Original Contribution

Maternal Intake of Supplemental Iron and Risk of Autism Spectrum Disorder

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Iron deficiency affects 40%–50% of pregnancies. Iron is critical for early neurodevelopmental processes that are dysregulated in autism spectrum disorder (ASD). We examined maternal iron intake in relation to ASD risk in California-born children enrolled in a population-based case-control study (the Childhood Autism Risks from Genetics and the Environment (CHARGE) Study) from 2003 to 2009 with a diagnosis of ASD ($n = 520$) or typical development ($n = 346$) that was clinically confirmed using standardized assessments. Mean maternal daily iron intake was quantified on the basis of frequency, dose, and brands of supplements and cereals consumed each month from 3 months before pregnancy through the end of pregnancy and during breastfeeding (the index period), as reported in parental interviews. Mothers of cases were less likely to report taking iron-specific supplements during the index period (adjusted odds ratio = 0.63, 95% confidence interval: 0.44, 0.91), and they had a lower mean daily iron intake (51.7 (standard deviation, 34.0) mg/day) than mothers of controls (57.1 (standard deviation, 36.6) mg/day; $P = 0.03$). The highest quintile of iron intake during the index period was associated with reduced ASD risk compared with the lowest (adjusted odds ratio = 0.49, 95% confidence interval: 0.29, 0.82), especially during breastfeeding. Low iron intake significantly interacted with advanced maternal age and metabolic conditions; combined exposures were associated with a 5-fold increased ASD risk. Further studies of this link between maternal supplemental iron and ASD are needed to inform ASD prevention strategies.

autism; case-control studies; child development; dietary supplements; iron; pregnancy; primary prevention; risk factors

Abbreviations: ASD, autism spectrum disorder; CHARGE, Childhood Autism Risks from Genetics and the Environment; CI, confidence interval; OR, odds ratio; TD, typical development.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by the presence of social deficits, language impairments, and stereotyped or repetitive behaviors and interests (1–3). ASD affects 1 in every 68 children in the United States, and prevalence is increasing (4). Combinations of multiple genetic and environmental factors likely play an etiological role in ASD. Evidence supports the hypothesis of prenatal origins for autism (5) and an influence of gestational nutrition (6, 7).

Iron deficiency, with its resultant anemia, is the most commonly measured nutrient deficiency, and it is especially common during pregnancy, affecting 40%–50% of women and their infants (8–10). The fetus depends on maternal iron as his or her only source of iron (11), and severe maternal iron deficiency can induce fetal and infant iron deficiency (12, 13).

Iron is crucial to early neurodevelopment. In the brain, iron contributes to neurotransmitter production, myelination, and immune function (14); dysregulation of all 3 of these pathways has been associated with ASD. Iron deficiency early in life has been shown to impair cognition, motor development, social orientation and engagement, and language development, with improvements being observed upon iron supplementation (15–19). Poor iron status is more prevalent in children with ASD (20–24) and does not necessarily correlate with low iron intake (22), suggesting that these children could absorb and/or metabolize iron less efficiently. However, to our knowledge, no study to date has examined gestational iron status in relation to development of ASD. We examined maternal intake of supplemental iron in relation to ASD risk.

Am J Epidemiol. 2014;180(9):890–900

METHODS

Study population

Participants in the Childhood Autism Risks from Genetics and the Environment (CHARGE) Study, a population-based case-control study (25), who had undergone interviews from the start of the study in 2003 until the questionnaire was revised in September 2011 were included in these analyses. Eligible children included those who were aged 24–60 months, had been born in California, were living with at least 1 biological parent who spoke English or Spanish, and resided in one of the catchment areas on a specified list of California Department of Developmental Services regional centers that coordinate services for children with autism and developmental delay. Children were excluded if they had impairments that would preclude a valid developmental assessment. Children with genetic syndromes were not excluded if they met other inclusion criteria.

Children with autism and developmental delays were identified through the Department of Developmental Services' regional centers, clinics and providers, self-referrals by parents, and public outreach. A stratified random sample of children from the general population, identified from state birth files, was generated by frequency-matching to the projected distribution of autism cases on age, sex, and regional center catchment area. The CHARGE Study protocol was approved by institutional review boards at the University of California, Davis, and the University of California, Los Angeles, and by the State of California Committee for the Protection of Human Subjects. Written informed consent was obtained before participation.

Diagnostic confirmation

All children were assessed at the Medical Investigation of Neurodevelopmental Disorders (MIND) Institute clinic (Sacramento, California) for confirmation of their diagnosis. Children were assessed for cognitive function using the Mullen Scales of Early Learning (26) and for adaptive function using the Vineland Adaptive Behavior Scales (27). The Autism Diagnostic Interview–Revised (28, 29) and the Autism Diagnostic Observation Schedule–Generic (30, 31) were used to confirm autism diagnoses. The children of families recruited from the general population or with developmental delays were screened for evidence of ASD using the Social Communication Questionnaire (32); if children scored above 14, they were evaluated for autism. Autism case status was defined as meeting criteria in the communication, social, and repetitive-behavior domains of the Autism Diagnostic Interview–Revised *and* scoring at or above the total cutoff point for autistic disorder on the Autism Diagnostic Observation Schedule–Generic, module 1, 2, or 3. A broader definition of impairment encompasses ASD as defined by Risi et al. (33). Because autism and ASD represent different symptom severities along the continuum of the disorder (33), we present results for the combined ASD group in accordance with the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (34). All interviews and clinical assessments were conducted in English or Spanish by bilingual staff.

Maternal supplemental iron intake

As previously described (35), trained telephone interviewers collected information from the mother on her intake of multivitamins, prenatal vitamins, iron-specific vitamins, cereals, and other supplements (including whether or not each item had been consumed and, if so, what brand and dose had been consumed, how frequently, and in which months) during a defined period (the index period) beginning 3 months before pregnancy and continuing throughout each month of pregnancy and while breastfeeding. From this information, we calculated average daily intake of iron (and other nutrients) for each product and summed these values to a total value for each month for each woman. Iron amounts were assigned to each brand/product based on information obtained from the manufacturer; if this information was not available, a standard amount was assigned based on the amount most commonly found in similar products.

Statistical analyses

Data were reviewed for outliers using univariate descriptive analyses. Logistic regression was used to calculate odds ratios (with 95% confidence intervals) as measures of association between iron intake categories and case status, using SAS software, version 9.3 (SAS Institute Inc., Cary, North Carolina). Stratified analyses and interaction terms were used to examine effect modification of maternal iron intake by child sex, interpregnancy interval (defined as elapsed time from the last livebirth or previous pregnancy with a gestational age of ≤20 weeks to the start of the current pregnancy), maternal and child race/ethnicity (non-Hispanic white, Hispanic, or other), maternal age, and maternal metabolic conditions (prepregnancy obesity, defined as body mass index (weight (kg)/height (m)^2 ≥30), hypertension, and/or diabetes), as defined previously (36). The above variables were also examined as potentially confounding factors because of their relationship with iron status, as were the following variables: child's birth year, paternal age, home ownership, month in which prenatal care began, number of prenatal-care visits, parity, maternal birthplace, education, folic acid intake during the first month of pregnancy (<600 μg/day or ≥600 μg/day), cigarette smoking, residing with a smoker, and alcohol consumption. Our analyses started with a full model containing potential confounders identified in the bivariate analyses as being broadly associated ($P < 0.2$) with both ASD and quintile categories of iron intake (based on control intake). Variables were then excluded using backward selection, retaining in the model any variables that caused at least a 10% change in the exposure parameter estimates. Maternal folic acid intake, home ownership, and child's birth year were the only variables meeting the confounder criteria.

In sensitivity analyses, we assessed the impact of missing data using multiple imputation via the Markov chain Monte Carlo algorithm (37). To ensure that the results represented the study base, we used survey research methods to fit the logistic regression models, with participants being assigned weights equal to the inverse of the estimated participation probability in strata defined by the entry case group and demographic factors (38). Estimates were also stratified by

Table 1. Characteristics of Children With Autism Spectrum Disorder or Typical Development and Their Mothers, CHARGE Study, California, 2003–2009

	TD (n = 346)		ASD (n = 520)		P Value[a]
	No.	%	No.	%	
Child sex					
Male	285	82.4	445	85.6	0.20
Female	61	17.6	75	14.4	
Child race/ethnicity					
Non-Hispanic white	179	51.7	265	51.0	0.12
Hispanic	103	29.8	166	31.9	
Non-Hispanic black	8	2.3	12	2.3	
Asian	9	2.6	28	5.4	
Mixed or other	47	13.6	49	9.4	
Child's birth year					
1998–2001	62	17.9	238	45.8	<0.0001
2002–2004	190	54.9	181	34.8	
2005–2006	65	18.8	91	17.5	
2007–2009	29	8.4	10	1.9	
Maternal age at child's birth, years					
<20	12	3.5	10	1.9	0.09
20–25	49	14.2	63	12.1	
25–29	75	21.7	138	26.5	
30–34	130	37.6	165	31.7	
35–39	63	18.2	122	23.5	
≥40	17	4.9	22	4.2	
Maternal birthplace					
United States	283	81.8	394	75.8	0.10
Mexico	23	6.7	41	7.9	
Other	40	11.6	85	16.4	
Maternal education					
High school graduation or less	54	15.6	75	14.4	0.04
Some college, vocational school, or associate's degree	109	31.5	208	40.0	
Bachelor's degree or higher	183	52.9	237	45.6	
Home ownership					
No	78	22.7	173	33.5	0.001
Yes	266	77.3	344	66.5	
Type of health insurance					
Private	49	14.2	97	18.7	0.08
Government	296	85.8	422	81.3	

Table continues

Table 1. Continued

	TD (n = 346)		ASD (n = 520)		P Value[a]
	No.	%	No.	%	
Intentionality of pregnancy					
Intended to become pregnant at that time	233	68.1	322	63.5	0.39
Indifferent about becoming pregnant at that time	42	12.3	75	14.8	
Intended to become pregnant later	20	5.8	41	8.1	
Did not intend to become pregnant at all	47	13.7	69	13.6	
Maternal cigarette smoking[b]					
No	322	93.9	455	89.6	0.03
Yes	21	6.1	53	10.4	
Maternal alcohol consumption[b]					
No	243	72.3	353	69.9	0.45
Yes	93	27.7	152	30.1	
Use of a prenatal vitamin[c]					
No	22	6.4	31	6.0	0.83
Yes	324	93.6	486	94.0	
Use of a multivitamin[c]					
No	304	88.6	432	83.7	0.04
Yes	39	11.4	84	16.3	
Use of an iron-specific vitamin[c]					
No	234	68.6	380	74.5	0.06
Yes	107	31.4	130	25.5	
Consumption of cereal[c]					
No	27	7.9	70	13.5	0.01
Yes	316	92.1	448	86.5	
Use of other supplements[c]					
No	306	88.7	456	87.9	0.71
Yes	39	11.3	63	12.1	

Abbreviations: ASD, autism spectrum disorder; CHARGE, Childhood Autism Risks from Genetics and the Environment; TD, typical development.

[a] P values were derived from χ^2 tests comparing category proportions between the ASD group and the TD group.

[b] Any reported intake during the period from 3 months before pregnancy through the end of pregnancy.

[c] Any reported intake during the index period (3 months before pregnancy, during pregnancy, or while breastfeeding).

maternal folic acid intake during the first month of pregnancy to further control for folic acid's correlated association. To assess the effect of recall bias using the length of time elapsed before mothers were asked to recall their intake, associations were also examined for children who were under the median age of controls at the start of the interview (completion of the interview could require multiple sessions) compared with those who were older at the start of the interview.

RESULTS

Characteristics of participants with ASD and those with typical development (TD) are shown in Table 1. Children with ASD were more likely to have been born earlier than

Table 2. Use of Iron Supplements During the Index Period[a] by Mothers of Children With Autism Spectrum Disorder and Mothers of Children With Typical Development, CHARGE Study, California, 2003–2009

Iron-Specific Vitamin Use[b]	TD (*n* = 341)		ASD (*n* = 510)		Odds Ratio[c]	95% Confidence Interval	*P* Value
	No.	%	No.	%			
No	234	68.6	380	74.5	1	Reference	
Yes	107	31.4	130	25.5	0.63	0.44, 0.91	0.01

Abbreviations: ASD, autism spectrum disorder; CHARGE, Childhood Autism Risks from Genetics and the Environment; TD, typical development.

[a] The index period was defined as the period from 3 months before pregnancy through breastfeeding.

[b] Use at any time during the index period.

[c] Adjusted for maternal periconceptional folic acid intake, child's year of birth, and home ownership.

children with TD, and their mothers were significantly more likely to have some college education but no bachelor's or higher degree, to have smoked cigarettes, and to have taken a multivitamin during the index period and less likely to have eaten cereal during the index period. Their mothers also tended to be older, were more likely to have been born outside of the United States, and were less likely to have private medical insurance. Parents of children with ASD were significantly less likely to own their homes.

Of the 520 ASD and 346 TD CHARGE participants eligible for these analyses, 510 (98%) ASD participants and 341 (99%) TD participants had information on maternal use of iron supplements during the index period, and 454 (87%) ASD participants and 307 (89%) TD participants had information on total average maternal iron intake from all sources.

Twenty-five percent of mothers of children with ASD and 31% of mothers of TD children reported taking an iron-specific supplement at any time during the index period (Table 2), producing a significant association between reported iron supplement use and ASD (odds ratio (OR) = 0.63,

95% confidence interval (CI): 0.44, 0.91) after adjustment for maternal folic acid intake, home ownership, and the child's birth year. More mothers reported taking an iron supplement in the latter half of pregnancy (Figure 1). Adjusted odds ratios for associations between ASD and reported use of iron supplements were consistently below the null across the index period but were nonsignificant except during breastfeeding (among those who breastfed) (Figure 2; also see Web Table 1, available at http://aje.oxfordjournals.org/). Adjusted odds ratios also differed by the child's birth year (Web Table 2, Web Figure 1).

Prenatal vitamins were the greatest source of iron for mothers in both diagnostic groups during the index period (Table 3). Mean daily maternal iron intake from cereal and total iron intake from all collected sources during the index period were significantly lower for children with ASD than
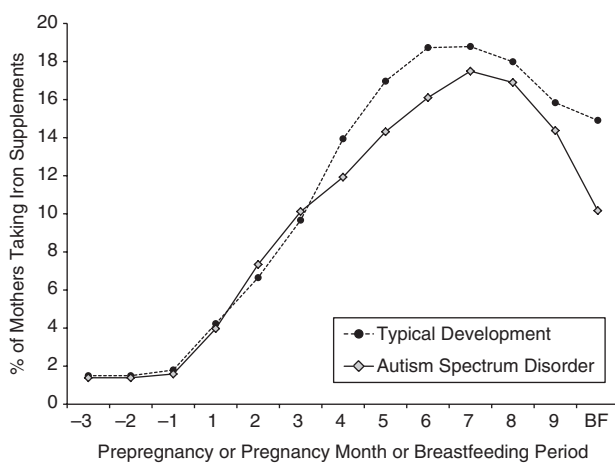
Figure 1. Percentages of mothers of children with autism spectrum disorder and mothers of children with typical development who took iron supplements during the index period (from 3 months before pregnancy through the end of pregnancy and during breastfeeding), Childhood Autism Risks from Genetics and the Environment (CHARGE) Study, California, 2003–2009. A trend towards a significant difference between groups was found during the breastfeeding (BF) period (*P* = 0.06).
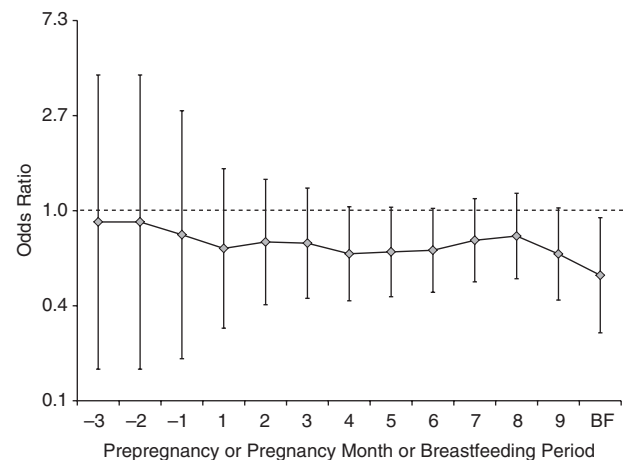
Figure 2. Adjusted odds ratios for associations between reported maternal iron supplement intake during the index period (from 3 months before pregnancy through the end of pregnancy and during breastfeeding) and autism spectrum disorder, Childhood Autism Risks from Genetics and the Environment (CHARGE) Study, California, 2003–2009. Odds ratios were adjusted for maternal folic acid intake during the first month of pregnancy, child's year of birth, and home ownership. Vertical bars represent 95% confidence intervals. Frequencies and *P* values are presented in Web Table 2. There was a significant association with autism spectrum disorder during the breastfeeding (BF) period (*P* < 0.05) and a borderline-significant association during months 4, 5, 6, and 9 of pregnancy (*P* < 0.10).

Table 3. Mean Maternal Iron Intake During the Index Period,[a] by Source, CHARGE Study, California, 2003–2009

Source of Iron	Maternal Iron Intake,[b] mg/day		Difference, mg/day	P Value[c]
	TD	ASD		
Prenatal vitamins	32.2 (20.9)	29.5 (15.8)	−2.7	0.39
Iron supplements	15.9 (28.5)	13.6 (28.4)	−2.4	0.08
Cereal	7.0 (7.5)	6.3 (7.6)	−0.6	0.047
Multivitamins	1.7 (6.7)	2.3 (6.0)	0.6	0.04
Other supplements	0.2 (0.8)	0.3 (1.2)	0.1	0.30
Total	57.1 (36.6)	51.7 (34.0)	−5.5	0.03

Abbreviations: ASD, autism spectrum disorder; CHARGE, Childhood Autism Risks from Genetics and the Environment; SD, standard deviation; TD, typical development.

[a] The index period was defined as the period from 3 months before pregnancy through breastfeeding.

[b] Values are presented as mean (standard deviation).

[c] Wilcoxon 2-sample test.

for children with TD, while maternal intake of iron from multivitamins was higher (Table 3).

Mean total iron intake was highest in the second half of pregnancy for both groups (Figure 3). Mean intake reported by case mothers was lower than that reported by control mothers, especially for the months before pregnancy and during early pregnancy (Figure 3). During this time (from 3 months before pregnancy through the second month of pregnancy), nonsignificantly more case mothers (74%) than control mothers (69%) ($P = 0.13$) had iron intakes below the Dietary Reference Intake for iron established by the Institute of Medicine for nonpregnant (18 mg/day) and pregnant (27 mg/day) women aged 19–50 years (39).

The highest category of maternal iron intake (≥86 mg/day) during the index period was associated with significantly reduced risk of ASD in the child, before and after adjustment for supplemental periconceptional folic acid intake, child's birth year, and home ownership (OR = 0.49, 95% CI: 0.29, 0.82) (Table 4). ASD risk decreased as mean maternal iron intake increased ($P_{\text{trend}} = 0.01$) (Table 4). These findings remained after imputation of missing values and when using survey weights (Table 4).

Adjusted odds ratios for the association between ASD and each quintile of maternal iron intake differed across the index period: Odds ratios were near the null during the months before pregnancy; above the null for the first 2 months of pregnancy (after adjustment for the highly correlated folic acid intake during this time); consistently below the null for the highest quintile from the third month of pregnancy onward; and below the null during breastfeeding (Figure 4, Web Table 3). Unadjusted odds ratios were more consistent across time (Web Figure 2). Post-hoc analysis showed that estimates for iron-specific supplement intake and the highest quintiles of iron intake during breastfeeding were not meaningfully different after adjustment for folic acid intake *during breastfeeding* (Web Table 4).

There were no significant differences in mean maternal iron intake on the basis of ASD-related characteristics such
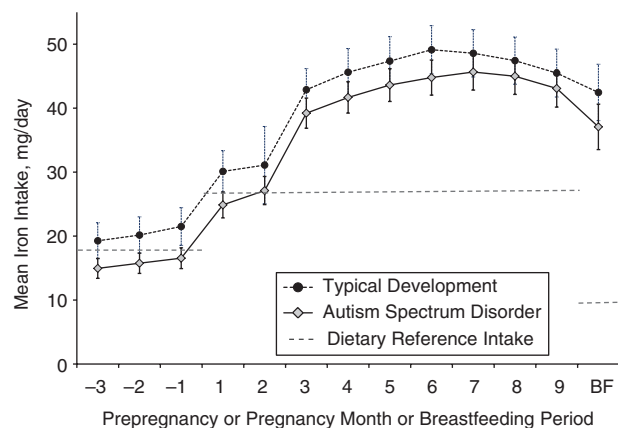
Figure 3. Mean iron intakes of mothers of children with autism spectrum disorder and mothers of children with typical development during the index period (from 3 months before pregnancy through the end of pregnancy and during breastfeeding), Childhood Autism Risks from Genetics and the Environment (CHARGE) Study, California, 2003–2009. Vertical bars represent 95% confidence intervals. Dietary Reference Intakes for iron in females aged 19–50 years are 18 mg/day for all women, 27 mg/day during pregnancy, and 9 mg/day during lactation (39). Significant differences between mothers of children with autism spectrum disorder and mothers of children with typical development were found for the 3 months before and the first month of pregnancy ($P < 0.01$) and for the second month of pregnancy ($P < 0.05$). A borderline-significant association was observed for months 3, 4, and 6 of pregnancy and during the breastfeeding (BF) period ($P < 0.10$).

as regression, delayed or atypical development, seizure, or verbal status (Web Table 5); however, children with ASD who had experienced early-onset symptoms and delayed or atypical development had larger differences from TD children in mean maternal iron intake than children with ASD who had experienced regression and who did not have delayed or atypical development.

No significant interactions were observed between maternal iron intake and child sex, child or maternal race/ethnicity, or interpregnancy interval. A significant multiplicative interaction ($P = 0.002$) was found between low maternal iron intake and older maternal age at delivery, with more than a 5-fold increased risk of ASD for mothers aged 35 years or older with iron intake in the lowest quintile as compared with younger mothers with iron intake in the highest quintile (OR = 5.01, 95% CI: 1.98, 12.69) (Table 5). The estimate for the combination of older maternal age and low iron intake was more than 5 times that expected from adding (OR = 0.98) or multiplying (OR = 0.80) their independent associations. There was also a significant multiplicative interaction ($P = 0.01$), with higher ASD risk being associated with the combination of mothers having metabolic conditions and iron intake in the lowest quintile (OR = 4.72, 95% CI: 1.69, 13.15), which was over twice that expected from adding (OR = 2.07) or multiplying (OR = 2.36) their independent associations (Table 6).

In analysis conducted to assess recall bias, the associations between reduced ASD risk and both use of an iron-specific supplement during the index period (OR = 0.52, 95% CI: 0.30, 0.90) and highest quintile of maternal iron intake versus

Table 4. Odds Ratios for Associations Between Quintile of Mean Maternal Iron Intake During the Index Period[a] and Child's Risk of Autism Spectrum Disorder, CHARGE Study, California, 2003–2009

Quintile of Mean Iron Intake, mg/day	TD (n = 307) No.	TD (n = 307) %	ASD (n = 454) No.	ASD (n = 454) %	Crude OR[b]	95% CI	P Value	Adjusted OR[c]	95% CI	P Value	Imputed-data OR[c,d]	95% CI	P Value	Weighted OR[c,e]	95% CI	P Value
<30	60	19.5	116	25.6	1	Reference		1	Reference		1	Reference		1	Reference	
30–<36	61	19.9	92	20.3	0.78	0.50, 1.23	0.28	0.92	0.57, 1.51	0.75	0.87	0.55, 1.39	0.57	1.08	0.64, 1.83	0.75
36–<52	63	20.5	88	19.4	0.72	0.46, 1.14	0.16	0.80	0.48, 1.33	0.38	0.82	0.51, 1.33	0.43	0.85	0.49, 1.49	0.38
52–<86	60	19.5	92	20.3	0.79	0.50, 1.25	0.32	0.76	0.46, 1.24	0.27	0.74	0.46, 1.19	0.21	0.76	0.45, 1.30	0.27
≥86	63	20.5	66	14.5	0.54	0.34, 0.87	0.01	0.49	0.29, 0.82	0.01	0.52	0.32, 0.84	0.01	0.46	0.27, 0.79	0.01
2-sided P for trend		0.02		0.02			0.02			0.01			0.01			0.004

Abbreviations: ASD, autism spectrum disorder; CHARGE, Childhood Autism Risks from Genetics and the Environment; CI, confidence interval; OR, odds ratio; TD, typical development.
[a] The index period was defined as the period from 3 months before pregnancy through breastfeeding.
[b] Unadjusted.
[c] Adjusted for maternal periconceptional folic acid intake, child's birth year, and home ownership.
[d] Missing values were imputed.
[e] To account for unequal sampling and response probabilities, estimated weights based on case group and demographic factors were used.

the lowest (OR = 0.38, 95% CI: 0.18, 0.83; $P_{trend} = 0.03$) were stronger when data were limited to children who were under 3.6 years of age (the median age of controls) at the time of interview start than in children who were older (for taking an iron-specific supplement, OR = 0.74, 95% CI: 0.45, 1.20; for highest quintile of maternal iron vs. lowest, OR = 0.65, 95% CI: 0.31, 1.37 ($P_{trend} = 0.11$)) (Web Table 6). There were no significant differences in associations for taking an iron-specific supplement during the index period or higher maternal iron intake when data were stratified by maternal folic acid intake during the first month of pregnancy (<600 µg/day, ≥600 µg/day) (Table 7). Finally, adjusted odds ratios for the association between quintile of iron intake and ASD differed somewhat by the child's birth year, but not for the highest quintile of iron intake compared with the lowest, for which odds ratios were consistently below the null (Web Table 7, Web Figure 3).

DISCUSSION

Findings of this study

To our knowledge, this study was the first to examine maternal iron intake in relation to ASD. We found that mothers of children with ASD had significantly lower iron intakes during the index period than mothers of children with TD. The highest quintile of maternal iron intake during the index period was associated with an approximately halved risk of ASD compared with the lowest quintile. The association between higher maternal iron intake and reduced ASD risk was strongest during breastfeeding, after adjustment for folic acid intake. Further, ASD risk associated with low maternal iron intake was much greater when the mother also was older at the time of the child's birth and/or had metabolic conditions during pregnancy.

Average iron intake from cereals and supplements throughout pregnancy for both groups in this study was above the Tolerable Upper Intake Limit for iron (40) but was in line with national estimates of intake from supplements for pregnant women (48 mg/day) (41) and women of childbearing age (18 mg/day) (42). Notably, the lowest iron quintile (<30 mg/day) for the index period included the amounts recommended for pregnant (27 mg/day), nonpregnant (18 mg/day), and lactating (9 mg/day) women (40). If the results of this study are replicated, it would suggest that the current Recommended Daily Allowance for pregnancy and/or lactation could be set too low to prevent adverse outcomes in the child. A randomized, double-blind trial demonstrated that during pregnancy, 40 mg/day of supplemental iron was needed to prevent iron deficiency in the majority of women and that up to 80 mg/day was well-tolerated (43). On the other hand, even though the amount of iron absorbed is regulated by the gut to correspond to needs and avoid accumulation of excess iron in healthy individuals (44), very high iron intake in supplemental or fortified forms is known to be toxic and has been associated with negative health impacts, including adverse neurodevelopmental outcomes, and could be especially harmful for certain genetically susceptible individuals (42, 45). The stronger association for higher maternal iron intake while breastfeeding is intriguing given the fact that maternal iron intake has been shown to not affect iron concentrations in breast milk
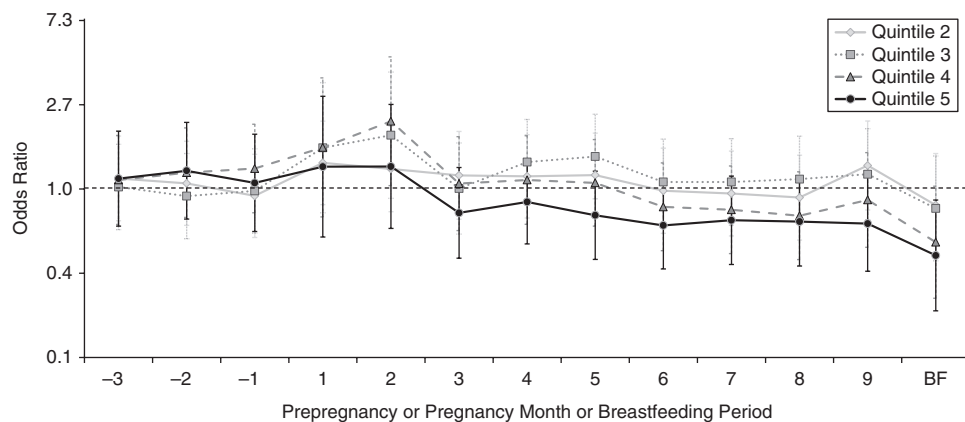
Figure 4. Adjusted odds ratios for associations between mean maternal iron intake during the index period (from 3 months before pregnancy through the end of pregnancy and during breastfeeding) and autism spectrum disorder, Childhood Autism Risks from Genetics and the Environment (CHARGE) Study, California, 2003–2009. Odds ratios were adjusted for maternal folic acid intake during the first month of pregnancy, child's year of birth, and home ownership. Vertical bars represent 95% confidence intervals. Frequencies and P values are presented in Web Table 3. There was a significant inverse association with autism spectrum disorder for the highest iron quintile (P = 0.02) during the breastfeeding (BF) period; a significant positive association for the fourth quintile (P = 0.04) and a borderline-significant association for the third quintile (P = 0.10) during the second month of pregnancy; and a borderline-significant association for the fourth quintile (P = 0.06) during breastfeeding.

Table 5. Odds Ratios for Autism Spectrum Disorder According to Maternal Age at the Child's Birth and Category of Mean Maternal Iron Intake During the Index Period,[a] CHARGE Study, California, 2003–2009

Category of Mean Iron Intake, mg/day	Maternal Age <35 Years					Maternal Age ≥35 Years				
	No. With TD	No. With ASD	OR[b]	95% CI	P Value	No. With TD	No. With ASD	OR[b]	95% CI	P Value
Highest quintile (≥86)	49	56	1	Reference		14	10	0.57	0.19, 1.72	0.31
Middle 3 quintiles (30–<86)	140	204	1.46	0.89, 2.38	0.13	44	68	1.85	0.98, 3.48	0.06
Lowest quintile (<30)	50	75	1.41	0.79, 2.51	0.24	10	41	5.01[c]	1.98, 12.69	0.001

Abbreviations: ASD, autism spectrum disorder; CHARGE, Childhood Autism Risks from Genetics and the Environment; CI, confidence interval; OR, odds ratio; TD, typical development.

[a] The index period was defined as the period from 3 months before pregnancy through breastfeeding.

[b] Adjusted for maternal periconceptional folic acid intake, child's birth year, and home ownership.

[c] The P value for multiplicative interaction was 0.002 for the lowest quintile of iron intake. The expected odds ratios for the combination of the lowest quintile of iron intake and maternal age ≥35 years were 0.98 and 0.80 in the additive and multiplicative models, respectively.

Table 6. Odds Ratios for Autism Spectrum Disorder According to Maternal Metabolic Conditions Present During Pregnancy[a] and Category of Maternal Iron Intake During the Index Period,[b] CHARGE Study, California, 2003–2009

Category of Mean Iron Intake, mg/day	No Maternal Metabolic Condition					Maternal Metabolic Condition				
	No. With TD	No. With ASD	OR[c]	95% CI	P Value	No. With TD	No. With ASD	OR[c]	95% CI	P Value
Highest quintile (≥86)	53	54	1	Reference		7	9	1.51	0.39, 5.84	0.55
Middle 3 quintiles (30–<86)	148	195	1.60	0.98, 2.61	0.06	23	41	2.21	1.01, 4.31	0.048
Lowest quintile (<30)	48	71	1.56	0.87, 2.78	0.13	6	27	4.72[d]	1.69, 13.15	0.003

Abbreviations: ASD, autism spectrum disorder; CHARGE, Childhood Autism Risks from Genetics and the Environment; CI, confidence interval; OR, odds ratio; TD, typical development.

[a] Metabolic conditions included obesity (prepregnancy body mass index (weight (kg)/height (m)^2) ≥30), hypertension, and/or diabetes.

[b] The index period was defined as the period from 3 months before pregnancy through breastfeeding.

[c] Adjusted for maternal education, race/ethnicity, and periconceptional folic acid intake; child's birth year and sex; home ownership; type of health insurance; and regional center catchment area.

[d] The P value for multiplicative interaction was 0.01 for the lowest quintile of iron intake. The expected odds ratios for the combination of the lowest quintile of iron intake and maternal metabolic conditions were 2.07 and 2.36 in the additive and multiplicative models, respectively.

Table 7.   Odds Ratios for Autism Spectrum Disorder According to Folic Acid Intake During the First Month of Pregnancy, Use of an Iron-Specific Supplement During the Index Period,[a] and Quintile of Mean Maternal Iron Intake During the Index Period, CHARGE Study, California, 2003–2009

	Folic Acid Intake <600 μg/day[b]					Folic Acid Intake ≥600 μg/day[b]				
	No. With TD	No. With ASD	OR[c]	95% CI	*P* Value	No. With TD	No. With ASD	OR[c]	95% CI	*P* Value
Maternal use of iron-specific supplement[d]										
No	73	155	1	Reference		124	173	1	Reference	
Yes	38	54	0.58	0.34, 0.99	0.046	47	49	0.68	0.41, 1.12	0.13
Quintile of mean iron intake, mg/day[e]										
<30	29	61	1	Reference		26	44	1	Reference	
30–<36	16	32	0.86	0.40, 1.86	0.71	38	55	0.98	0.50, 1.92	0.10
36–<52	19	32	0.72	0.34, 1.54	0.40	38	48	0.88	0.43, 1.80	0.73
52–<86	20	46	0.85	0.41, 1.75	0.65	33	40	0.72	0.35, 1.48	0.37
≥86	22	27	0.48	0.22, 1.01	0.054	33	30	0.51	0.25, 1.07	0.07
2-sided *P* for trend					0.09					0.04

Abbreviations: ASD, autism spectrum disorder; CHARGE, Childhood Autism Risks from Genetics and the Environment; CI, confidence interval; OR, odds ratio; TD, typical development.

[a] The index period was defined as the period from 3 months before pregnancy through breastfeeding.

[b] The Dietary Reference Intake established by the Institute of Medicine for folic acid during pregnancy is 600 μg/day (39).

[c] Adjusted for child's birth year and home ownership.

[d] *P* for interaction = 0.63.

[e] *P* for interaction: second quintile, *P* = 0.81; third quintile, *P* = 0.74; fourth quintile, *P* = 0.83; fifth quintile, *P* = 0.84.

(46); however, the very low concentrations of iron in breast milk (relative to those in serum) present measurement problems that could have obscured a relationship, and concentrations in the early postnatal period (before 9 months) have not been examined using modern iron indicators. Alternatively, maternal iron intake could influence breast milk production or composition in other ways.

This study examined maternal iron intake, not maternal or child iron status. Maternal circulation constitutes the only source of iron for the developing fetus (11), and maternal iron intake can influence both the mother's iron status and her child's status (47) during brain development. Our findings may reflect compensation through iron supplementation for poorer iron status resulting from inadequate intake, inefficient uptake or metabolism, or increased needs for iron, producing a functional iron deficiency. It has been demonstrated, primarily in animal studies, that reduced iron supply at several stages of development generates enduring changes in dopamine neurotransmission (48–53) that outlast the iron-deficient periods (52, 54). Long-term effects are observed in adulthood, long after iron repletion, in hippocampal structure and function, monoamine metabolism, and myelination (55–60), indicating that early developmental periods are critical and that prevention of iron deficiency might be key for protecting against adverse neurodevelopmental outcomes. Iron deficiency can also impair the function of several enzymes that are directly involved in antioxidant and nucleic acid metabolism, which could affect genomic stability during periods of DNA synthesis and cell proliferation during development (39). In addition, mothers of children with ASD have been shown to have elevated markers of inflammation (61), and prenatal inflammation is an independent risk

factor for ASD (62). Prenatal inflammation can produce a cytokine-mediated reduction of circulating nonheme iron, or hypoferremia (48), that can disrupt fetal brain development and lead to persistent structural and functional brain defects (55–60). Maternal iron supplementation has been shown to prevent effects of inflammation-induced hypoferremia (48).

Metabolic conditions like diabetes and obesity lead to iron deficiency (63, 64), and as their prevalence continues to rise dramatically (65), suboptimal iron status during pregnancy can be expected to increase as well. Notably, metabolic conditions are independently associated with a 1.7-fold increased risk of ASD and nearly a 2-fold risk of developmental delays (36). Our study shows that the combination of maternal metabolic conditions and low supplemental iron intake is associated with a nearly 5-fold increased risk of ASD, and that the ASD risk associated with maternal metabolic conditions was nearly null for persons with the highest supplemental iron intake. This interaction effect, if replicated, implies that maternal supplemental iron is associated with prevention of ASD in children of mothers with metabolic conditions during pregnancy.

The significant interaction between older maternal age and lower supplemental iron intake seems biologically plausible given changes in iron metabolism and storage with age, especially in women (66, 67). If this finding is replicated, more work would be needed to delineate the mechanistic pathways behind this interaction.

Limitations and strengths of this study

The retrospective reporting of vitamin and supplement information after the child's developmental status was known,

whereby mothers were asked to recall a period several years before the interview, raises the issues of recall accuracy and bias in this study. A scenario in which recall bias explained part of the association between maternal iron intake and ASD would involve case mothers underreporting or control mothers overreporting their intake of supplements containing iron. Notably, the association between iron supplementation and reduced ASD risk was stronger when women recalled the information for a more recent pregnancy versus a less recent pregnancy, which argues against recall bias in this direction. However, we cannot rule out some role for differential recall across case status.

In addition, during these study years data were not collected on other dietary sources of iron, so information was not available with which to completely assess dietary iron intake. However, fortified cereals, which were included in this study, are the largest source of total dietary iron consumed in the United States (68). In addition, the amount of iron in supplements tends to outweigh the amount of iron found in the diet. Finally, iron supplementation is probably more amenable to prevention strategies than diet is.

We did not collect information on why the mothers took iron supplements, which are often taken after physician recommendations when iron deficiency anemia is detected during pregnancy. However, case mothers reported lower intake of iron from sources other than iron-specific supplements (breakfast cereal) that would not have been as likely to increase in response to an anemia diagnosis. In addition, differences in iron intake between groups were observed not only in late pregnancy, when anemia is more likely to be diagnosed, but throughout the index period. This provides evidence that the association was not entirely due to confounding by indication.

Supplemental intakes of folic acid and iron tend to be correlated, and thus it is difficult to examine their independent contributions. Odds ratios for quintiles of iron intake during the months before pregnancy and during early pregnancy were attenuated after adjustment for folic acid intake, as expected given that periconceptional folic acid is associated with reduced ASD risk (35, 69). However, the association with maternal iron intake during the index period remained strong after adjustment for and stratification across folic acid intake. In addition, the association with iron-specific supplements, which was not correlated with folic acid, was consistently below the null and significant during breastfeeding. Differences in associations by year were probably artifactual, given that the associations were not influenced by year in a consistent pattern. Still, other unmeasured confounding factors associated with taking supplements could have played a role in our findings.

The efficiency of iron uptake and metabolism differs vastly between individuals on the basis of genetic differences (46). It is likely that genetically determined metabolic efficiency could modify the need for and the effects of iron supplementation. These genetic differences were not considered here, and they deserve further evaluation.

Strengths of this study include detailed information systematically collected on numerous potentially confounding variables, clinical confirmation of all ASD diagnoses, and confirmation of typical social and cognitive development for the population-based controls. Additionally, the study's large sample size allowed for stratification of results by case subgroup and by maternal and child factors likely to modify the association.

Conclusions

This study provides initial evidence for an association between increased maternal supplemental iron intake and reduced risk of ASD. Researchers should attempt to replicate this association in additional studies and to further delineate who is metabolically susceptible, clarify what dose of iron during pregnancy and breastfeeding is ideal for neurodevelopment, and identify and refine strategies for prevention of ASD through supplemental iron intake.

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