Web Appendix: Maternal exposure to nitrogen dioxide, intake of methyl nutrients and congenital heart defects in offspring

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Web Appendix 1

Similar to previous research within this population (1), two-stage hierarchical regression(2) was used to estimate adjusted odds ratios and 95% confidence intervals representing the relationship between nitrogen dioxide (NO_2), methyl nutrient intake and congenital heart defects (CHDs). Computer code was adapted from previous work by Witte et al.(3) Separate models were constructed for each of the five methyl nutrients (methionine, choline, dietary folate equivalents, vitamin B_6 and vitamin B_{12}).

The first-stage model, an unconditional, multinomial logistic regression model, is represented by Equation 1. In this equation, *X* represents the seven indicator variables corresponding to the combinations between the four levels of NO_2 exposure and two levels of nutrient intake (i.e. above/below the 25th centile of intake in the population), *w* is the full adjustment set of confounders, and *d* is an individual CHD phenotype. $\boldsymbol{\beta}_d$ is the vector of regression coefficients for the NO_2-methyl intake exposure variables and CHD phenotype, and $\boldsymbol{\gamma}_d$ corresponds to the regression coefficients for confounders and the CHD phenotype. In this analysis, *m*, the total number of CHD phenotypes, was equal to 12.

$$\Pr(Y = d|x, w) = \frac{\exp(\alpha_d + \boldsymbol{x\beta}_d + \boldsymbol{w\gamma}_d)}{1 + \sum_{k=1}^{m} \exp(\alpha_k + \boldsymbol{x\beta}_k + \boldsymbol{w\gamma}_k)} \quad (1)$$

The second stage model is shown in Equation 2, and defines the relationships between the beta-coefficients obtained from the first-stage model ($\boldsymbol{\beta}$).

$$\beta_i = Z_i\pi + \delta_i \quad (2)$$

Z is the design-matrix that contains the variables that describe the associations between the first-stage betas, while π is the vector of regression coefficients for the design-matrix

variables and δ are independent, normal random variables with a mean of zero and a variance of τ^2. τ^2, the second-stage variance, represents the residual variation in the first-stage betas that is not described by the variables in the design-matrix. In this study, the design-matrix was an 84x21 matrix with columns corresponding to a variable for the intercept, three indicator variables for the level of NO_2 exposure, an indicator for low nutrient intake, eleven indicators for CHD phenotype and five indicators for the broader defect groupings. The 84 rows represent the beta coefficients from the first-stage model (i.e. betas for 7 indicator variables corresponding to the combinations of NO_2 exposure and methyl-nutrient intake for each of the 12 CHD phenotypes). As an illustrative example, the row in the design matrix that corresponds to the beta-coefficient for NO_2 exposure greater than the 90th centile, low nutrient intake and a perimembranous ventricular septal defect is shown in (3):

$$Z_i = \begin{bmatrix} 1 & 0\,0\,1\,1\,0\,1\,0\,0\,0\,0\,0\,0\,0\,0\,1\,0\,0\,0\,0 \\ \vdots & \vdots \end{bmatrix} \quad (3)$$

Once the second-stage coefficients are obtained, they are used to calculate the values towards which the first-stage estimates will be shrunk. The magnitude of that shrinkage depends upon the precision of the first-stage beta estimate and the value of τ^2.(2)

REFERENCES

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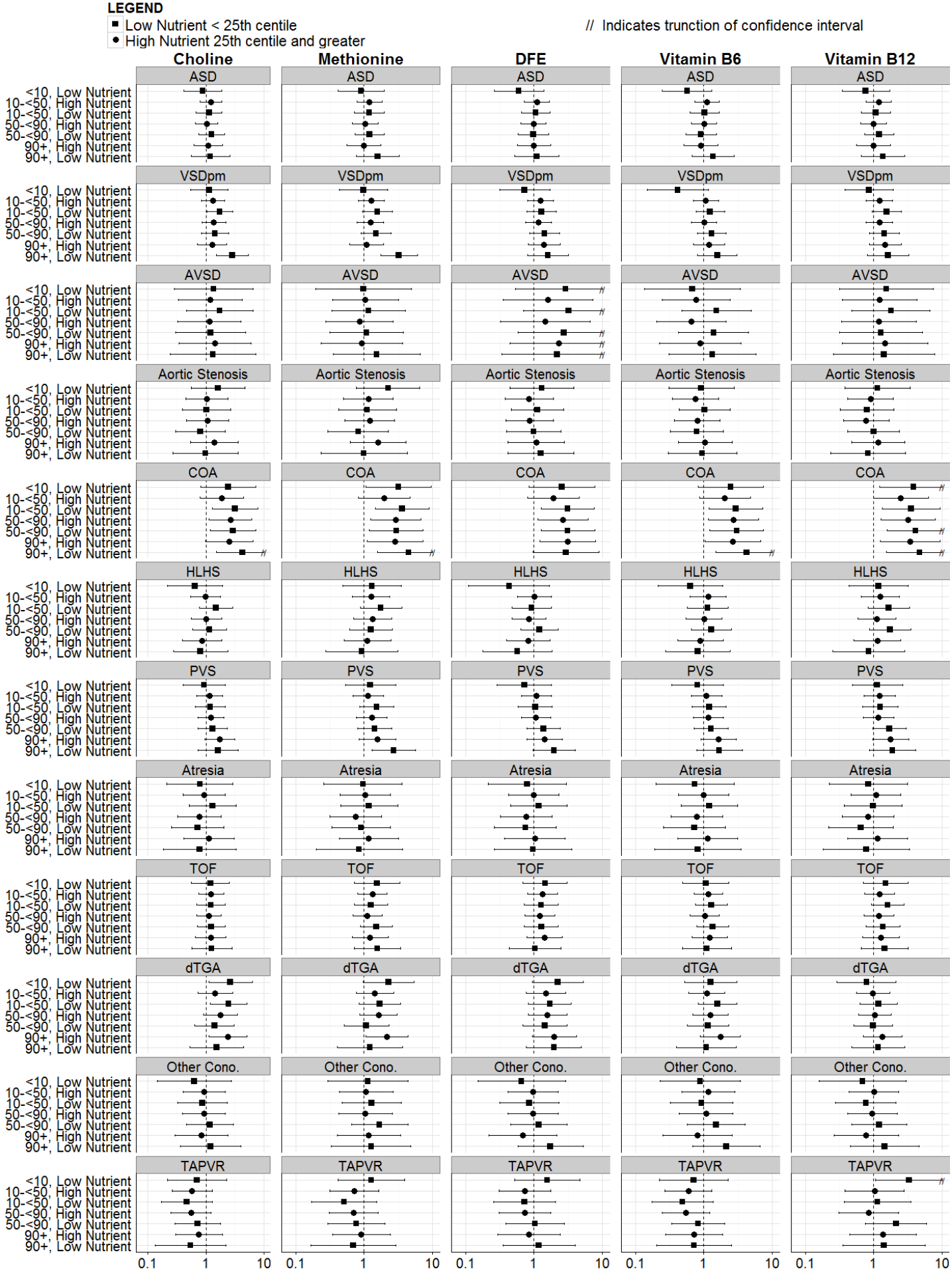

LEGEND
Low Nutrient < 25th centile
High Nutrient 25th centile and greater
// Indicates trunction of confidence interval
Choline
Methionine
DFE
Vitamin B6
Vitamin B12
ASD
VSDpm
AVSD
Aortic Stenosis
COA
HLHS
PVS
Atresia
TOF
dTGA
Other Cono.
TAPVR
<10, Low Nutrient
10-<50, High Nutrient
10-<50, Low Nutrient
50-<90, High Nutrient
50-<90, Low Nutrient
90+, High Nutrient
90+, Low Nutrient
0.1
1
10

Web Figure 1: Estimated adjusted odds ratios and 95% confidence intervals between congenital heart defects and categories of nitrogen dioxide (NO_2) exposure and dietary intake of methyl nutrients, National Birth Defects Prevention Study 1997-2006. Referent group for all comparisons is NO_2 exposure less than the 10^{th} centile and nutrient intake at or greater than the 25^{th} centile (high nutrient). Models adjusted for maternal race/ethnicity, age, education, household income, tobacco and alcohol use during pregnancy, use of folic-acid supplements one month prior to conception and site-specific ratio of septal cases to total congenital heart defect cases. Abbreviations: ASD-atrial septal defect; AVSD-atrioventricular septal defect; COA-coarctation of the aorta; DFE-dietary folate equivalents; dTGA-d-transposition of the great arteries; HLHS-hypoplastic left heart syndrome; Other Cono.-other conotruncal defects;PVS-pulmonary valve stenosis;TAPVR-total anomalous pulmonary venous return;TOF-tetralogy of Fallot;VSDpm-perimembranous ventricular septal defect.

Web Table 1: Adjusted[a] Odds Ratios (OR) and 95% Confidence Intervals (CI) between Congenital Heart Defects and Categories of NO_2 Exposure and Dietary Intake of Methyl Nutrients, National Birth Defects Prevention Study 1997-2006.

Defect	Nutrient	Ref[b]	<10th centile NO_2, Low Nutrient		10-<50th centile NO_2, High Nutrient		10-<50th centile NO_2, Low Nutrient		50-<90th centile NO_2, High Nutrient		50-<90th centile NO_2, Low Nutrient		≥90th centile NO_2, High Nutrient		≥90th centile NO_2, Low Nutrient	
			OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI
ASD																
	Choline	1	0.88	0.42,1.87	1.22	0.79,1.88	1.14	0.68,1.89	1.03	0.66,1.61	1.25	0.75,2.08	1.11	0.63,1.95	1.20	0.55,2.60
	Methionine	1	0.91	0.42,1.98	1.20	0.78,1.84	1.19	0.72,1.96	1.04	0.67,1.61	1.21	0.73,1.99	1.00	0.56,1.76	1.59	0.77,3.28
	DFE	1	0.60	0.26,1.39	1.10	0.72,1.67	1.06	0.65,1.73	0.99	0.65,1.51	0.99	0.59,1.64	1.00	0.57,1.75	1.10	0.52,2.30
	Vitamin B_6	1	0.57	0.25,1.32	1.11	0.73,1.69	1.03	0.63,1.68	1.01	0.66,1.55	0.91	0.55,1.52	0.90	0.51,1.60	1.37	0.68,2.77
	VitaminB_{12}	1	0.77	0.34,1.71	1.18	0.77,1.81	1.08	0.65,1.77	0.99	0.64,1.53	1.21	0.74,1.99	0.99	0.56,1.75	1.37	0.66,2.82
VSDpm																
	Choline	1	1.14	0.54,2.41	1.33	0.83,2.12	1.73	1.03,2.89	1.37	0.86,2.19	1.43	0.84,2.44	1.28	0.72,2.29	2.86	1.50,5.45
	Methionine	1	0.98	0.44,2.22	1.28	0.82,1.99	1.56	0.95,2.57	1.24	0.80,1.94	1.49	0.91,2.45	1.10	0.62,1.94	3.23	1.74,6.01
	DFE	1	0.73	0.32,1.70	1.25	0.80,1.93	1.30	0.79,2.13	1.16	0.75,1.80	1.43	0.87,2.36	1.41	0.82,2.41	1.60	0.81,3.17
	Vitamin B_6	1	0.42	0.15,1.16	1.07	0.70,1.63	1.24	0.77,2.00	1.01	0.66,1.54	1.30	0.81,2.10	1.19	0.70,2.01	1.58	0.82,3.05
	VitaminB_{12}	1	0.85	0.38,1.93	1.22	0.78,1.9	1.55	0.95,2.54	1.21	0.78,1.90	1.43	0.86,2.36	1.47	0.86,2.52	1.62	0.80,3.26
AVSD																
	Choline	1	1.36	0.28,6.57	1.19	0.34,4.18	1.74	0.47,6.48	1.15	0.33,4.04	1.20	0.30,4.81	1.43	0.34,6.01	1.32	0.24,7.22
	Methionine	1	0.98	0.19,4.91	1.05	0.35,3.17	1.16	0.34,3.97	0.86	0.28,2.65	1.08	0.32,3.72	0.92	0.23,3.64	1.54	0.36,6.65
	DFE	1	2.91	0.54,15.8	1.61	0.36,7.26	3.23	0.71,14.7	1.47	0.32,6.63	2.75	0.59,12.9	2.30	0.45,11.9	2.19	0.34,14.2
	Vitamin B_6	1	0.68	0.14,3.43	0.78	0.25,2.44	1.53	0.48,4.88	0.66	0.21,2.10	1.39	0.43,4.50	0.90	0.23,3.53	1.34	0.31,5.78
	VitaminB_{12}	1	1.53	0.32,7.38	1.23	0.35,4.32	1.80	0.48,6.69	1.19	0.34,4.18	1.28	0.32,5.12	1.46	0.35,6.13	1.41	0.26,7.71
Aortic Stenosis																
	Choline	1	1.62	0.56,4.68	1.05	0.45,2.43	1.02	0.39,2.67	1.07	0.46,2.47	0.81	0.30,2.18	1.39	0.54,3.62	0.98	0.27,3.60
	Methionine	1	2.26	0.78,6.54	1.16	0.50,2.68	1.12	0.42,2.96	1.21	0.53,2.78	0.82	0.30,2.28	1.61	0.63,4.13	0.99	0.23,4.24
	DFE	1	1.31	0.45,3.80	0.85	0.38,1.91	1.13	0.47,2.73	0.86	0.39,1.91	1.00	0.40,2.47	1.08	0.42,2.79	1.26	0.41,3.82
	Vitamin B_6	1	0.92	0.31,2.75	0.75	0.35,1.63	1.03	0.44,2.40	0.80	0.37,1.72	0.79	0.32,1.94	1.04	0.42,2.57	0.95	0.30,3.02
	VitaminB_{12}	1	1.14	0.38,3.40	0.90	0.42,1.93	0.80	0.32,1.98	0.77	0.36,1.67	1.00	0.42,2.38	1.16	0.48,2.81	0.83	0.24,2.90
COA																
	Choline	1	2.44	0.81,7.35	1.88	0.79,4.45	3.17	1.29,7.80	2.65	1.14,6.19	2.94	1.19,7.25	2.55	0.99,6.58	4.28	1.52,12.0
	Methionine	1	3.18	1.06,9.59	1.97	0.83,4.66	3.61	1.47,8.85	2.92	1.25,6.80	2.96	1.20,7.33	2.83	1.11,7.26	4.50	1.57,12.9
	DFE	1	2.57	0.85,7.72	1.93	0.81,4.57	3.13	1.28,7.66	2.67	1.15,6.24	3.12	1.27,7.71	3.11	1.23,7.89	2.94	0.98,8.82
	Vitamin B_6	1	2.49	0.83,7.50	2.03	0.86,4.80	2.93	1.18,7.24	2.71	1.16,6.33	3.04	1.23,7.51	2.66	1.03,6.84	4.24	1.51,11.9
	VitaminB_{12}	1	3.81	1.23,11.8	2.50	0.98,6.35	3.51	1.33,9.30	3.18	1.27,7.99	4.12	1.57,10.8	3.41	1.25,9.34	4.69	1.54,14.2

Defect	Nutrient	Ref	<10th centile NO_2, Low	10-<50th centile NO_2,	10-<50th centile NO_2,	50-<90th centile NO_2,	50-<90th centile NO_2,	≥90th centile NO_2, High	≥90th centile NO_2, Low

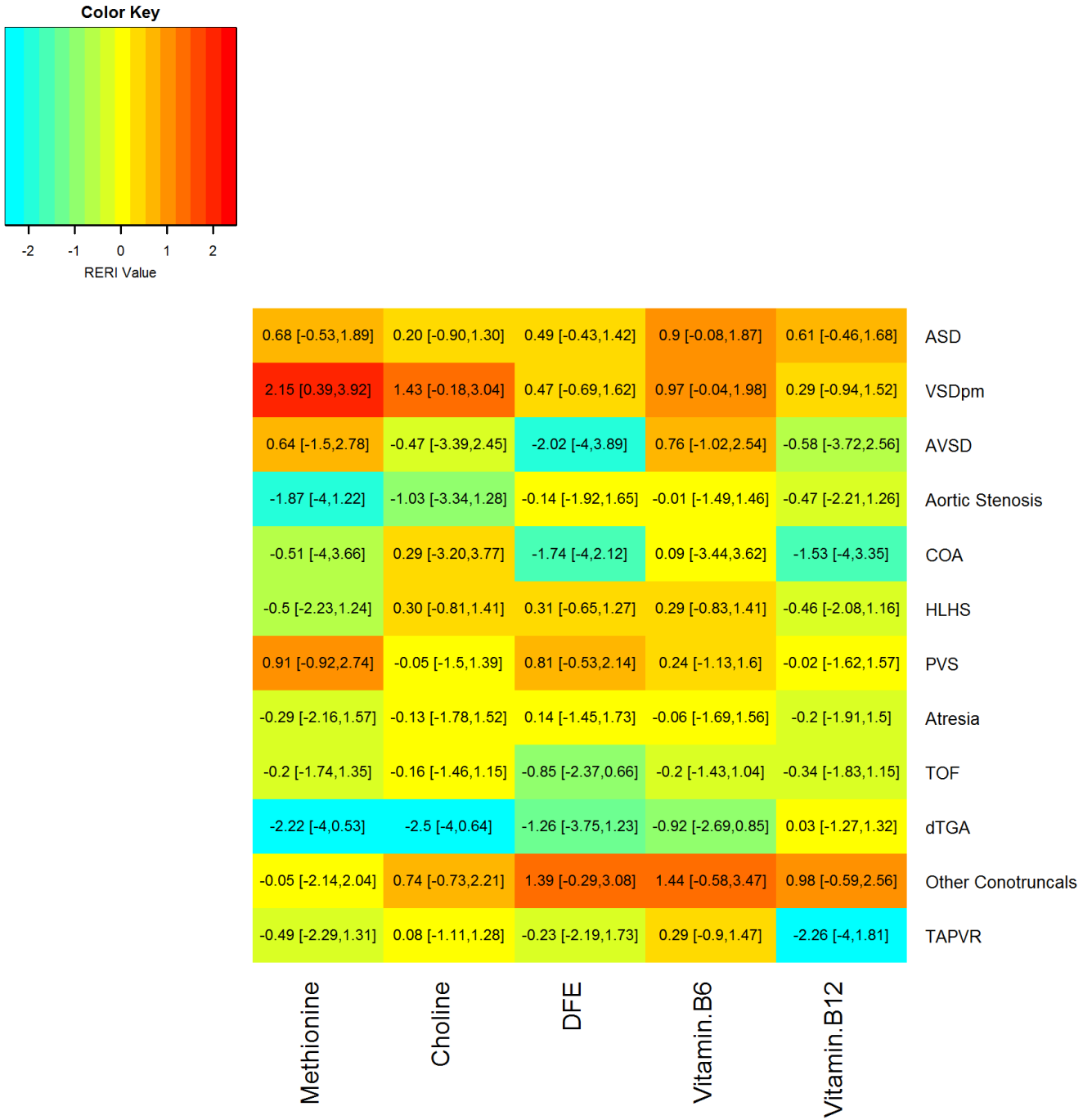
			Nutrient		High Nutrient		Low Nutrient		High Nutrient		Low Nutrient		Nutrient		Nutrient	
			OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI
HLHS																
	Choline	1	0.65	0.22,1.93	0.99	0.54,1.80	1.50	0.78,2.90	1.02	0.56,1.86	1.15	0.58,2.28	0.86	0.40,1.86	0.81	0.27,2.38
	Methionine	1	1.30	0.49,3.50	1.26	0.67,2.39	1.77	0.88,3.54	1.33	0.71,2.51	1.26	0.61,2.59	1.12	0.51,2.47	0.93	0.28,3.09
	DFE	1	0.43	0.11,1.69	1.02	0.58,1.80	0.92	0.48,1.79	0.84	0.48,1.50	1.20	0.64,2.27	0.83	0.40,1.74	0.57	0.18,1.85
	Vitamin B_6	1	0.63	0.21,1.89	1.15	0.64,2.10	1.14	0.57,2.25	1.01	0.55,1.84	1.29	0.66,2.52	0.89	0.41,1.93	0.82	0.28,2.41
	VitaminB_{12}	1	1.18	0.44,3.16	1.25	0.66,2.36	1.66	0.83,3.32	1.12	0.59,2.13	1.75	0.88,3.49	1.13	0.51,2.49	0.85	0.25,2.82
PVS																
	Choline	1	0.94	0.40,2.18	1.16	0.69,1.95	1.19	0.66,2.14	1.22	0.73,2.04	1.30	0.72,2.34	1.73	0.95,3.17	1.62	0.73,3.57
	Methionine	1	1.24	0.53,2.88	1.15	0.68,1.94	1.53	0.86,2.73	1.29	0.77,2.17	1.44	0.81,2.56	1.57	0.85,2.91	2.72	1.31,5.65
	DFE	1	0.73	0.29,1.81	1.09	0.66,1.80	1.05	0.59,1.86	1.08	0.65,1.77	1.38	0.79,2.42	1.43	0.79,2.61	1.97	0.96,4.02
	Vitamin B_6	1	0.81	0.34,1.93	1.10	0.66,1.83	1.20	0.68,2.13	1.17	0.71,1.95	1.28	0.72,2.28	1.64	0.91,2.98	1.69	0.78,3.63
	VitaminB_{12}	1	1.13	0.48,2.63	1.22	0.73,2.06	1.25	0.69,2.25	1.17	0.70,1.97	1.71	0.97,3.01	1.77	0.97,3.25	1.88	0.87,4.07
Atresia																
	Choline	1	0.79	0.21,2.94	0.93	0.40,2.18	1.31	0.52,3.33	0.77	0.33,1.84	0.72	0.25,2.06	1.12	0.41,3.05	0.78	0.18,3.35
	Methionine	1	0.97	0.26,3.59	1.05	0.45,2.43	1.18	0.46,3.08	0.75	0.31,1.80	0.91	0.34,2.45	1.18	0.43,3.19	0.85	0.20,3.63
	DFE	1	0.80	0.22,2.97	0.99	0.42,2.30	1.18	0.46,3.02	0.78	0.33,1.84	0.75	0.26,2.14	1.03	0.37,2.86	0.98	0.27,3.58
	Vitamin B_6	1	0.74	0.20,2.75	0.99	0.43,2.30	1.20	0.47,3.09	0.79	0.33,1.88	0.73	0.26,2.08	1.14	0.42,3.10	0.82	0.19,3.50
	VitaminB_{12}	1	0.84	0.23,3.12	1.08	0.47,2.50	0.98	0.37,2.59	0.83	0.35,1.95	0.65	0.22,1.92	1.14	0.42,3.10	0.78	0.18,3.33
TOF																
	Choline	1	1.20	0.56,2.56	1.23	0.76,2.01	1.22	0.70,2.14	1.12	0.68,1.83	1.23	0.70,2.16	1.21	0.66,2.22	1.26	0.57,2.81
	Methionine	1	1.55	0.72,3.31	1.33	0.82,2.16	1.27	0.72,2.23	1.12	0.68,1.83	1.52	0.89,2.62	1.23	0.67,2.25	1.58	0.72,3.44
	DFE	1	1.46	0.69,3.08	1.33	0.81,2.20	1.28	0.72,2.25	1.21	0.74,2.00	1.29	0.73,2.28	1.43	0.78,2.61	1.04	0.44,2.47
	Vitamin B_6	1	1.07	0.49,2.32	1.16	0.72,1.88	1.29	0.75,2.22	1.03	0.64,1.68	1.36	0.79,2.32	1.23	0.68,2.21	1.10	0.48,2.51
	VitaminB_{12}	1	1.51	0.71,3.18	1.22	0.74,2.01	1.61	0.93,2.79	1.19	0.72,1.97	1.37	0.78,2.41	1.28	0.69,2.36	1.44	0.66,3.16
dTGA																
	Choline	1	2.64	1.11,6.29	1.44	0.73,2.87	2.46	1.19,5.10	1.78	0.90,3.51	1.41	0.64,3.08	2.40	1.13,5.13	1.55	0.53,4.47
	Methionine	1	2.29	0.96,5.43	1.44	0.76,2.71	1.70	0.84,3.45	1.64	0.87,3.06	1.08	0.51,2.30	2.16	1.06,4.38	1.23	0.41,3.69
	DFE	1	2.24	0.95,5.29	1.50	0.78,2.90	1.71	0.83,3.52	1.57	0.82,3.02	1.45	0.69,3.05	1.99	0.94,4.18	1.96	0.78,4.94
	Vitamin B_6	1	1.26	0.52,3.06	1.10	0.61,2.01	1.58	0.82,3.04	1.24	0.68,2.24	1.15	0.58,2.30	1.75	0.89,3.45	1.09	0.4,2.98
	VitaminB_{12}	1	0.79	0.29,2.12	0.98	0.56,1.71	1.18	0.63,2.20	1.03	0.60,1.79	0.98	0.51,1.88	1.35	0.70,2.59	1.16	0.48,2.82
Other Cono.																
	Choline	1	0.63	0.15,2.74	0.92	0.40,2.14	0.87	0.32,2.36	0.92	0.40,2.14	1.17	0.46,2.98	0.84	0.30,2.41	1.21	0.36,4.06
	Methionine	1	1.15	0.30,4.40	1.07	0.44,2.63	1.29	0.47,3.51	1.05	0.43,2.58	1.69	0.65,4.36	1.17	0.40,3.40	1.27	0.33,4.83
	DFE	1	0.66	0.15,2.88	0.97	0.42,2.25	0.86	0.32,2.31	0.97	0.42,2.24	1.18	0.46,3.03	0.69	0.22,2.14	1.74	0.58,5.23
	Vitamin B_6	1	0.89	0.23,3.40	1.17	0.48,2.84	0.93	0.32,2.67	1.08	0.44,2.63	1.52	0.58,4.00	0.81	0.25,2.60	2.14	0.68,6.67
Defect	Nutrient	Ref	<10th centile NO_2, Low Nutrient		10-<50th centile NO_2, High Nutrient		10-<50th centile NO_2, Low Nutrient		50-<90th centile NO_2, High Nutrient		50-<90th centile NO_2, Low Nutrient		≥90th centile NO_2, High Nutrient		≥90th centile NO_2, Low Nutrient	
			OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI

	VitaminB_{12}	1	0.69	0.16,2.99	1.01	0.44,2.32	0.77	0.28,2.12	0.95	0.41,2.21	1.21	0.48,3.06	0.78	0.26,2.31	1.45	0.46,4.57
TAPVR																
	Choline	1	0.70	0.22,2.25	0.58	0.26,1.27	0.47	0.17,1.30	0.55	0.25,1.22	0.72	0.29,1.80	0.76	0.30,1.94	0.54	0.13,2.23
	Methionine	1	1.27	0.42,3.89	0.72	0.32,1.63	0.51	0.17,1.53	0.7	0.31,1.60	0.77	0.30,2.01	0.91	0.35,2.40	0.69	0.17,2.90
	DFE	1	1.56	0.52,4.69	0.74	0.31,1.77	0.73	0.26,2.06	0.74	0.31,1.75	1.04	0.39,2.76	0.85	0.30,2.44	1.19	0.35,3.96
	Vitamin B_6	1	0.71	0.22,2.28	0.60	0.27,1.32	0.49	0.18,1.36	0.55	0.25,1.21	0.83	0.34,2.02	0.72	0.28,1.88	0.72	0.21,2.52
	VitaminB_{12}	1	3.30	1.06,10.3	1.04	0.38,2.79	1.14	0.37,3.49	0.85	0.31,2.32	2.12	0.76,5.93	1.37	0.45,4.23	1.41	0.36,5.64

Abbreviations: ASD-atrial septal defect; AVSD-atrioventricular septal defect; COA-coarctation of the aorta; DFE-dietary folate equivalents; dTGA-d-transposition of the great arteries; HLHS-hypoplastic left heart syndrome; NO_2-nitrogen dioxide; Other Cono.-other conotruncals; PVS-pulmonary valve stenosis; TAPVR-total anomalous pulmonary venous return; TOF-tetralogy of Fallot; VSDpm-perimembranous ventricular septal defect.

[a] Models adjusted for maternal race/ethnicity, age, education, household income, tobacco and alcohol use during pregnancy, use of folic acid supplements one month prior to conception and site-specific ratio of septal cases to total congenital heart defect cases.

[b]Referent group for all comparisons is NO_2 exposure less than the 10^{th} centile and nutrient intake at or greater than the 25^{th} centile (high nutrient).



Web Figure 2: Patterns in Relative Excess Risk due to Interaction (RERI) between maternal nitrogen dioxide (NO_2) exposure and methyl nutrient intake, National Birth Defects Prevention Study, 1997-2006. RERIs calculated by comparing odds ratios of women with NO_2 exposure $\geq 90^{th}$ centile and nutrient $< 25^{th}$ centile, women with NO_2 exposure $\geq 90^{th}$ centile and nutrient intake $\geq 25^{th}$ centile and women with NO_2 exposure $< 10^{th}$ centile and nutrient intake $< 25^{th}$ centile (referent: women with NO_2 exposure $< 10^{th}$ centile and nutrient intake $\geq 25^{th}$ centile). The color gradient extends from red indicating more than additive interaction to blue which indicates less than additive interaction, with yellow indicating no departure from additivity. Abbreviations: ASD-atrial septal defect, AVSD-atrioventricular septal defect, COA-coarctation of the aorta, DFE-dietary folate equivalents, dTGA-d-transposition of the great arteries, HLHS-hypoplastic left heart syndrome, PVS-pulmonary valve stenosis, TAPVR-total anomalous pulmonary venous return, TOF-tetralogy of Fallot, VSD_{pm}-perimembranous ventricular septal defect

Web Table 2: Adjusted[a] Odds Ratios (OR) and 95% Confidence Intervals (CI) between Congenital Heart Defects and Categories of NO_2 Exposure and Dietary Intake of Methionine, Stratified by Use of a Folic-Acid Supplement, National Birth Defects Prevention Study 1997-2006.

Defect	Use of a Folic-Acid Supplement[b]	Ref[c]	<10th centile NO_2, Low Methionine		10-<50th centile NO_2, High Methionine		10-<50th centile NO_2, Low Methionine		50-<90th centile NO_2, High Methionine		50-<90th centile NO_2, Low Methionine		≥90th centile NO_2, High Methionine		≥90th centile NO_2, Low Methionine	
		OR	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI
ASD																
	Supplement Use	1	2.06	0.35,12.0	2.49	0.86,7.20	2.04	0.62,6.67	1.74	0.60,5.10	2.44	0.77,7.73	2.34	0.67,8.23	8.30	2.19,31.52
	No Use of Supplements	1	0.61	0.24,1.59	0.95	0.59,1.53	1.00	0.56,1.77	0.90	0.55,1.47	0.93	0.52,1.66	0.71	0.36,1.40	0.55	0.16,1.92
VSDpm																
	Supplement Use	1	1.05	0.28,3.98	0.91	0.47,1.77	1.56	0.75,3.23	0.78	0.40,1.53	1.33	0.63,2.81	0.77	0.30,1.98	2.55	0.90,7.20
	No Use of Supplements	1	0.82	0.25,2.61	1.64	0.89,3.02	1.60	0.80,3.20	1.73	0.94,3.19	1.66	0.83,3.30	1.36	0.64,2.90	4.23	1.85,9.67
Aortic Stenosis																
	Supplement Use	1	1.82	0.31,10.6	0.92	0.29,2.90	0.41	0.07,2.29	0.71	0.22,2.28	1.02	0.27,3.78	1.11	0.26,4.75	1.05	0.11,10.1
	No Use of Supplements	1	3.95	0.85,18.4	1.47	0.42,5.18	1.95	0.49,7.72	1.94	0.56,6.66	0.25	0.03,2.48	2.39	0.61,9.34	NE[d]	
COA																
	Supplement Use	1	3.56	0.67,18.8	1.30	0.37,4.59	3.47	0.95,12.58	1.92	0.57,6.51	1.66	0.43,6.48	3.06	0.79,11.92	2.38	0.37,15.3
	No Use of Supplements	1	2.91	0.57,14.9	2.46	0.74,8.18	3.78	1.07,13.42	3.75	1.14,12.3	4.23	1.21,14.82	2.44	0.63,9.41	7.1	1.70,29.7
HLHS																
	Supplement Use	1	1.32	0.13,13.4	1.40	0.40,4.92	2.35	0.61,8.95	2.08	0.62,7.03	1.56	0.39,6.25	1.92	0.45,8.07	NE[d]	
	No Use of Supplements	1	1.23	0.36,4.18	1.20	0.57,2.52	1.61	0.70,3.69	1.00	0.47,2.15	1.15	0.48,2.74	0.77	0.28,2.12	0.75	0.16,3.63
PVS																
	Supplement Use	1	1.79	0.42,7.67	1.35	0.54,3.36	1.98	0.73,5.37	1.20	0.48,2.97	1.47	0.54,4.04	1.57	0.53,4.68	2.57	0.66,10.0
	No Use of Supplements	1	0.86	0.27,2.75	1.02	0.54,1.94	1.36	0.66,2.79	1.29	0.68,2.43	1.38	0.67,2.82	1.47	0.69,3.16	2.89	1.15,7.26

Defect	Use of a Folic-Acid Supplement[b]	Ref[c]	<10th centile NO_2, Low Methionine		10-<50th centile NO_2, High Methionine		10-<50th centile NO_2, Low Methionine		50-<90th centile NO_2, High Methionine		50-<90th centile NO_2, Low Methionine		≥90th centile NO_2, High Methionine		≥90th centile NO_2, Low Methionine	
			OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI
Atresia																
	Supplement Use	1	1.25	0.12,12.7	1.31	0.37,4.72	0.85	0.17,4.36	0.39	0.09,1.67	0.46	0.08,2.88	0.82	0.13,5.18	NE[d]	
	No Use of Supplements	1	0.69	0.07,6.33	0.87	0.28,2.71	1.51	0.43,5.28	1.04	0.34,3.22	1.22	0.34,4.44	1.48	0.41,5.40	0.79	0.09,7.31
TOF																
	Supplement Use	1	1.07	0.28,4.12	1.06	0.54,2.10	0.96	0.42,2.17	0.75	0.37,1.50	1.23	0.57,2.66	1.05	0.42,2.65	0.73	0.15,3.54
	No Use of Supplements	1	2.30	0.82,6.48	1.72	0.83,3.53	1.66	0.73,3.78	1.63	0.79,3.39	1.96	0.89,4.35	1.56	0.66,3.69	2.58	0.91,7.32
dTGA																
	Supplement Use	1	2.82	0.59,13.5	1.66	0.56,4.92	2.10	0.64,6.96	1.51	0.51,4.42	1.2	0.34,4.26	2.92	0.88,9.68	NE[d]	
	No Use of Supplements	1	2.28	0.76,6.89	1.29	0.59,2.83	1.51	0.62,3.71	1.69	0.78,3.67	0.90	0.34,2.40	1.82	0.74,4.47	1.41	0.36,5.55
Other Cono.																
	Supplement Use	1	NE[d]		2.14	0.26,17.7	2.53	0.26,25.0	2.06	0.25,16.9	5.44	0.64,45.9	2.60	0.22,30.0	4.58	0.27,77.9
	No Use of Supplements	1	1.07	0.20,5.70	0.88	0.32,2.40	1.04	0.32,3.35	0.90	0.32,2.48	1.12	0.36,3.50	0.91	0.26,3.24	0.66	0.07,5.83
TAPVR																
	Supplement Use	1	2.06	0.18,24.1	0.92	0.19,4.54	0.40	0.04,4.47	1.03	0.22,4.88	1.37	0.24,7.79	0.50	0.04,5.85	NE[d]	
	No Use of Supplements	1	1.59	0.38,6.64	0.62	0.23,1.64	0.40	0.10,1.64	0.54	0.20,1.49	0.55	0.15,1.98	1.03	0.33,3.19	0.54	0.06,4.69

Abbreviations: ASD-atrial septal defect; AVSD-atrioventricular septal defect; COA-coarctation of the aorta; DFE-dietary folate equivalents; dTGA-d-transposition of the great arteries; HLHS-hypoplastic left heart syndrome; NO_2-nitrogen dioxide; Other Cono.-other conotruncal defects; PVS-pulmonary valve stenosis; TAPVR-total anomalous pulmonary venous return; TOF-tetralogy of Fallot; VSDpm-perimembranous ventricular septal defect.

[a]Estimates result from first-stage logistic regression model, adjusted for maternal race/ethnicity, age, education, household income, tobacco and alcohol use during pregnancy, use of folic acid supplements one month prior to conception and site-specific ratio of septal cases to total congenital heart defect cases.

[b] Time period of exposure was month prior to conception

[c] Referent group for all comparisons is NO_2 exposure less than the 10th centile and methionine intake at or greater than the 25th centile.

[d]Non-estimable due to limited sample size

Web Table 3: Adjusted[a] Odds Ratios (OR) and 95% Confidence Intervals (CI) between Congenital Heart Defects and Categories of NO_2 Exposure and Combined Dietary Intake of All Nutrients, National Birth Defects Prevention Study 1997-2006.

Defect	Ref[b]	<10th centile NO_2, Low in at least one Nutrient		10-<50th centile NO_2, High in all Nutrients		10-<50th centile NO_2, Low in at least one Nutrient		50-<90th centile NO_2, High in all Nutrients		50-<90th centile NO_2, Low in at least one Nutrient		≥90th centile NO_2, High in all Nutrients		≥90th centile NO_2, Low in at least one Nutrient	
	OR	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI
ASD	1	0.72	0.36,1.44	1.09	0.67,1.80	1.06	0.64,1.76	0.96	0.58,1.59	1.00	0.60,1.67	0.91	0.47,1.79	1.14	0.57,2.27
VSDpm	1	1.12	0.55,2.28	1.47	0.84,2.56	1.47	0.84,2.58	1.30	0.74,2.28	1.58	0.90,2.77	1.18	0.58,2.40	2.41	1.25,4.65
AVSD	1	1.35	0.22,8.24	1.06	0.22,5.07	1.71	0.38,7.73	1.08	0.23,5.11	1.25	0.27,5.85	1.52	0.25,9.43	1.20	0.16,8.76
Aortic Stenosis	1	2.15	0.65,7.15	1.30	0.43,3.89	1.26	0.42,3.80	1.38	0.47,4.06	1.07	0.35,3.24	1.80	0.53,6.16	1.32	0.34,5.07
COA	1	2.03	0.60,6.88	1.93	0.67,5.55	2.80	0.99,7.96	2.91	1.04,8.18	2.93	1.03,8.28	2.80	0.88,8.89	3.73	1.18,11.7
HLHS	1	0.56	0.20,1.56	0.99	0.50,1.97	1.06	0.53,2.12	0.85	0.42,1.70	1.09	0.55,2.17	0.70	0.28,1.80	0.75	0.28,1.99
PVS	1	0.75	0.33,1.67	0.90	0.49,1.64	1.21	0.67,2.18	1.02	0.56,1.84	1.24	0.69,2.23	1.38	0.68,2.83	1.81	0.89,3.69
Atresia	1	0.54	0.13,2.19	0.88	0.35,2.24	0.86	0.33,2.23	0.65	0.25,1.71	0.58	0.21,1.59	1.19	0.39,3.63	0.42	0.08,2.11
TOF	1	1.68	0.79,3.57	1.52	0.81,2.86	1.57	0.83,2.97	1.33	0.71,2.53	1.55	0.82,2.94	1.50	0.70,3.22	1.55	0.70,3.42
dTGA	1	1.73	0.70,4.29	1.33	0.61,2.89	1.73	0.80,3.75	1.59	0.74,3.42	1.40	0.64,3.05	2.25	0.95,5.33	1.56	0.60,4.07
Other Cono.	1	0.62	0.15,2.66	0.89	0.33,2.42	0.95	0.34,2.63	1.03	0.38,2.78	0.98	0.36,2.68	0.76	0.20,2.91	1.17	0.33,4.15
TAPVR	1	2.15	0.63,7.33	1.12	0.37,3.37	0.59	0.17,1.99	0.60	0.19,1.96	1.44	0.48,4.30	1.34	0.37,4.87	0.88	0.19,4.03

Abbreviations: ASD-atrial septal defect; COA-coarctation of the aorta; dTGA-d-transposition of the great arteries; HLHS-hypoplastic left heart syndrome; NO_2-nitrogen dioxide; Other Cono.-other conotruncal defects; PVS-pulmonary valve stenosis; TAPVR-total anomalous pulmonary venous return; TOF-tetralogy of Fallot; VSDpm-perimembranous ventricular septal defect.

[a]Estimates result from first-stage logistic regression model, adjusted for maternal race/ethnicity, age, education, household income, tobacco and alcohol use during pregnancy, use of folic acid supplements one month prior to conception and site-specific ratio of septal cases to total congenital heart defect cases.

[b]Referent group for all comparisons is NO_2 exposure less than the 10th centile and all nutrients (choline, methionine, dietary folate equivalents, vitamin B_6 and vitamin B_{12}) intake at or greater than the 25th centile.

Web Table 4: Adjusted[a] Odds Ratios (OR) and 95% Confidence Intervals (CI) between Congenital Heart Defects and Categories of NO_2 Exposure and Dietary Intake of Methionine by Value of Second-stage Variance (τ^2), National Birth Defects Prevention Study 1997-2006.

Defect	Value of second-stage variance	Ref[b]	<10th centile NO_2, Low Methionine		10-<50th centile NO_2, High Methionine		10-<50th centile NO_2, Low Methionine		50-<90th centile NO_2, High Methionine		50-<90th centile NO_2, Low Methionine		≥90th centile NO_2, High Methionine		≥90th centile NO_2, Low Methionine	
		OR	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI
ASD																
	0.25	1	0.96	0.46,2.01	1.20	0.78,1.84	1.19	0.72,1.96	1.04	0.67,1.61	1.21	0.73,1.98	1.01	0.58,1.77	1.54	0.77,3.10
	0.50	1	0.91	0.42,1.98	1.20	0.78,1.84	1.19	0.72,1.96	1.04	0.67,1.61	1.21	0.73,1.99	1.00	0.56,1.76	1.59	0.77,3.28
	0.83	1	0.90	0.41,1.97	1.20	0.78,1.84	1.19	0.72,1.97	1.04	0.67,1.61	1.21	0.73,1.99	0.99	0.56,1.76	1.60	0.77,3.36
VSDpm																
	0.25	1	1.07	0.49,2.31	1.28	0.82,1.99	1.56	0.95,2.56	1.24	0.80,1.94	1.49	0.91,2.45	1.12	0.64,1.97	3.05	1.66,5.60
	0.50	1	0.98	0.44,2.22	1.28	0.82,1.99	1.56	0.95,2.57	1.24	0.80,1.94	1.49	0.91,2.45	1.10	0.62,1.94	3.23	1.74,6.01
	0.83	1	0.95	0.42,2.18	1.28	0.82,1.99	1.56	0.95,2.57	1.25	0.80,1.94	1.49	0.90,2.46	1.09	0.61,1.94	3.30	1.77,6.15
Aortic Stenosis																
	0.25	1	2.04	0.73,5.69	1.16	0.50,2.67	1.15	0.45,2.98	1.21	0.53,2.76	0.90	0.33,2.39	1.58	0.63,3.98	1.19	0.34,4.14
	0.50	1	2.26	0.78,6.54	1.16	0.50,2.68	1.12	0.42,2.96	1.21	0.53,2.78	0.82	0.30,2.28	1.61	0.63,4.13	0.99	0.23,4.24
	0.83	1	2.35	0.80,6.91	1.16	0.50,2.68	1.11	0.42,2.96	1.21	0.53,2.79	0.80	0.29,2.23	1.62	0.63,4.18	0.90	0.19,4.27
COA																
	0.25	1	3.18	1.09,9.23	1.99	0.84,4.71	3.58	1.47,8.74	2.90	1.25,6.76	2.97	1.21,7.31	2.86	1.13,7.27	4.32	1.55,12.0
	0.50	1	3.18	1.06,9.59	1.97	0.83,4.66	3.61	1.47,8.85	2.92	1.25,6.80	2.96	1.20,7.33	2.83	1.11,7.26	4.50	1.57,12.9
	0.83	11	3.18	1.04,9.72	1.96	0.83,4.64	3.62	1.48,8.89	2.92	1.25,6.80	2.96	1.20,7.34	2.82	1.10,7.25	4.58	1.58,13.3
HLHS																
	0.25	1	1.32	0.52,3.37	1.26	0.67,2.38	1.74	0.87,3.47	1.33	0.70,2.50	1.27	0.62,2.59	1.15	0.53,2.50	1.11	0.38,3.20
	0.50	1	1.30	0.49,3.50	1.26	0.67,2.39	1.77	0.88,3.54	1.33	0.71,2.51	1.26	0.61,2.59	1.12	0.51,2.47	0.93	0.28,3.09
	0.83	1	1.30	0.47,3.55	1.27	0.67,2.39	1.78	0.89,3.57	1.33	0.71,2.52	1.26	0.61,2.60	1.11	0.50,2.47	0.86	0.24,3.03
PVS																
	0.25	1	1.29	0.58,2.89	1.16	0.69,1.94	1.54	0.87,2.72	1.29	0.77,2.16	1.44	0.81,2.56	1.57	0.86,2.89	2.57	1.26,5.22
	0.50	1	1.24	0.53,2.88	1.15	0.68,1.94	1.53	0.86,2.73	1.29	0.77,2.17	1.44	0.81,2.56	1.57	0.85,2.91	2.72	1.31,5.65
	0.83	1	1.21	0.51,2.87	1.15	0.68,1.94	1.53	0.86,2.74	1.29	0.77,2.17	1.44	0.81,2.57	1.57	0.85,2.92	2.77	1.33,5.80
Atresia																
	0.25	1	1.00	0.30,3.28	1.04	0.45,2.39	1.16	0.46,2.97	0.76	0.32,1.81	0.92	0.35,2.43	1.16	0.44,3.05	0.98	0.28,3.42
	0.50	1	0.97	0.26,3.59	1.05	0.45,2.43	1.18	0.46,3.08	0.75	0.31,1.80	0.91	0.34,2.45	1.18	0.43,3.19	0.85	0.20,3.63
	0.83	1	0.95	0.24,3.74	1.05	0.45,2.44	1.19	0.46,3.12	0.75	0.31,1.80	0.90	0.33,2.46	1.19	0.43,3.25	0.79	0.17,3.74
TOF																
	0.25	1	1.53	0.73,3.19	1.33	0.81,2.16	1.27	0.73,2.23	1.12	0.68,1.83	1.52	0.89,2.61	1.24	0.68,2.26	1.57	0.75,3.31
	0.50	1	1.55	0.72,3.31	1.33	0.82,2.16	1.27	0.72,2.23	1.12	0.68,1.83	1.52	0.89,2.62	1.23	0.67,2.25	1.58	0.72,3.44
	0.83	1	1.55	0.72,3.35	1.33	0.82,2.16	1.26	0.72,2.23	1.12	0.68,1.83	1.53	0.89,2.63	1.22	0.67,2.25	1.58	0.72,3.48

Defect	Value of second-stage variance	Ref[b]	<10th centile NO_2, Low Methionine		10-<50th centile NO_2, High Methionine		10-<50th centile NO_2, Low Methionine		50-<90th centile NO_2, High Methionine		50-<90th centile NO_2, Low Methionine		≥90th centile NO_2, High Methionine		≥90th centile NO_2, Low Methionine	
			OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI
dTGA																
	0.25	1	2.19	0.94,5.07	1.44	0.77,2.71	1.70	0.85,3.43	1.63	0.87,3.05	1.13	0.54,2.37	2.13	1.05,4.29	1.40	0.52,3.80
	0.50	1	2.29	0.96,5.43	1.44	0.76,2.71	1.70	0.84,3.45	1.64	0.87,3.06	1.08	0.51,2.30	2.16	1.06,4.38	1.23	0.41,3.69
	0.83	1	2.32	0.97,5.57	1.44	0.76,2.71	1.70	0.84,3.46	1.64	0.88,3.07	1.07	0.50,2.27	2.17	1.07,4.41	1.16	0.37,3.64
Other Cono																
	0.25	1	1.20	0.35,4.09	1.07	0.44,2.63	1.29	0.48,3.45	1.05	0.43,2.57	1.63	0.64,4.19	1.20	0.43,3.36	1.34	0.40,4.49
	0.50	1	1.15	0.30,4.40	1.07	0.44,2.63	1.29	0.47,3.51	1.05	0.43,2.58	1.69	0.65,4.36	1.17	0.40,3.40	1.27	0.33,4.83
	0.83	1	1.12	0.28,4.54	1.07	0.44,2.63	1.29	0.47,3.53	1.05	0.43,2.58	1.70	0.66,4.42	1.17	0.40,3.42	1.24	0.31,5.00
TAPVR																
	0.25	1	1.14	0.40,3.29	0.72	0.32,1.62	0.58	0.21,1.61	0.70	0.31,1.58	0.78	0.31,1.96	0.90	0.35,2.29	0.79	0.23,2.68
	0.50	1	1.27	0.42,3.89	0.72	0.32,1.63	0.51	0.17,1.53	0.70	0.31,1.60	0.77	0.30,2.01	0.91	0.35,2.40	0.69	0.17,2.90
	0.83	1	1.33	0.42,4.18	0.72	0.32,1.64	0.49	0.16,1.49	0.70	0.31,1.60	0.77	0.29,2.03	0.92	0.35,2.44	0.65	0.14,3.01

Abbreviations: ASD-atrial septal defect;; COA-coarctation of the aorta;; dTGA-d-transposition of the great arteries; HLHS-hypoplastic left heart syndrome; NO_2-nitrogen dioxide; Other Cono.-other conotruncal defects; PVS-pulmonary valve stenosis; TAPVR-total anomalous pulmonary venous return; TOF-tetralogy of Fallot; VSDpm-perimembranous ventricular septal defect.

[a] Models adjusted for maternal race/ethnicity, age, education, household income, tobacco and alcohol use during pregnancy, use of folic acid supplements one month prior to conception and site-specific ratio of septal cases to total congenital heart defect cases.

[b] Referent group for all comparisons is NO_2 exposure less than the 10th centile and methionine intake at or greater than the 25th centile.