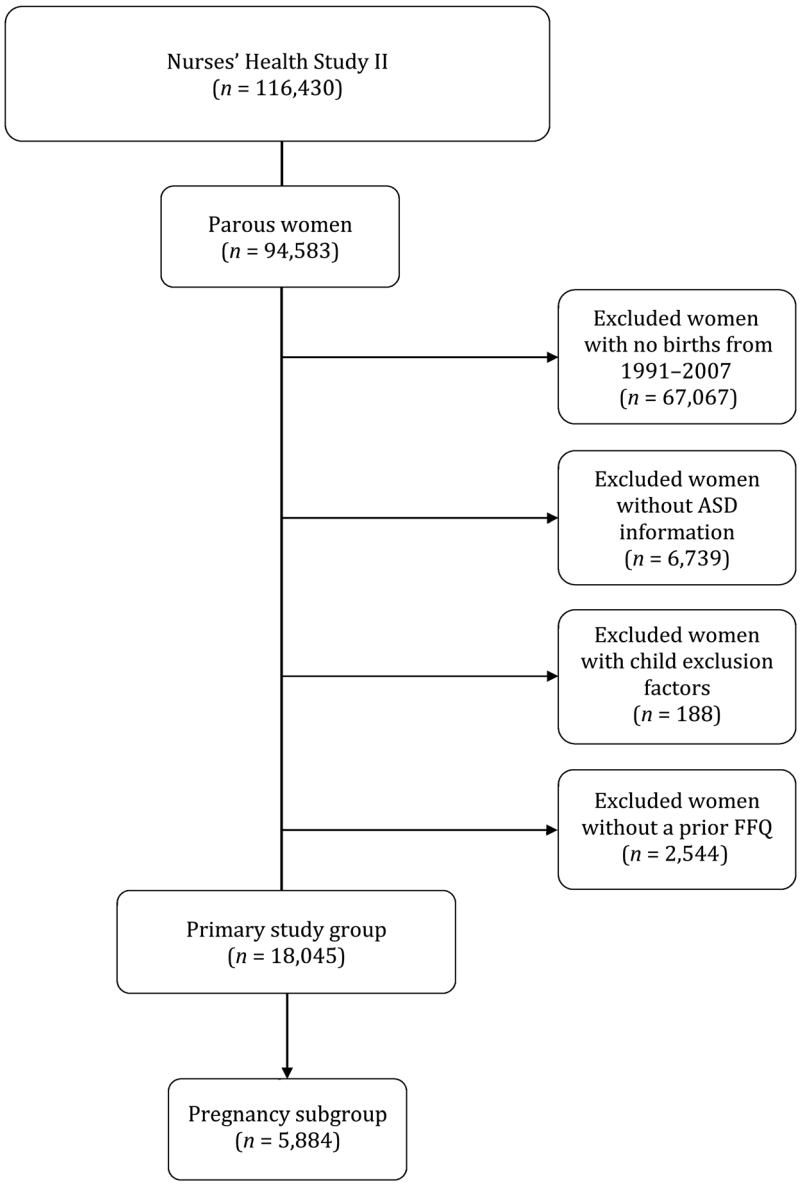

Figure 1. Exclusions for the primary study group, which included 317 mothers who reported a child with autism spectrum disorder (ASD) and 17,728 comparison mothers from the Nurses' Health Study II. Parous women were those who had had a pregnancy lasting >6 months in 1991–2007. For women who answered the 2005 but not the 2009 questionnaire, only births through 2003 were included. Women who provided ASD information were those who returned the long version of the questionnaire in 2005 or 2009 and did not skip the ASD question. Child exclusion factors were as follows: missing year of birth ($n = 51$), indication that the child was adopted ($n = 6$), failure to confirm ASD in a nested case-control follow-up study ($n = 6$), or report of a competing diagnosis (Down syndrome, fragile X syndrome, trisomy 18) ($n = 6$); remaining individuals were excluded on the basis of not meeting the case definition because of reporting the diagnosis on only 1 questionnaire. Primary study group includes 317 cases; pregnancy subgroup includes 86 cases.

correlation between dietary factors, we further considered adjustment for intake of protein, whole grains, alcohol, fruit, and vegetables, as well as for multivitamin use, physical activity, child birth order, and maternal pregnancy complications, including gestational diabetes. Because adjustment for these factors did not materially alter results, they were not included in final models. In models assessing total PUFA and ω-3 and ω-6 fatty acids, adjustment for *trans*-fat intake was also considered because *trans*-fat can affect PUFA metabolism (24). Likewise, because of the high correlation between linoleic acid and α-linolenic acid, models assessing these fatty acids considered adjustment for the other.

In a second set of models, we used continuous exposure variables created as a percentage of total energy intake. We included in such models total fat, total calories, PUFA, and monounsaturated fat (in addition to confounders listed

Table 1. Basic Characteristics of the Study Population by Quartile of Energy-Adjusted Total Fat, ω-3, and ω-6 Fatty Acid Intake[a] in the Nurses' Health Study II[b]

	Quartile 1			Quartile 4		
	No.	%	Mean	No.	%	Mean
Total fat	4,495			4,493		
Nurse age at baseline, years			30.1			30.1
Physical activity, METs/week			28.4			17.8*
Body mass index[c]			23.7			25.1*
Alcohol intake, g/day[d]			3.1			2.6*
Maternal age, years			34.7			33.7*
Maternal age categories						
20–29		5.5			8.5	
30–34		36			40	
≥35		58			52*	
Household income[e]						
<$40,000		3.9			5.5	
≥$150,000		14			9*	
Race						
White		96			98	
Other		4			2*	
Multivitamin use		70			59*	
Current smoker		6			12*	
Index birth–firstborn[f]		37			29*	
Gestational diabetes		5.5			9*	
ω-3 fatty acid	4,494			4,470		
Nurse age at baseline, years			29.6			30.7*
Physical activity, METs/week			20.8			23.5*
Body mass index			24.3			24.3
Alcohol intake, g/day			2.6			3.4*
Maternal age, years			34.1			34.6
Maternal age categories						
20–29		9			5.6	
30–34		41			36	
≥35		50			59.5*	
Household income						
<$40,000		4.5			4.5	
≥$150,000		10			13*	

Table continues

previously). In these "substitution models," the coefficient for PUFA represents the effect of replacing saturated fat in the diet with PUFA on the risk of having a child with ASD.

Sensitivity analyses

To examine diet more specifically during pregnancy, we also conducted analyses within the subgroup of women whose FFQs corresponded to diet during pregnancy ($n = 5,884$). Information from this pregnancy subgroup was also used to examine stability of diet over time. Furthermore, we examined the effect of adding to the pregnancy subgroup women with FFQs collected *after* the birth of the index child but within the range of reported breastfeeding, for an "index period" analysis. We also tested the effect of stratifying by prior pregnancy complications, which could potentially influence dietary intake in future pregnancies.

RESULTS

The primary analyses included 317 case mothers and 17,728 noncase mothers. Table 1 shows basic characteristics of the study population for 3 of the main exposures of interest: total fat, total ω-3 (i.e., *n*-3) fatty acid, and total ω-6 fatty

Table 1. Continued

	Quartile 1			Quartile 4		
	No.	%	Mean	No.	%	Mean
Race						
White		98			97	
Other		2			3	
Multivitamin use		66			61*	
Current smoker		9.4			9.5*	
Index birth–firstborn		30			33*	
Gestational diabetes		7			7	
ω-6 fatty acid	4,513			4,504		
Nurse age at baseline, years			30			30.3*
Physical activity, METs/week			24.8			19.6*
Body mass index			24.1			24.5*
Alcohol intake, g/day			3.1			2.9
Maternal age, years			34			34.2
Maternal age categories						
20–29		8			7	
30–34		41			37	
≥35		51			56*	
Household income						
<$40,000		4			5	
≥$150,000		12			10*	
Race						
White		96			98	
Other		3.5			2*	
Multivitamin use		68			60*	
Current smoker		8			10*	
Index birth–firstborn		34			29*	
Gestational diabetes		7			8	

Abbreviation: MET, metabolic equivalents.

* $P < 0.05$ in t test or χ^2 test (as appropriate) comparing quartiles of the fat shown as follows: total fat quartile 1 = 48.2; total fat quartile 4 = 73.7; ω-3 quartile 1 = 0.80; ω-3 quartile 4 = 1.51; ω-6 quartile 1 = 6.8; and ω-6 quartile 4 = 12.2.

[a] Only the lowest quartile (1) and highest (4) quartile are shown (median intake for each quartile).

[b] The primary study group includes index births in 1991–2007 from the US Nurses' Health Study II cohort.

[c] Weight (kg)/height $(m)^2$.

[d] Energy adjusted.

[e] Based on categorically assessed income; median value in the cohort is approximately $75,000–$99,000, though nearly 25% were missing income information.

[f] Mean birth order in the study group was 2.1; this did not differ across quartiles of these exposures.

acid intake. As expected, unhealthy characteristics, including lower physical activity, higher body mass index, higher alcohol intake, and smoking were associated with higher total fat intake and generally lower ω-3 intake. Demographic characteristics of our study population are representative of the NHS II and were overall similar by exposure category, though the prevalence of lower income and nonwhite race was slightly higher in the highest total fat quartile and the lowest ω-3 quartile. These variables were included in multivariable analyses.

In adjusted analyses, saturated fat and *trans*-fat intakes, as well as fat intake from animal, vegetable, and dairy sources, were not associated with risk of having a child with ASD. In contrast, a decreasing risk was observed for higher intake of total PUFAs (P for trend = 0.008; Table 2).

Results for specific fatty acids are shown in Table 3. Women in the highest quartile of intake of ω-6 fatty acids had a 34% reduction in risk of having a child with ASD compared with those in the lowest quartile (risk ratio = 0.66, 95% confidence interval (CI): 0.47, 0.92); results were similar for

Table 2. Maternal Dietary Fat Intake and Risk of Autism Spectrum Disorder in Offspring in the Nurses' Health Study II[a]

Fat	Median Intake[b]	No. Cases	No. Noncases	Energy Adjusted[c]		Fully Adjusted[d]		*P* Value[e]
				Risk Ratio	95% CI	Risk Ratio	95% CI	
Total fat								
Q1	48.2	100	4,395	1.0		1.0		0.05
Q2	57.6	72	4,456	0.71	0.53, 0.96	0.74	0.55, 1.00	
Q3	64.5	71	4,458	0.70	0.52, 0.95	0.73	0.54, 0.99	
Q4	73.8	74	4,419	0.74	0.55, 1.00	0.75	0.55, 1.01	
Saturated fat								
Q1	16.3	90	4,422	1.0		1.0		0.33
Q2	20.4	82	4,481	0.90	0.67, 1.21	0.95	0.71, 1.29	
Q3	23.3	70	4,367	0.79	0.58, 1.08	0.84	0.62, 1.16	
Q4	27.4	75	4,458	0.83	0.61, 1.12	0.88	0.64, 1.20	
Monounsaturated fat								
Q1	17.9	99	4,466	1.0		1.0		0.12
Q2	21.6	73	4,397	0.75	0.56, 1.01	0.77	0.57, 1.04	
Q3	24.6	65	4,458	0.66	0.49, 0.90	0.67	0.49, 0.92	
Q4	28.8	80	4,407	0.82	0.62, 1.10	0.81	0.60, 1.09	
Total PUFA								
Q1	7.8	95	4,499	1.0		1.0		0.008*
Q2	9.5	85	4,215	0.95	0.71, 1.28	0.97	0.73, 1.30	
Q3	11.0	76	4,564	0.79	0.59, 1.07	0.82	0.61, 1.11	
Q4	13.4	61	4,450	0.65	0.48, 0.90	0.67	0.49, 0.92	
Trans fat								
Q1	1.9	90	4,417	1.0		1.0		0.36
Q2	2.5	74	4,463	0.81	0.60, 1.10	0.84	0.62, 1.14	
Q3	3.2	81	4,391	0.90	0.67, 1.21	0.94	0.70, 1.27	
Q4	4.3	72	4,457	0.80	0.59, 1.08	0.83	0.61, 1.13	
Substitution for saturated fat[f]								
Total fat–5%				0.88	0.65, 1.19	0.99	0.73, 1.35	0.97
PUFA–5%				0.52	0.30, 0.93	0.52	0.30, 0.91	0.02*

Abbreviations: CI, confidence interval; PUFA, polyunsaturated fatty acid; Q, quartile.

* Indicates test for trend significant, $P < 0.05$.

[a] Index births in 1991–2007 from the US Nurses' Health Study II cohort.

[b] Energy-adjusted intake. Values are in grams per day.

[c] Adjusted for total energy intake only.

[d] Adjusted for total energy intake, maternal age, child's year of birth, income level, race, body mass index, and prepregnancy smoking status. Removal of adjustment for smoking did not affect results. Additional adjustment for child birth order, maternal physical activity level, spouse's education level, or multivitamin use, or for *trans*-fat in PUFA model, did not materially alter estimates.

[e] *P* value from test of trend for primary models; Wald *P* for substitution models.

[f] Models for effect of substituting for saturated fat; models include major types of fat and total fat as continuous variables for percentage of total energy intake, with saturated fat left out of the model. Thus, the coefficient for polyunsaturated fat represents the effect of substituting that percent of energy intake from polyunsaturated for saturated fat.

linoleic acid (risk ratio = 0.66, 95% CI: 0.48, 0.92). Intakes of other types of fatty acids, including eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid, were not associated with risk of having a child with ASD.

In analyses of extreme deciles of fat intake compared with the middle 80% of the distributions, ω-6 fatty acid and linoleic acid continued to show significant associations with ASD (Figure 2). These associations were somewhat stronger than those seen in analyses in which quartiles were used. Furthermore, when the lowest 5% of the distribution of intake was assessed, women with the lowest ω-3 fatty acid intake had a 53% increase in risk of having a child with ASD as compared with women in the middle 90% of the distribution (95% CI: 1.00, 2.32).

Although numbers were small and power reduced in the pregnancy subgroup, there was a statistically significant

Table 3. Maternal Dietary Fatty Acid Intake and Risk of Autism Spectrum Disorder in Offspring in the Nurses' Health Study II[a]

Fat	Median Intake[b]	No. Cases	No. Noncases	Energy Adjusted[c]		Fully Adjusted[d]		*P* Value[e]
				Risk Ratio	95% CI	Risk Ratio	95% CI	
ω-3 fatty acids								
Total ω-3 fatty acid								
Q1	0.8	87	4,407	1.0		1.0		0.12
Q2	1.0	83	4,337	0.97	0.72, 1.31	0.98	0.73, 1.32	
Q3	1.2	69	4,592	0.76	0.56, 1.04	0.78	0.57, 1.06	
Q4	1.5	78	4,392	0.90	0.67, 1.22	0.90	0.66, 1.22	
α-Linolenic acid								
Q1	0.7	92	4,526	1.0		1.0		0.14
Q2	0.9	75	4,175	0.88	0.65, 1.20	0.91	0.67, 1.23	
Q3	1.0	79	4,662	0.83	0.62, 1.12	0.86	0.64, 1.16	
Q4	1.2	71	4,365	0.80	0.59, 1.09	0.80	0.58, 1.08	
Eicosapentaenoic acid								
Q1	0.01	52	3,078	1.0		1.0		0.97
Q2	0.02	108	5,975	1.06	0.76, 1.47	1.11	0.80, 1.54	
Q3	0.05	72	3,897	1.08	0.76, 1.54	1.13	0.79, 1.61	
Q4	0.11	85	4,778	1.04	0.74, 1.47	1.07	0.76, 1.51	
Docosahexaenoic acid								
Q1	0.04	87	4,780	1.0		1.0		0.74
Q2	0.08	79	4,328	1.00	0.74, 1.35	1.05	0.78, 1.42	
Q3	0.13	65	3,964	0.90	0.66, 1.24	0.95	0.69, 1.31	
Q4	0.22	86	4,656	1.02	0.76, 1.37	1.07	0.79, 1.45	
ω-6 fatty acids								
Total ω-6 fatty acid								
Q1	6.8	85	4,428	1.0		1.0		0.01*
Q2	8.4	87	4,423	1.03	0.76, 1.38	1.01	0.75, 1.36	
Q3	9.9	87	4,431	1.02	0.76, 1.37	1.01	0.75, 1.36	
Q4	12.2	58	4,446	0.68	0.49, 0.95	0.66	0.47, 0.92	
Linoleic acid								
Q1	6.6	94	4,549	1.0		1.0		0.008*
Q2	8.1	85	4,141	0.99	0.74, 1.33	1.01	0.76, 1.35	
Q3	9.5	80	4,674	0.83	0.62, 1.12	0.86	0.64, 1.16	
Q4	11.8	58	4,364	0.65	0.47, 0.90	0.66	0.48, 0.92	
Arachidonic acid								
Q1	0.09	89	4,184	1.0		1.0		0.09
Q2	0.12	81	4,078	0.93	0.69, 1.25	0.98	0.73, 1.32	
Q3	0.15	77	4,966	0.73	0.54, 0.99	0.78	0.57, 1.06	
Q4	0.21	70	4,500	0.74	0.54, 1.01	0.79	0.58, 1.09	

Abbreviations: CI, confidence interval; Q, quartile.

Total ω-3 and ω-6 intakes do not include use of vitamin supplements, though estimates did not materially differ when intake from supplements was included.

* $P < 0.05$.

[a] Index births in 1991–2007 from the US Nurses' Health Study II cohort.

[b] Energy-adjusted intake. Values are in grams per day.

[c] Adjusted for total energy intake only.

[d] Adjusted for total energy intake, maternal age, child's year of birth, income level, race, body mass index, and prepregnancy smoking status. Removal of adjustment for smoking did not affect results. Additional adjustment for child birth order, maternal physical activity level, spouse's education level, multivitamin use, or *trans*-fat did not materially alter estimates.

[e] *P* for trend.

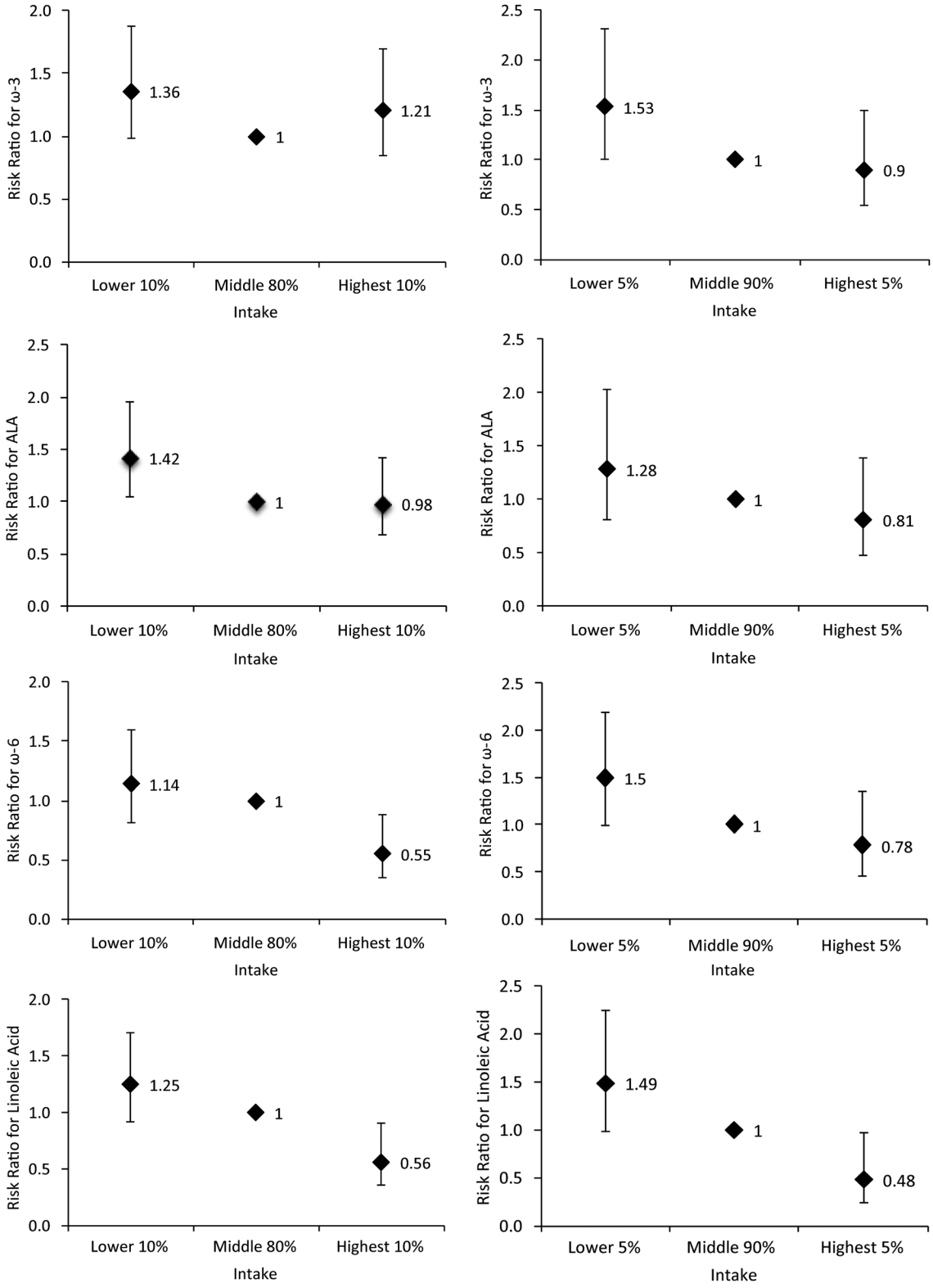

Figure 2. Association of maternal dietary fats with risk of autism spectrum disorder in offspring, according to extreme deciles and 5% of intake, in mothers who reported a child with autism spectrum disorder and comparison mothers from the US Nurses' Health Study II (index births in 1991–2007). Risk ratios for the association between maternal dietary ω-3, α-linolenic acid, ω-6, and linoleic acid are shown. Error bars represent 95% confidence intervals. The first column of graphs shows high and low deciles of maternal intake compared with the referent middle 80% of the distribution in association with risk of ASD; the second column compares those in the highest 5% and lowest 5% of maternal intake to those in the remaining 90% of the distribution. Total polyunsaturated fat demonstrated similar associations as ω-6 fatty acids; other fats not shown for these analyses of deciles and extremes of intake did not exhibit significant associations.

association with low ω-3 fatty acid intake (logistic regression was used because of small case *n*; odds ratio for the lowest 5% of the distribution of intake = 2.42, 95% CI: 1.19, 4.91; see Web Table 2, available at http://aje.oxfordjournals.org/). In addition, risk was significantly elevated for those with the lowest 10% of intake of α-linolenic acid (odds ratio = 2.23, 95% CI: 1.30, 3.84) and the lowest 5% of intake of linoleic acid (odds ratio = 2.20, 95% CI: 1.09, 4.46). No other statistically significant associations were seen in the small pregnancy subgroup, though the point estimates for top quartiles were similar to those seen in the primary study group. Correlations in nutrient values from FFQs collected during pregnancy with values from FFQs collected before and after pregnancy were fair for most nutrients, and these correlations between FFQs were the same as for women without an FFQ collected in pregnancy (nutrient correlations in both the pregnancy subgroup and the primary study group across FFQs were approximately 0.5).

When associations in the index period group were examined (which added 9,372 women with diet information during lactation to the pregnancy subgroup), results were consistent overall with the primary analysis. Women in the lowest 5% of intake of linoleic fatty acid had significantly increased risks of having a child with ASD (risk ratio = 1.64, 95% CI: 1.04, 2.59), and women with the lowest 5% of ω-3 fatty acid intake had a 50% increase in risk, though confidence intervals were just overlapping the null.

In assessment of maternal fish intake in association with ASD, no significant associations were seen in either crude or adjusted analyses (Web Table 1). Sensitivity analyses that examined alternative case groups and covariates and stratified by prior pregnancy complications yielded similar primary findings.

DISCUSSION

In this subcohort of the NHS II, we found significant associations between maternal PUFA intake and risk of having a child with ASD. Our preliminary findings suggest that increased intake of ω-6 fatty acids could decrease risk of ASD, but these results require replication and further consideration.

Several limitations should be considered in interpreting these findings. Although multiple validation studies, including those in which biological measurements were used, have demonstrated that the reported intake of linoleic acid and other specific fatty acids in this population correlates with tissue composition and several health outcomes (15, 25–28), some degree of error is expected. In univariate analyses, this error would tend to bias relative risk estimates toward the null, but bias in either direction is possible in multivariate analyses because of residual confounding (29). For this reason, although our results support the importance of maternal dietary fat consumption as a risk factor for ASD, the attribution of this association to specific fatty acids should be interpreted cautiously. In addition, because we cannot rule out measurement error in the exposures and confounders, risk ratios provided should be interpreted cautiously. Although results were materially unchanged by adjustment for birth order, residual confounding by birth order cannot be ruled out. However, in analyses stratified by prior pregnancy complications, significant associations persisted in women with no history of prior complications, which suggests that the observed associations were not due to residual confounding by the influence of prior complications on diet. An additional limitation of this study is that maternal dietary information was not collected in pregnancy for all participants. Error from this source is likely to be modest because we had dietary information in close proximity to pregnancy for the majority of our study group. The average lag time between FFQ and birth of the index child was 1.35 years, and exclusion of women with longer lag times did not materially alter the results. Furthermore, evidence indicated that the diet among participants in our cohort was fairly stable both before and during pregnancy (27, 30).

The overall prevalence of ASD among women who have ever had a child in the NHS II cohort is approximately 1.5%, which is within the range of estimates reported by the Autism Developmental Disabilities Monitoring Network (ADDM) (31). We cannot rule out the potential for diagnostic misclassification of the outcome because we relied on maternal report. However, the effect of any such misclassification is expected to be small, given the high degree of validity suggested by results from our Autism Diagnostic Interview–Revised diagnostic confirmation subgroup and scores on Social Responsiveness Scale forms. Our results might not be generalizable to all groups, inasmuch as our study population is primarily white and of middle to high income. In this cohort, however, we had the ability to adjust for many potential confounders and to use a validated FFQ in an educated, reliable population.

We analyzed these continuous dietary variables according to quartiles and extremes of the distribution of intake, as well as continuously as a percentage of energy intake. Across these analyses, not all fatty acids demonstrated significant associations according to all categorizations, and we cannot rule out chance findings; however, the pattern of results across analyses suggested an association between maternal PUFA intake, particularly ω-6 fatty acids, and offspring ASD. A significant reduction of approximately 40% in the risk of having a child with ASD was seen for mothers in the highest quartiles of total PUFA intake and ω-6 intake, as compared with mothers in the lowest quartiles of intake of these fats. Increases in risk of having a child with ASD approached statistical significance for very low intake of ω-6, as well. Significant associations with total ω-3 fatty acids, which we had hypothesized to be of primary relevance given their role in brain development, anti-inflammatory processes, and immune function, were seen only when very low intakes were assessed. For specific fatty acids, the only significant associations were seen with linoleic and α-linolenic acids, essential fatty acids that are required from dietary sources. Intakes of these fats, however, are highly correlated, and in analyses in which each of these fats was adjusted for the other, only linoleic acid remained significant. We did not see any association with eicosapentaenoic acid or docosahexaenoic acid, which are 2 ω-3 fatty acids essential to fetal brain development (32), or arachidonic acid, which is also important in brain development (32). Limited variability in the intake of these specific fatty acids in this population, measurement errors, and high correlation between the fatty acids could have contributed to the nonsignificant results.

Our finding of a significant increase in risk associated with very low ω-3 intake, but no decrease in risk with higher intake, suggests that once minimal ω-3 requirements are satisfied, higher intake might provide little benefit. To our knowledge, no prior study has examined maternal fatty acid intake in association with ASD specifically. However, in several studies of maternal ω-3 supplementation, moderate gains were found in offspring neurodevelopmental outcomes, including intelligence quotient and Bayley Scales of Infant Development scores (6, 9). Other studies have suggested that docosahexaenoic acid or arachidonic acid supplementation in lactating mothers or in formula has no impact on child outcomes (33, 34), though it has been suggested that differences in results might be due to the timing of supplementation, given that the uptake of fatty acids is highest during brain growth in late pregnancy (35).

Maternal intake of fish, a key source of fatty acids, has been investigated in association with child neurodevelopmental outcomes in several studies. As reviewed by Oken and Bellinger (35), in the majority of these studies, higher maternal fish intake was associated with higher child development scores, though the effect of mercury levels should be considered. In another study, it was found that low maternal fish intake might increase risk of suboptimal behavioral and cognitive outcomes in offspring (36). However, in the Seychelles Child Development Study, no significant associations were identified between fish intake and various measures of child development (10). We also did not see any associations between maternal fish intake and ASD in our exploratory analyses in the primary study group or pregnancy subgroup, though our pregnancy group could have been underpowered to detect differences. Furthermore, intake in our highest category (>1 serving per week) might not have been high enough, given that prior associations were for those with intakes of >2 servings per week. Thus, future studies with large numbers and greater variability in fish intake should examine potential associations with ASD in more detail.

Though speculative at this time, the inverse association seen for those in the highest quartiles of intake of ω-6 fatty acids could be due to biological effects of these fatty acids on brain development. PUFAs have been shown to be important in retinal and brain development in utero (37) and to play roles in signal transduction and gene expression and as components of cell membranes (38, 39). Maternal stores of fatty acids in adipose tissue are utilized by the fetus toward the end of pregnancy and are necessary for the first 2 months of life in a crucial period of development (37). The complex effects of fatty acids on inflammatory markers and immune responses could also mediate an association between PUFA and ASD. Activation of the maternal immune system and maternal immune aberrations have been previously associated with autism (5, 40, 41), and findings suggest that increased interleukin-6 could influence fetal brain development and increase risk of autism and other neuropsychiatric conditions (42–44). Although results for effects of ω-6 intake on interleukin-6 levels are inconsistent (45, 46), maternal immune factors potentially could be affected by PUFA intake (47).

Although intake of total fat has decreased in the United States from ≈1970 to 2000, relative intakes of types of fat in Western cultures have also changed, with increases in polyunsaturated fats, including linoleic acid (48, 49). This trend is an argument against a strong independent protective effect of linoleic acid, but the increase in PUFA intake preceded increases in autism prevalence, which suggests other factors might be interacting. Additional research will be needed to determine whether the associations seen in the present study with ω-6 and other PUFAs persist in other studies, and, if so, what underlies them.

Our results provide preliminary evidence that increased maternal intake of ω-6 fatty acids could reduce risk of offspring ASD and that very low intakes of ω-3 fatty acids and linoleic acid could increase risk. Future studies with larger sample sizes and more detailed timing information should consider maternal intake of these fats in association with child neurodevelopment and autism.

ACKNOWLEDGMENTS

Author affiliations: Department of Nutrition, Harvard School of Public Health, Harvard University, Boston Massachusetts (Kristen Lyall, Kassandra L. Munger, Éilis J. O'Reilly, Alberto Ascherio); Department of Epidemiology, Harvard School of Public Health, Harvard University, Boston, Massachusetts (Susan L. Santangelo, Alberto Ascherio); Department of Public Health Sciences, University of California, Davis, Davis, California (Kristen Lyall); Department of Psychiatry, Harvard Medical School, Harvard University, Boston, Massachusetts (Susan L. Santangelo); Department of Psychiatry, Maine Medical Center/Maine Medical Center Research Institute, Portland, Maine (Susan L. Santangelo); Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts (Alberto Ascherio).

Conflict of interest: none declared.

REFERENCES

1. Miles JH. Autism spectrum disorders—a genetics review. *Genet Med*. 2011;13(4):278–294.
2. Newschaffer CJ, Croen LA, Daniels J, et al. The epidemiology of autism spectrum disorders. *Annu Rev Public Health*. 2007;28:235–258.
3. Acosta MT, Pearl PL. Imaging data in autism: from structure to malfunction. *Semin Pediatr Neurol*. 2004;11:205–213.
4. Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. *Trends Neurosci*. 2008;31(3):137–145.
5. Gardener H, Spiegelman D, Buka S. Prenatal risk factors for autism: comprehensive meta-analysis. *Br J Psychiatry*. 2009;195:7–14.
6. Helland IB, Smith L, Saarem K, et al. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics*. 2003;111(1):e39–e44.
7. Lucas M, Dewailly E, Muckle G, et al. Gestational age and birth weight in relation to n-3 fatty acids among Inuit (Canada). *Lipids*. 2004;39(6):617–626.

8. Szajewska H, Horvath A, Koletzko B. Effect of n-3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2006;83(6):1337–1344.
9. Cohen JT, Bellinger DC, Connor WE, et al. A quantitative analysis of prenatal intake of n-3 polyunsaturated fatty acids and cognitive development. *Am J Prev Med*. 2005;29(4):366–374.
10. Davidson PW, Myers GJ, Cox C, et al. Methylmercury and neurodevelopment: longitudinal analysis of the Seychelles child development cohort. *Neurotoxicol Teratol*. 2006;28(5):529–535.
11. Oken E, Radesky JS, Wright RO, et al. Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. *Am J Epidemiol*. 2008;167(10):1171–1181.
12. Solomon CG, Willett WC, Carey VJ, et al. A prospective study of pregravid determinants of gestational diabetes mellitus. *JAMA*. 1997;278(13):1078–1083.
13. Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview–Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord*. 1994;24(5):659–685.
14. Constantino JN, Davis SA, Todd RD, et al. Validation of a brief quantitative measure of autistic traits: comparison of the social responsiveness scale with the Autism Diagnostic Interview–Revised. *J Autism Dev Disord*. 2003;33(4):427–433.
15. Chavarro JE, Rich-Edwards JW, Rosner BA, et al. Dietary fatty acid intakes and the risk of ovulatory infertility. *Am J Clin Nutr*. 2007;85(1):231–237.
16. Iso H, Rexrode KM, Stampfer MJ, et al. Intake of fish and omega-3 fatty acids and risk of stroke in women. *JAMA*. 2001;285(3):304–312.
17. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol*. 1985;122(1):51–65.
18. London SJ, Sacks FM, Caesar J, et al. Fatty acid composition of subcutaneous adipose tissue and diet in postmenopausal US women. *Am J Clin Nutr*. 1991;54(2):340–345.
19. Willett W, Stampfer M, Chu NF, et al. Assessment of questionnaire validity for measuring total fat intake using plasma lipid levels as criteria. *Am J Epidemiol*. 2001;154(12):1107–1112.
20. Willett WC, Lenart E. Reproducibility and validity of food-frequency questionnaires. In: Willett WC, ed. *Nutritional Epidemiology*. 2nd ed. New York, NY: Oxford University Press; 1998:101–147.
21. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr*. 1997;65(4 suppl):1220S–1228S.
22. Spiegelman D, Hertzmark E. Easy SAS calculations for risk or prevalence ratios and differences. *Am J Epidemiol*. 2005;162(3):199–200.
23. Wacholder S. Binomial regression in GLIM: estimating risk ratios and risk differences. *Am J Epidemiol*. 1986;123(1):174–182.
24. Ascherio A, Willett WC. Health effects of trans fatty acids. *Am J Clin Nutr*. 1997;66(4 suppl):1006S–1010S.
25. Mozaffarian D, Pischon T, Hankinson SE, et al. Dietary intake of trans fatty acids and systemic inflammation in women. *Am J Clin Nutr*. 2004;79(4):606–612.
26. Munger KL, Zhang SM, O'Reilly E, et al. Vitamin D intake and incidence of multiple sclerosis. *Neurology*. 2004;62(1):60–65.
27. Zhang C, Liu S, Solomon CG, et al. Dietary fiber intake, dietary glycemic load, and the risk for gestational diabetes mellitus. *Diabetes Care*. 2006;29(10):2223–2230.
28. Zhang SM, Willett WC, Hernan MA, et al. Dietary fat in relation to risk of multiple sclerosis among two large cohorts of women. *Am J Epidemiol*. 2000;152(11):1056–1064.
29. Spiegelman D, McDermott A, Rosner B. Regression calibration method for correcting measurement-error bias in nutritional epidemiology. *Am J Clin Nutr*. 1997;65(4 suppl):1179S–1186S.
30. Rifas-Shiman SL, Rich-Edwards JW, Willett WC, et al. Changes in dietary intake from the first to the second trimester of pregnancy. *Paediatr Perinat Epidemiol*. 2006;20(1):35–42.
31. Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2008. *MMWR Surveill Summ*. 2012;61(3):1–19.
32. Greenberg JA, Bell SJ, Ausdal WV. Omega-3 fatty acid supplementation during pregnancy. *Rev Obstet Gynecol*. 2008;1(4):162–169.
33. Beyerlein A, Hadders-Algra M, Kennedy K, et al. Infant formula supplementation with long-chain polyunsaturated fatty acids has no effect on Bayley developmental scores at 18 months of age—IPD meta-analysis of 4 large clinical trials. *J Pediatr Gastroenterol Nutr*. 2010;50(1):79–84.
34. van Goor SA, Dijck-Brouwer DA, Erwich JJ, et al. The influence of supplemental docosahexaenoic and arachidonic acids during pregnancy and lactation on neurodevelopment at eighteen months. *Prostaglandins Leukot Essent Fatty Acids*. 2011;84(5-6):139–146.
35. Oken E, Bellinger DC. Fish consumption, methylmercury and child neurodevelopment. *Curr Opin Pediatr*. 2008;20(2):178–183.
36. Hibbeln JR, Davis JM, Steer C, et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *Lancet*. 2007;369(9561):578–585.
37. Haggarty P. Effect of placental function on fatty acid requirements during pregnancy. *Eur J Clin Nutr*. 2004;58(12):1559–1570.
38. Casper RC. Nutrients, neurodevelopment, and mood. *Curr Psychiatry Rep*. 2004;6(6):425–429.
39. Deckelbaum RJ, Worgall TS, Seo T. n-3 Fatty acids and gene expression. *Am J Clin Nutr*. 2006;83(suppl):1520S–1525S.
40. Braunschweig D, Ashwood P, Krakowiak P, et al. Autism: maternally derived antibodies specific for fetal brain proteins. *Neurotoxicology*. 2008;29(2):226–231.
41. Croen LA, Grether JK, Yoshida CK, et al. Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. *Arch Pediatr Adolesc Med*. 2005;159(2):151–157.
42. Elovitz MA, Brown AG, Breen K, et al. Intrauterine inflammation, insufficient to induce parturition, still evokes fetal and neonatal brain injury. *Int J Dev Neurosci*. 2011;29(6):663–667.
43. Jyonouchi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *J Neuroimmunol*. 2001;120(1-2):170–179.

44. Smith SE, Li J, Garbett K, et al. Maternal immune activation alters fetal brain development through interleukin-6. *J Neurosci*. 2007;27(40):10695–10702.
45. Kiecolt-Glaser JK, Belury MA, Porter K, et al. Depressive symptoms, omega-6:omega-3 fatty acids, and inflammation in older adults. *Psychosom Med*. 2007;69(3): 217–224.
46. Simopoulos AP. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomed Pharmacother*. 2006;60(9): 502–507.
47. Kremmyda LS, Tvrzicka E, Stankova B, et al. Fatty acids as biocompounds: their role in human metabolism, health and disease—a review. Part 2: Fatty acid physiological roles and applications in human health and disease. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2011;155(3): 195–218.
48. Briefel RR, Johnson CL. Secular trends in dietary intake in the United States. *Annu Rev Nutr*. 2004;24:401–431.
49. Stephen AM, Wald NJ. Trends in individual consumption of dietary fat in the United States, 1920–1984. *Am J Clin Nutr*. 1990;52(3):457–469.