DeVilbiss et al. Prenatal nutritional supplementation and autism spectrum disorders in the Stockholm Youth Cohort: population-based cohort study

Methods Supplement I and supplementary tables

Methods Supplement I

Supplement recommendations and use

The official recommendation from the National Board of Health and Welfare since 1996 is to take a folic acid supplement with 400 μg per day, beginning one month prior to conception and continuing through the end of the first trimester.[1] Regarding iron supplementation, guidelines from the Swedish Society of Obstetrics and Gynecology from 1991 through the study period recommended initiation of iron supplementation of 100 mg per day in weeks 20-24 (this recommendation was removed in 2008), while women with anemia should start iron supplementation immediately at 200 mg daily.[2] We were unable to find any guidelines regarding multivitamin supplement use in Sweden during the study period. As a reviewer points out, use of multivitamins in pregnant Swedish women may vary by time and place. A large study in southeast Sweden for children born 1997-1999 found that 18% of pregnant women take multivitamins,[3] supporting that estimates of use of multivitamins in the present study do not appear to be unreasonably low.

Actual recommendations regarding diet and nutrition vary depending on the health practitioner seen. For example, a 2003 study in Sweden found that 41% of midwives who were responsible for individual counseling provided recommendations regarding use of iron supplements that were consistent with the policy.[4] Prenatal supplements are purchased at the pharmacy and this purchase is subsidized by the universal health care system. Because of repeated antenatal visits, it is possible that a woman's intake might change after this point. We acknowledge that timing, among other aspects of supplement use such as dose, are necessary to investigate in further studies.

While there is no tradition of using omega 3 fatty acid (e.g., cod liver oil) supplements in Sweden,[3] women in Sweden reported a wide variety of supplement use during pregnancy. For example,

in a study of >15,000 women in Sweden giving birth during the timeframe of our study, 89% of women used some sort of supplement during pregnancy.[5] Thus, we are careful to clarify that the 'no MV/FA/iron' group is likely to be taking other supplements at some point during pregnancy. Because of the prior studies on folic acid, iron, and multivitamins and ASD, we restricted our study to these exposures *a priori*.

Free text coding of nutritional supplementation

Free text coding of supplements and other drugs was performed in R. Manual coding was performed on the most common 1,002 (2.4%) of 41,020 free text responses. Since the 1,002 manually coded values occurred 338,555 times from a total of 414,907 free text responses, manual examination of these values represented 81.6% of the free text data.

Manual coding informed an automated exact and fuzzy pattern matching process. Exact matching was used to account for known generic, Swedish, and international brand names, abbreviations, and non-standard terms, whereas fuzzy (approximate) matching was integrated to account for punctuation and spacing differences, and misspellings.[6] Automated matches were reviewed, false positives were removed as necessary, and coding was optimized accordingly. R code for pattern matching is included in Methods Supplement II.

Covariate categorization

Maternal education, smoking, and BMI categorical covariates include levels for missing data. The creation of missing categories is preferred to exclusion due to the degree of missingness for smoking (13.4%) and BMI (20.7%). Consequently, any difference in estimates might not suggest that inclusion of a missing category introduces bias but rather could be due completely to the fact that the sample has changed appreciably.

Propensity score analyses

Propensity score (PS) matching can create a sample matched on all observed covariates, including maternal health indicators. The goal of matching is to make exposed and unexposed groups more comparable, or balanced, on these characteristics. PS matching aims to reduce bias due to confounding by comparing the outcomes of matched groups that primarily differ in their exposure. As such, PS matching reduces dependence on model-based assumptions and increases robustness of resulting statistical models.[7]

PS analyses were conducted separately for each exposure and were compared against non-users of folic acid, iron and multivitamins. k:1 nearest neighbor greedy matching was implemented with a caliper of 0.05 - 0.1 standardized difference using the MatchIt package in R.[8] Exact matching on birth year was specified for multivitamin models to improve balance. Unexposed to exposed matching ratios were a function of size of the exposed group, with ratios ranging from 1:1 to 5:1. Balance was assessed through comparison of standardized mean difference of covariates before and after matching. PS matching resulted in well-balanced comparison groups (eFigure 1).

Sensitivity analysis

Unmeasured confounding: The method of Lin et al[9] was used to examine robustness of an observed association to unmeasured confounding. The goal of this analysis was to assess whether an observed association between multivitamin use and risk of ASD with ID was likely to be qualitatively changed under reasonable assumptions about unmeasured confounding. A hypothetical binary confounder that reduced the risk of ASD was assumed to be more prevalent among multivitamin users than in non-users across a range of plausible parameters. Because the association from the propensity score analysis is theoretically the most robust to measured confounding, this association was the basis of this test of unmeasured confounding.

Population preventive fraction

Given the low prevalence of ASD with ID, the estimated unadjusted odds ratio (uOR) of 0.54 should approximate a relative risk. The estimated prevalence of ASD with ID among mothers not taking multivitamin supplements (963/207,193 = 0.46%) and uOR suggest that if the association between multivitamins and ASD with ID could be assumed to be causal, absolute risk among children of mothers not taking supplements could be reduced from 465 to 214 per 100,000 with supplementation[10]. Assumptions that may be unsupported are implicit in this calculation and given that underlying mechanisms are unknown, this estimate must be cautiously interpreted.[11] Moreover, assuming a causal link could be established between multivitamin use and ASD with ID, it is uncertain how this magnitude of absolute effect would translate to other populations that have different backgrounds of nutritional supplement use, fortification, dietary patterns, and health risks.

Supplementary Tables

eTable1. Analytic sample sizes

	Sibling[1]		Propensity score		Multivariate	
	Mothers	Children	Mothers	Children	Mothers	Children
ASD with ID						
Folic acid	77,395	169,760	15,575	16,086	173,839	268,695
Iron			102,974	127,860		
Folic acid and Iron	Discordant: 642	Discordant: 1536	71,645	84,473		
Multivitamin			72,696	85,568		
ASD without ID						
Folic acid	78,903	173,209	15,810	16,353	176,952	271,944
Iron			103,994	129,582		
Folic acid and Iron	Discordant: 2447	Discordant: 5615	72,373	85,495		
Multivitamin			73,533	86,686		
Any ASD						
Folic acid	79,404	174,428	15,892	16,432	178,083	273,107
Iron			104,280	130,230		
Folic acid and Iron	Discordant: 3066	Discordant: 7122	72,695	85,871		
Multivitamin			73,730	86,994		

[1] While the larger figures represent the samples utilized in sibling analyses, only strata (mothers) having discordant supplement usage contribute to the analyses mathematically. The N's in the Mothers column thus represents the number of sets of siblings with discordant exposures. The N's in the Children column represents the number of siblings total in the discordant sets.

eTable 2. Derivation of supplement user groups from ATC codes

Supplement use	Logic				
Folic acid only[a]	*Contains* B03BB (B03BB, B03BB01, B03BB51)	*AND*	*Does not contain* B03A *OR* A11A *OR* A11B		
Iron only[b]	*Contains* B03A (B03A, B03AA, B03AB, B03AC, B03AD, B03AE)	*AND*	*Does not contain* B03BB *OR* A11A *OR* A11B		
Iron and Folic acid	*Contains* B03BB	*AND*	*Contains* B03A	*AND*	*Does not contain* A11A *OR* A11B
Multivitamins[c]	*Contains* A11A (A11A, A11AA, A11AB)	*OR*	*Contains* A11B (A11B, A11BA)		
Non-use (ref)	*Does not contain* B03BB, B03A, A11A, *OR* A11B				

[a] Folic acid use is recorded as B03BB, "Folic acid and derivatives", including ATC codes B03BB, B03BB01, and B03BB51.
[b] Iron use is recorded as B03A, "Iron preparations", which includes B03A, B03AA, B03AB, B03AC, B03AD, and B03AE.
[c] Multivitamin use is recorded as A11A, "Multivitamins, combinations" or A11B, "Multivitamins, plain". This includes ATC codes A11A, A11AA, A11AB, A11B, and A11BA.

eTable 3. ICD-8, ICD-9, and ICD-10 codes used to identify history of specific neuropsychiatric conditions before birth

ICD version	Nordic ICD-8	Nordic ICD-9	ICD-10
Neuropsychiatric condition			
Anxiety disorders (any)	300 [0,2,9]	300 [00,01,02] 300 [2,9]	F40-41 (all)
Autism		299 (all)	F84
Bipolar disorder	296 (all)	296 [.0, .1, .4, .5, .6, .7, .8, .9]	F30-31
Depression/ mood disorder	300.4	296 [2,3] 300.4 311	F32-39
Epilepsy	345 (all)	345 (all)	G40 (all) G41
Intellectual disability	310-315	317 318 319	F70-79
Non-affective psychotic disorder (schizophrenia)	F20-29	295 298	295 (all) 298 [3,9] 299
Stress related disorders	298 [0,1,2,9]	308 (all) 309 (all)	F43 (all)

eTable 4. Adjusted[a] odds ratios and 95% confidence intervals[b] for complete multivariable sample: any multivitamins (multivitamins with or without use of additional iron or folic acid) compared to use of multivitamins alone

ASD with ID	
Multivitamin	**0.69 (0.57 to 0.84)**
Multivitamin only	**0.60 (0.44 to 0.84)**

[a] Adjusted for child characteristics (sex, birth year, and years resided in Stockholm County), socioeconomic indicators (education, family income, and maternal birth country), maternal health indicators (age, BMI, parity, smoking status), medication use during pregnancy (anti-depressants or anti-epileptics), and specific neuropsychiatric diagnoses before birth (anxiety disorders, autism, bipolar disorder, depression, epilepsy, intellectual disability, non-affective psychotic disorder, and stress disorder)

[b] Calculated using generalized estimating equation logistic regression, grouped by birth mother

eTable 5. Adjusted odds ratios and 95% confidence intervals for sibling and multivariate analyses using indicator variables for supplement use.

Sample	Sibling[a]	Multivariable[b]
Model	CLR[c]	GEE[d]
ASD with ID		
Folic acid	0.91 (0.63 to 1.31)	1.08 (0.91 to 1.28)
Iron	1.01 (0.77 to 1.32)	0.99 (0.87 to 1.13)
Multivitamin	**0.80 (0.55 to 1.17)**	**0.71 (0.59 to 0.84)**

[a] Adjusted for child sex, parity, and birth year

[b] Adjusted for child characteristics (sex, birth year, and years resided in Stockholm County), socioeconomic indicators (education, family income, and maternal birth country), maternal health indicators (age, BMI, parity, smoking status), medication use during pregnancy (anti-depressants or anti-epileptics), and specific neuropsychiatric diagnoses before birth (anxiety disorders, autism, bipolar disorder, depression, epilepsy, intellectual disability, non-affective psychotic disorder, and stress disorder)

[c] Conditional logistic regression, matched on birth mother

[d] Generalized estimating equation logistic regression, grouped by birth mother

eTable 6. Adjusted[a] odds ratios and 95% confidence intervals[b] for multivariate analysis stratified by hospital stays during pregnancy

Sample	Hospital stays	
	None n = 121,689	1 or more n = 148,324
ASD with ID		
Folic acid	0.70 (0.25 to 2.00)	1.58 (0.86 to 2.89)
Iron	1.05 (0.85 to 1.31)	0.87 (0.72 to 1.06)
Folic acid and Iron	1.17 (0.87 to 1.57)	0.96 (0.73 to 1.26)
Multivitamin	**0.64 (0.47 to 0.87)**	**0.75 (0.58 to 0.97)**
ASD without ID		
Folic acid	0.88 (0.55 to 1.43)	**1.59 (1.16 to 2.16)**
Iron	1.04 (0.92 to 1.17)	0.92 (0.83 to 1.02)
Folic acid and Iron	0.94 (0.78 to 1.13)	0.87 (0.74 to 1.01)
Multivitamin	1.01 (0.88 to 1.17)	0.89 (0.79 to 1.01)
Any ASD		
Folic acid	0.84 (0.54 to 1.30)	**1.59 (1.21 to 2.10)**
Iron	1.04 (0.93 to 1.16)	0.91 (0.83 to 1.00)
Folic acid and Iron	0.99 (0.85 to 1.17)	0.89 (0.78 to 1.02)
Multivitamin	0.94 (0.82 to 1.07)	**0.87 (0.78 to 0.97)**

[a] Adjusted for child characteristics (sex, birth year, and years resided in Stockholm County), socioeconomic indicators (education, family income, and maternal birth country), maternal characteristics (age, BMI, parity, smoking status), medication use during pregnancy (anti-depressants or anti-epileptics), and specific neuropsychiatric diagnoses before birth (anxiety disorders, autism, bipolar disorder, depression, epilepsy, intellectual disability, non-affective psychotic disorder, and stress disorder)

[b] Calculated using generalized estimating equation logistic regression, grouped by birth mother

eTable 7. Potential impact of unmeasured confounding on the propensity score estimate for maternal multivitamin supplementation and risk of ASD with ID

Reduced risk of ASD with ID due to confounder[a]	Prevalence of confounder		Adjusted OR (95% CI)
	Multivitamin users	Non-users	
-	0%	0%	**0.69 (0.57 to 0.84)[b]**
25%	20%	10%	**0.71 (0.59 to 0.86)**
25%	40%	20%	**0.73 (0.60 to 0.89)**
25%	60%	30%	**0.75 (0.62 to 0.91)**
25%	80%	40%	**0.78 (0.64 to 0.95)**
50%	20%	10%	**0.73 (0.60 to 0.89)**
50%	40%	20%	**0.78 (0.64 to 0.95)**
50%	60%	30%	0.84 (0.69 to 1.02)
50%	80%	40%	0.92 (0.76 to 1.12)

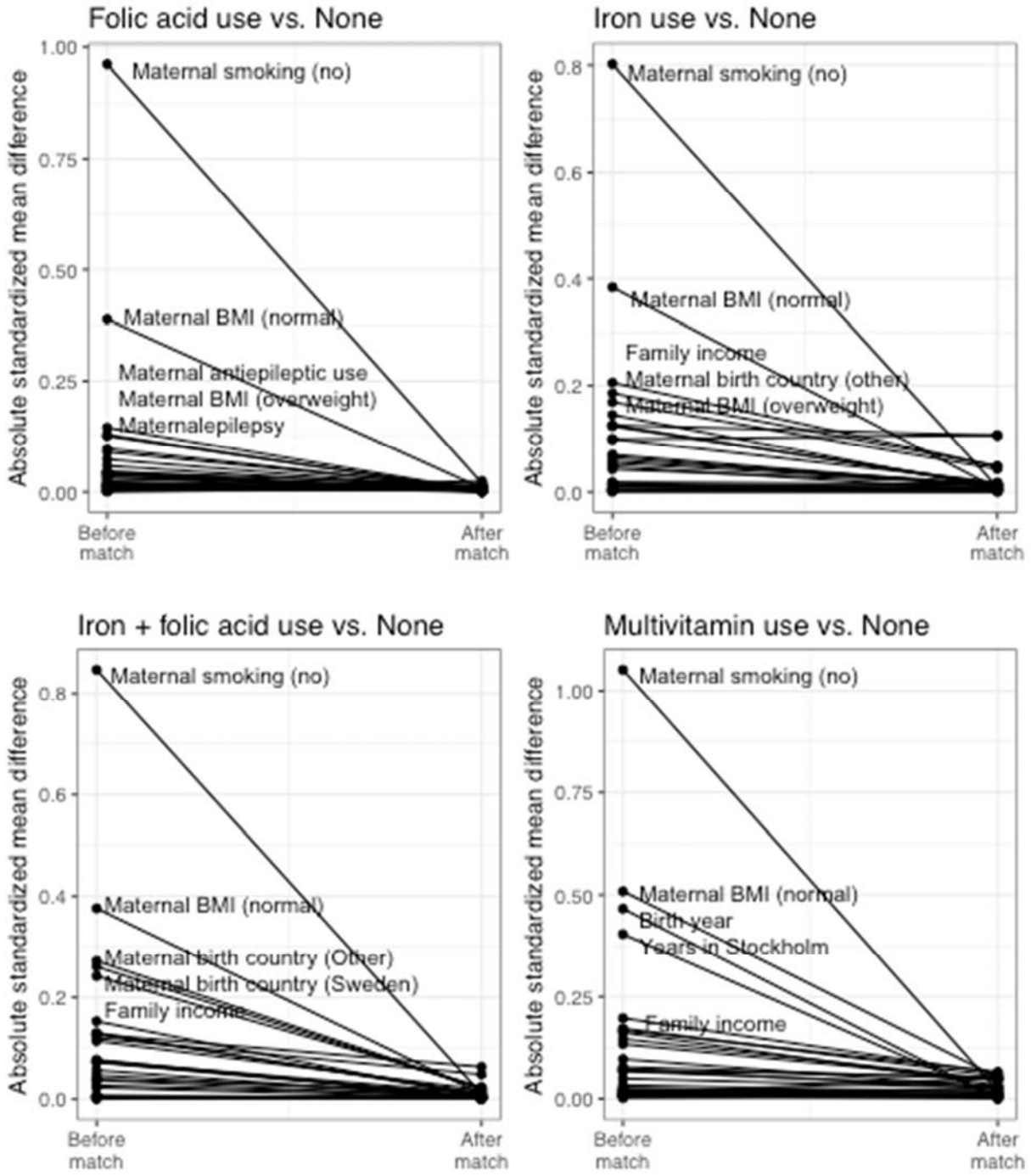
[a] Assumes that the elevated risk of ASD with ID due to the unmeasured confounder is the same in both multivitamin users and non-users

[b] The original reported estimate in Table 2

Sensitivity analysis of the multivitamin propensity score finding did not support that the presence of unmeasured confounding was likely to be wholly responsible for observed associations. For example, even if there were an unmeasured confounder that reduced the risk of ASD with ID by 50%, and had 40% prevalence among multivitamin users and 20% prevalence in non-users, the corrected PS estimate would remain significant (OR: 0.78 [0.64 to 0.95]). The unmeasured confounder would have to exert such an effect above and beyond the covariates matched in propensity score analyses.

We believe the ranges of the parameters represent a realistic range of plausible values. While stronger influences, such as having an affected first-degree family member, are in theory plausible, shared genetic familial factors are controlled for in the sibling analyses, which produced a similar adjusted OR among matched siblings.

eFigure 1. Absolute standard biases[a] for covariates in ASD propensity score models[b]



Each graph incorporates data from propensity score models for any ASD; graphs for ASD with ID and ASD without ID were largely similar

[a] The purpose of these plots is to indicate the relative improvement in covariate balance after matching. Absolute standardized bias is the absolute value of the weighted difference in covariate means between the treatment (supplement users) and control group (non-supplement users) divided by the standard deviation in the exposed group. If treated and control groups were perfectly balanced on all measured covariates, absolute standard bias would be equal to zero, indicating perfect matching. Absolute standard biases were calculated for all covariates used to calculate the propensity score.

[b] Propensity scores were calculated using logistic regression.

Supplement References

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