Web Material

One-Carbon Cofactor Intake and Risk of Neural Tube Defects Among Women Who Meet Folic Acid Recommendations: A Multicenter Case-Control Study

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Web Appendix 1

The One-Carbon Cycle

This description is provided to supplement Figure 1 in the main text. In brief, saturation of one of folate/folic acid's carbon-carbon double bonds results in dihydrofolate (DHF). Following, the addition of a hydrogen molecule leads to trahydrofolate (THF). Through the conversion of 5-methyl-THF to THF, homocysteine (Hcy) acquires a methyl group, resulting in methionine, a process mediated by S-adenosylmethionine (SAM); cobalamin (i.e., vitamin B12) is a cofactor of this pathway and interacts with the 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR) gene. BHMT also converts homocysteine to methionine (in liver and kidney); betaine, which is oxidized from choline, is the methyl-donor for this reaction, and pyridoxine (vitamin B6) is a cofactor in the BHMT pathway.

Categorization of One-Carbon Cofactor Intake

To categorize dietary intake (after adjustment for estimated total caloric intake), unadjusted cubic-restricted splines with three and five knots were used to identify cutoff values for each one-carbon cofactor. Cutoffs were identified where the crude odds ratio (OR) for neural tube defects (NTDs) between the intake categories of interest differed by at least 20%. The spline curve for dietary intake for dietary vitamin B6 is provided as an example (Web Figure 1). We explored the impact of total calorie adjustment by reconstructing the splines on values before energy adjustment and identified similar cutoffs.

Web Table 1. Distribution for and Correlations Between Periconceptional Intake of One-Carbon Cofactors From Diet[a]

Average Daily Estimates[b]	Stat	B6 (mg)		B12 (µg)		Choline (mg)		Betaine (mg)		Methionine (g)		Folate (µg)		Total Calories	
Central Tendency		Case	Cont	Case	Cont	Case	Cont	Case	Cont	Case	Cont	Case	Cont	Case	Cont
	mean (SD)	1.82 (0.42)	1.88 (0.50)	4.36 (3.34)	4.26 (3.25)	272.9 (67.3)	275.0 (68.2)	84.7 (36.5)	80.1 (33.8)	1.60 (0.36)	1.63 (0.35)	373.4 (120.8)	384.2 (123.2)	1590 (570)	1536 (482)
	median (SD)	1.84 (0.42)	1.83 (0.53)	3.58 (2.08)	3.79 (1.81)	262.4 (80.9)	267.6 (75.7)	82.4 (46.9)	75.1 (40.2)	1.57 (0.49)	1.63 (0.45)	361.8 (162.3)	364.4 (155.7)	1473 (545)	1486 (609)
Correlations															
B6 (mg)	r	1.00		0.33		0.28		0.08		0.23		0.62		0.00	
B12 (µg)	r			1.00		0.21		0.00		0.19		0.22		0.00	
Choline (mg)	r					1.00		0.11		0.65		0.04		0.00	
Betaine (mg)	r							1.00		0.05		0.26		-0.02	
Methionine (g)	r									1.00		-0.07		-0.01	
Folate (µg)	r											1.00		0.03	
Total Calories	r													1.00	

Case cases; Cont controls; iqr interquartile range; r Pearson Correlation Coefficient; SD standard deviation

[a] Data restricted to mothers reporting periconceptional folic acid intake ≥ 400 µg, Slone Birth Defects Study (1998-2015)

[b] Daily estimates shown above have been adjusted for total caloric intake.

Web Table 2. Distributions and Associations Between Individual Intakes of One-Carbon Cofactors and Neural Tube Defect and Spina Bifida Outcomes[a]

One-Carbon Cofactor Comparison[b]		Cases (n exposed/ n Total)	Controls n Exposed/ n Total	Crude OR	95% CI	Adj. OR1[c]	95% CI	Adj. OR2[d]	95% CI
Vitamin B6									
≥2.2 mg vs < 2.2 mg	All NTDs	98/164	2008/2831	0.61	0.44, 0.84	0.76	0.54, 1.07	0.79	0.55, 1.14
	SB	73/124		0.59	0.41, 0.85	0.73	0.50, 1.08	0.77	0.51, 1.18
Vitamin B12									
≥3 µg vs <3 µg	All NTDs	140/164	2548/2831	0.65	0.41, 1.02	0.76	0.47, 1.22	0.92	0.54, 1.55
	SB	105/124		0.61	0.37, 1.02	0.72	0.43, 1.22	0.87	0.48, 1.55
Choline									
≥200 mg vs <200 mg	All NTDs	143/164	2562/2831	0.72	0.45, 1.15	0.73	0.44, 1.20	0.78	0.43, 1.40
	SB	109/124		0.76	0.44, 1.33	0.75	0.42, 1.32	0.74	0.38, 1.46
Methionine									
≥1.3 g vs <1.3 g	All NTDs	133/164	2383/2831	0.81	0.54, 1.21	0.82	0.54, 1.25	0.95	0.57, 1.58
	SB	103/124		0.92	0.57, 1.49	0.91	0.55, 1.49	1.07	0.59, 1.94
Betaine									
40 to <70 mg vs ≥70 mg	All NTDs	47/152	982/2610	0.74	0.52, 1.06	0.86	0.59, 1.24	0.86	0.59, 1.25
	SB	33/115		0.67	0.44, 1.01	0.73	0.48, 1.12	0.74	0.48, 1.13
<40 mg vs ≥70 mg	All NTDs	12/117	221/1849		nc		nc		nc
	SB	9/91			nc		nc		nc

Adj. adjusted; CI confidence interval; nc not calculated; NTDs neural tube defects; OR odds ratio; SB spina bifida; vs versus

[a]Data are from the Slone Birth Defects Study (1998-2015), restricted to mothers with periconceptional folic acid ≥400 µg.

[b]Intake categorization based on intake from diet and supplementation; cutoff based on unadjusted restricted cublic splines.

[c]Adj. OR1 controls for maternal age, race, and study center; Adj. OR1 and 95% CI displayed in Web Figure 3.

[d]Adj. OR2 controls for maternal age, race, study center and the other FPC intake listed in the table.

Web Table 3. Sources of One-Carbon Cofactor Intake from Diet and Supplements[a]

Micronutrient Group	N	N (%) meeting cutoff based on			Top 5 Sources	
		Diet only	Supplements only	Both	Diet[b]	Supplementation
Higher B6	2106	272 (12.9%)	1519 (72.1%)	313 (14.9%)	Cold Cereal Dark / Wheat Bread Rice / Pasta Poultry Eggs	Centrum Multivitamin CVS Prenatal Nature Made Prenatal Flintstones Multivitamin Maternal Prenatal
Higher B12	2688	911 (33.9%)	497 (18.5%)	1279 (47.6%)	Liver Milk Cold Cereal Cheese Poultry	
Higher Choline	2705	2476 (91.5%)	18 (0.7%)	211 (7.8%)	Milk Cold Cereal Cheese Poultry Eggs	Rainbow Light Prenatal Flintstones Multi w/ Choline VitaFusion Prenatal Whole Foods Prenatal Up and Up Prenatal
Middle Betaine	1029	1029 (100.0%)	n/a	n/a	Rice / Pasta Potato Chips French Fries Cake Mixed Dish (Lasagna, Casserole, Stew)	n/a
High Methionine	2516	2508 (99.7%)	2 (0.1%)	6 (0.2%)	Cheese Poultry Eggs Beef, pork, lamb Fish	Materna Prenatal (New) Vita-Vim Natural Factors Ultra Multi Quest Prenatal Opti-natal
All 5	622	65 (10.5%)	n/a	n/a	-	-

n/a not applicable

[a]Data are from the Slone Epidemiology Center Birth Defects Study (years 1998-2015), restricted to mothers reporting and meeting the spline-based cutoffs for each one-carbon cofactor listed in the table.

[b]Among those meeting the cutoff based on dietary intake.

Web Table 4. Comparison of Methodologies to Categorize One-Carbon Cofactor Intake[a]

Cofactor	Modeling Method	Periconceptional Supplementation[b]	Comparison for OR[c] (among non-supplementers)	Cases (n=164)	Controls (n=2831)	OR (95% CI) unadjusted	OR (95% CI) Adjusted[d]
B6		Unknown		1	2	nc	nc
		Any		86	1746	0.67 (0.40-1.10)	0.87 (0.51-1.47)
	Quartiles	None	≥2.19 mg (Q4)	12	271	0.60 (0.29-1.25)	0.71 (0.33-1.52)
			<1.64 mg (Q1)	20	270	Ref	Ref
		Any		86	1746	0.61 (0.44-0.85)	0.78 (0.55-1.11)
	Spline	None	≥2.2 mg	11	261	0.43 (0.18-1.00)	0.50 (0.21-1.19)
			<2.2 mg	66	822	Ref	Ref
		Any		86	1746	0.85 (0.30-2.38)	1.00 (0.35-2.89)
	RDA[e]	None	≥1.3 mg	73	1014	1.24 (0.44-3.50)	1.20 (0.41-3.49)
			<1.3 mg	4	69	Ref	Ref
	Continuous	None	1-unit increase in SD	77	1083	0.67 (0.40-1.11)	0.74 (0.43-1.29)
B12		Unknown		0	1	nc	nc
		Any		87	1689	0.61 (0.38-0.98)	0.77 (0.47-1.26)
	Quartiles	None	≥4.93 µg (Q4)	16	285	0.67 (0.35-1.28)	0.68 (0.35-1.34)
			<3.00 µg (Q1)	24	285	Ref	Ref
		Any		87	1689	0.61 (0.38-0.97)	0.76 (0.46-1.25)
	Spline	None	≥3 µg	53	858	0.73 (0.44-1.20)	0.76 (0.45-1.28)
			<3 µg	24	283	Ref	Ref
		Any		87	1689	0.54 (0.29-1.02)	0.63 (0.33-1.23)
	RDA[e]	None	≥2.4 µg	65	1015	0.67 (0.35-1.28)	0.64 (0.33-1.25)
			<2.4 µg	12	126	Ref	Ref
	Continuous	None	1-unit increase in SD	77	1141	1.00 (0.93-1.06)	0.99 (0.93-1.05)
Choline		Unknown		0	0	nc	nc
		Any		12	217	0.95 (0.49-1.85)	1.11 (0.56-2.22)
	Quartiles	None	≥306.25 mg	42	653	1.11 (0.70-1.74)	1.20 (0.74-1.93)

Cofactor	Modeling Method	Periconceptional Supplementation[b]	Comparison for OR[c] (among non-supplementers)	Cases (n=164)	Controls (n=2831)	OR (95% CI) unadjusted	OR (95% CI) Adjusted[d]
			<230.84 mg	38	653	Ref	Ref

Cofactor	Modeling Method	Periconceptional Supplementation[a]	Comparison for OR[b] (among non-supplementers)	Cases (n=164)	Controls (n=2831)	OR (95% CI) unadjusted	OR (95% CI) adjusted[c]
Choline		Any		12	217	0.71 (0.34-1.47)	0.80 (0.38-1.72)
	Spline	None	≥200 mg	131	2345	0.72 (0.44-1.15)	0.72 (0.44-1.19)
			<200 mg	21	269	Ref	Ref
		Any		12	217	0.95 (0.52-1.74)	1.07 (0.57-2.00)
	RDI[e]	None	≥425 mg	5	85	1.01 (0.40-2.53)	0.95 (0.37-2.44)
			<425 mg	147	2529	Ref	Ref
	Continuous	None	1-unit increase in SD	152	2614	1.00 (1.00-1.00)	1.00 (1.00-1.00)
Betaine		Unknown		0	0	nc	nc
		Any		1	24	nc	nc
	Quartiles	None	≥96.72 mg (Q4)	51	702	1.24 (0.81-1.90)	1.06 (0.68-1.67)
			<56.50 mg (Q1)	41	701	Ref	Ref
		Any		1	24	nc	nc
	Spline	None	≥70 mg	104	1604	Ref	Ref
			40 to <70 mg	47	982	0.74 (0.52-1.05)	0.85 (0.59-1.24)
			< 40 mg	12	221	0.84 (0.45-1.55)	0.92 (0.48-1.74)
	Continuous	None	1-unit increase in SD	163	2807	1.00 (1.00-1.01)	1.00 (1.00-1.01)
Methionine		Unknown		0	0	nc	nc
		Any		1	7	nc	nc
	Quartiles	None	≥1.85 g (Q4)	42	706	0.96 (0.62-1.48)	1.15 (0.73-1.82)
			<1.41 g (Q1)	44	706	Ref	Ref
		Any		1	7	nc	nc
	Spline	None	≥1.3 g	132	2376	0.80 (0.54-1.20)	0.83 (0.54-1.26)
			<1.3 g	31	448	Ref	Ref
	Continuous	None	1-unit increase in SD	163	2824	0.77 (0.49, 1.20)	0.93 (0.58-1.49)

CI confidence interval; nc unable to calculate; NTD neural tube defect; OR odds ratio; Q1 quartile 1 (lowest); Q4 quartile 4 (highest); RDA recommended dietary allowance; RDI recommended dietary intake; Ref reference group; SD standard deviation

[a] Data are from the Slone Epidemiology Center Birth Defects Study (years 1998-2015), restricted to women with periconceptional folic acid ≥400 µg daily.

[b]Supplementation categorization is only for the nutrient of interest (e.g., for B12, supplementation is defined as any reported use of a vitamin supplement containing B12 during the periconceptional period).

[c]Dietary intake adjusted for total caloric intake

[d]OR calculated using conventional (unconditional) logistic regression and adjusted for restricted sufficient set of covariates (i.e., study center, maternal race (white, black, Hispanic, other) and maternal age (<25, 25 to <35, 35+ years)).

[e]RDA and RDI based on 1998 IOM guidelines for non-pregnant adult females 19-50 years.

Web Table 5. Distributions and Associations Between Concurrent Intakes of One-Carbon Cofactors and Neural Tube Defect and Spina Bifida Outcomes[a]

	Number of Concurrent One-Carbon Cofactor Intakes[b]				
	0—1	2	3	4	5
All NTDs (n=164)					
Cases/Controls	11/101	22/227	39/609	68/1284	24/598
OR (95% CI)	Ref	0.89 (0.42-1.90)	0.59 (0.29-1.19)	0.49 (0.25-0.95)	0.37 (0.18-0.78)
aOR (95% CI)[c]	Ref	0.85 (0.39-1.89)	0.64 (0.31-1.33)	0.56 (0.28-1.13)	0.49 (0.23-1.08)
Spina Bifida (n=124)					
Cases/Controls	7/101	17/227	33/609	50/1284	17/598
OR (95% CI)	Ref	1.08 (0.44-2.69)	0.78 (0.34-1.82)	0.56 (0.25-1.27)	0.41 (0.17-1.01)
aOR (95% CI)[c]	Ref	1.03 (0.40-2.64)	0.83 (0.35-1.97)	0.62 (0.27-1.45)	0.51 (0.20-1.32)

aOR adjusted odds ratio; CI confidence interval; FFQ food frequency questionnaire; NTDs neural tube defects; OR crude odds ratio; Ref reference group

[a]Data are from the Slone Birth Defects Study (1998-2015), restricted to women with periconceptional folic acid ≥400 µg daily.

[b]Intake categorization based on intake from diet (FFQ) and supplementation; cutoff based on unadjusted restricted cubic splines. Counts represent number of intakes within the respective primary comparison range for each one-carbon cofactor (B6 ≥2.2 mg, B12 ≥3 µg, choline ≥200 mg, methionine ≥1.3 g, and betaine 40 to <70 mg). n=12 in lowest intake group for betaine and reference intake for other one-carbon cofactors in a holdout group which is not reported above; this holdout group comprised controls only.

[c]aOR controlls for maternal age, race, and study center (correspond with estimates plotted in Figure 2 in the main text).

Web Table 6. Models Adjusted for Estimated Total Folate Intake[a]

	INDIVIDUAL INTAKE				
	B6	**B12**	**Choline**	**Methionine**	**Betaine**
aOR (95% CI)[b]	0.79 (0.56-1.13)	0.76 (0.54-1.08)	0.73 (0.45-1.21)	0.83 (0.55-1.27)	0.84 (0.58-1.22)
	COUNTS OF INTAKE[c,d]				
	0-1	**2**	**3**	**4**	**5**
aOR (95% CI)[b]	Ref	1.06 (0.42-2.73)	0.86 (0.36-2.05)	0.68 (0.29-1.59)	0.55 (0.21-1.42)

aOR adjusted odds ratio; CI confidence interval; NTDs neural tube defects; Ref reference group

[a]Adjusted models for individual and counts of concurrent index one-carbon cofactor intake and NTDs. Data are from the Slone Epidemiology Center Birth Defects Study (years 1998-2015), restricted to mothers reporting folic acid intake of ≥ 400 µg

[b]aORs controll for maternal age, race, and study center, and estimated total daily folate (supplements + diet, natural discounted by 30%) during the periconceptional period.

[c]Counts represent number of intakes within the respective higher range for B6, B12, choline, and methionine, and middle intake for betaine

[d]n=12 (controls only) who had intake in the lower group for betaine and the other one-carbon cofactors served as a holdout group which is not reported above.

Web Table 7. Models with Supplementers Excluded[a]

	INDIVIDUAL INTAKE				
	B6	**B12**	**Choline**	**Methionine**	**Betaine**
Cases/Controls	11/255	53/795	65/942	60/862	17/327
OR_c (95% CI)	0.54 (0.28-1.04)	0.79 (0.47-1.32)	0.78 (0.39-1.56)	0.90 (0.50-1.61)	0.63 (0.36-1.11)
aOR_1 (95% CI)[b]	0.64 (0.33-1.26)	0.82 (0.48-1.41)	0.83 (0.40-1.72)	0.92 (0.54-1.25)	0.64 (0.36-1.16)
aOR_2 (95% CI)[c]	0.66 (0.33-1.30)	0.89 (0.50-1.60)	0.76 (0.32-1.80)	1.25 (0.59-2.63)	0.66 (0.37-1.19)
	COUNTS OF INTAKE[d]				
	0-1	**2**	**3**	**4**	**5**
Cases/Controls	11/101	16/151	27/425	20/302	1/64
OR (95% CI)[b]	Ref	0.97 (0.43-2.18)	0.58 (0.28-1.22)	0.61 (0.28-1.31)	nc
aOR_1 (95% CI)[c]	Ref	0.82 (0.35-1.92)	0.61 (0.28-1.32)	0.69 (0.31-1.56)	nc

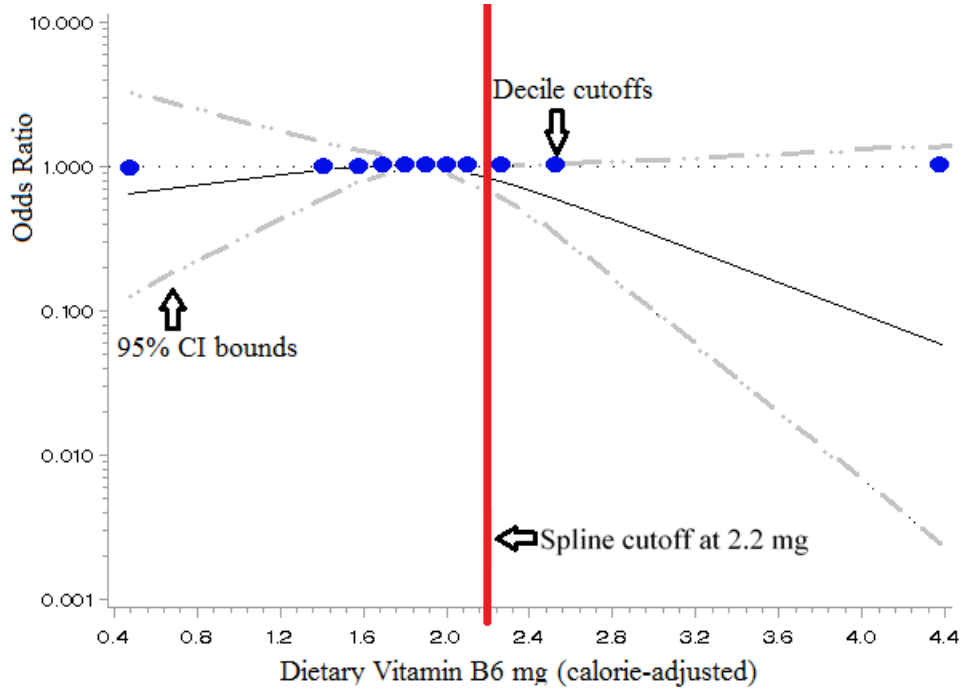
aOR adjusted odds ratio; CI confidence interval; NTDs neural tube defects; OR crude odds ratio; Ref reference group

[a]Crude and adjusted models for individual and counts of concurrent index one-carbon cofactor intake and NTDs. Data are from the Slone Epidemiology Center Birth Defects Study (years 1998-2015), restricted to mothers reporting folic acid intake of ≥ 400 µg and who did not report any periconceptional supplementation (n=75 cases, n=1055 controls).

[b]aOR_1 controls for maternal age, race, and study center

[c]aOR_2 additionally controls for intake of the other one-carbon cofactors listed in the table.

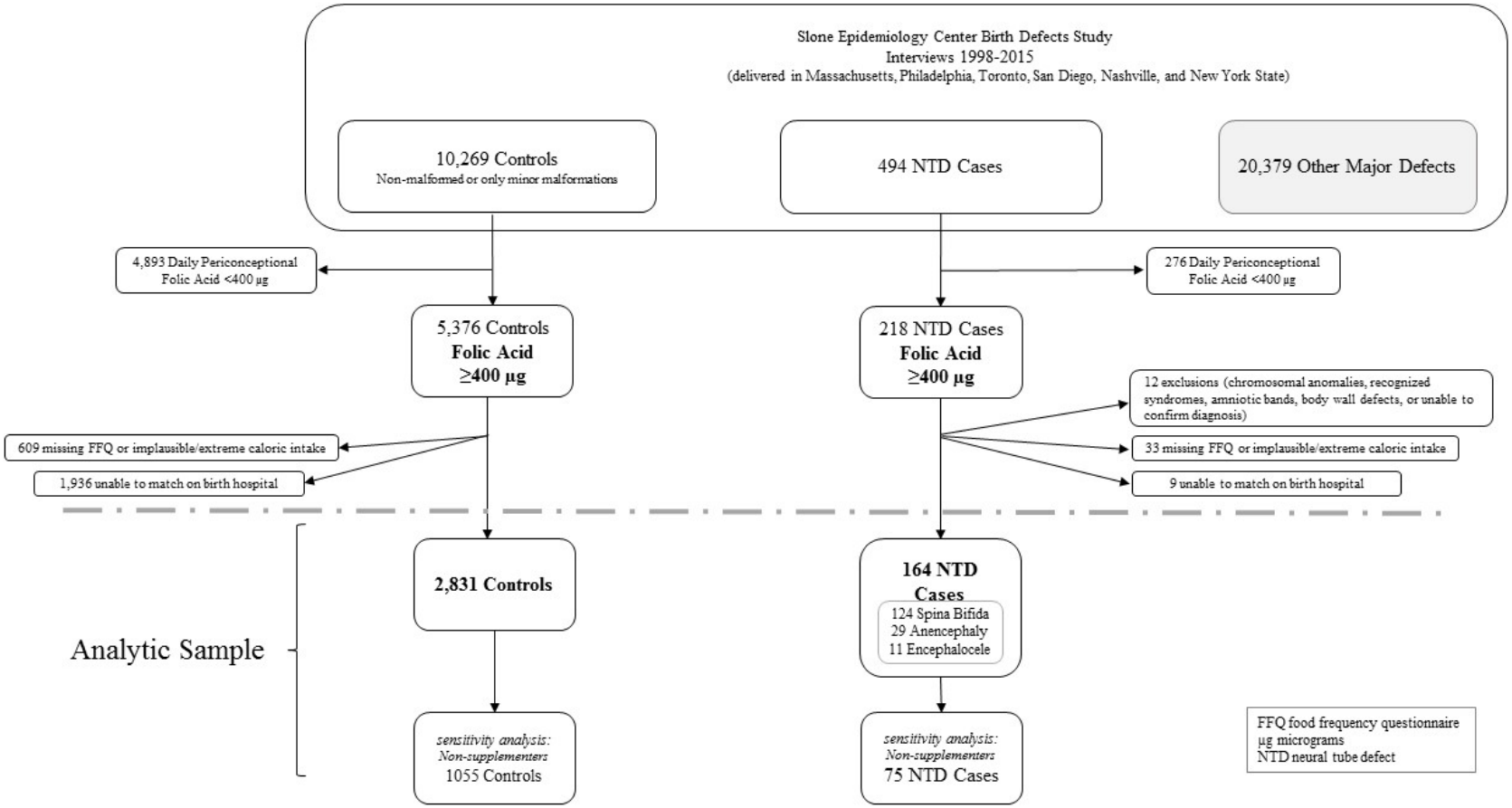
[d]Counts represent number of intakes within the respective higher range for B6, B12, choline, and methionine, and middle intake for betaine. n=12 (controls only) who had intake in the lower group for betaine and the other one-carbon cofactors served as a holdout group which is not reported above.



Web Figure 1. Spline Representing Association Between Dietary Vitamin B6 Intake and Neural Tube Defect Outcomes

Data are from the Slone Epidemiology Center Birth Defects Study (1998-2015), **r**estricted to mothers reporting periconceptional folic acid ≥400 µg and no periconceptional B6 supplementation. Figure displays restricted, cubic spline regression model with 3 knots. Daily vitamin B6 intake from diet was estimated from food frequency questionnaire data using nutrient matrices and has been adjusted for estimated total caloric intake.

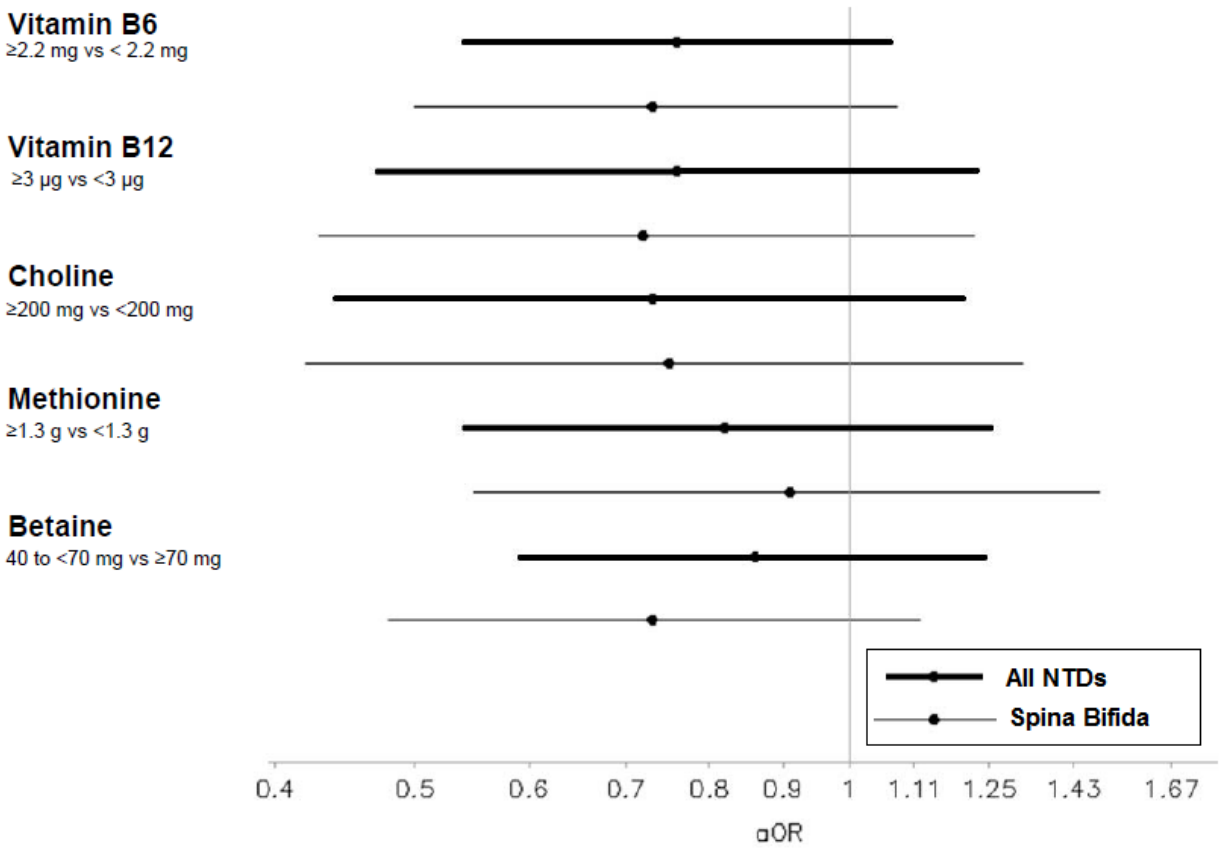
CI confidence interval



Web Figure 2. Flow of Inclusion in Study of One-Carbon Cofactors and Neural Tube Defect Risk

Data are from the Slone Epidemiology Center Birth Defects Study (1998-2015). Sensitivity analysis restricted to women who did not report periconceptional supplementation of vitamins B6, vitamin B12, choline, betaine, or methionine.

FFQ food frequency questionnaire; NTD neural tube defect



Web Figure 3. Adjusted Associations Between Individual Intakes of One-Carbon Cofactors and Neural Tube Defect and Spina Bifida Outcomes

Figure shows adjusted odds ratios control for maternal age, race, and study center; the dots represent the odds ratio point estimates and the whiskers represent the values within each 95% confidence interval. The numbers for these effect estimates are provided in Web Table 2.

aOR adjusted odds ratios; NTDs neural tube defects