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One-carbon metabolite levels in mid-pregnancy and risks of conotruncal heart defects

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Abstract

Background—Evidence exists for an association between use of vitamin supplements with folic acid in early pregnancy and reduced risk for offspring with conotruncal heart defects. A few observations have been made about nutrients related to one-carbon metabolism other than folate. Our prospective study attempted to extend information on nutrition and conotruncal heart defects by measuring analytes in mid-pregnancy sera.

Methods—This study included data from a repository of women's mid-pregnancy serum specimens based on screened pregnancies in California from 2002–07. Each woman's specimen was linked with delivery information to determine whether her fetus had a conotruncal heart defect or another structural malformation, or was nonmalformed. We identified 140 conotruncal cases and randomly selected 280 specimens as nonmalformed controls. Specimens were tested for a variety of analytes including: homocysteine, methylmalonic acid, folate, vitamin B_{12}, pyridoxal phosphate, pyridoxal, pyridoxic acid, riboflavin, total choline, betaine, methionine, cysteine, cystathionine, arginine, asymmetric and symmetric dimethylarginine.

Results and Conclusions—We did not observe statistical evidence for substantial differences between cases and controls for any of the measured analytes. Analyses specifically targeting B-vitamins also did not reveal differences between cases and controls.

Keywords

heart defects; nutrition; pregnancy; folic acid; B vitamins

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Introduction

Congenital heart defects comprise the most common group of human malformations, affecting about 1% of births (1,2). Despite their sizable contribution to child morbidity and infant mortality, etiologies for the majority of heart defect phenotypes remain unknown. Within the overall group of heart defects, those known as conotruncal defects are some of the more common phenotypes.

One promising clue to etiologies of conotruncal heart defects has been that women who use vitamins containing folic acid in early pregnancy are at approximately a 30% reduced risk to deliver offspring with these heart defects (3–7), but not in all studies (8). Folic acid has been the focus for the preponderance of inquiries about periconceptional nutrition and risks of heart defects, and birth defects more generally. There is some support from four small studies (9–14) that indicate compromised homocysteine remethylation and lack of methyl group nutrients may contribute to risks of certain heart defects.

Methylation of DNA is influenced by dietary contributions of methyl donors such as folate and vitamin B_{12}. A less than optimal methyl-donor supply and DNA methylation has certainly been a suggested area for research efforts for certain birth defects (15). A Netherlands study (9) observed that median fasting plasma homocysteine was higher and that mean B_{12} levels were lower, six months post delivery in mothers of children with heart defects. Similarly, an Arkansas study (10–12) observed post-partum plasma concentrations that were substantially higher in homocysteine, substantially lower in methionine, and lower in folate and $B_{12,}$ in mothers who had delivered babies with heart defects compared to mothers who delivered babies without birth defects. These observations included all heart defects combined, as well as specific phenotypes including conotruncal and septal defects. A third study (13) reported an increased risk for heart defects among Dutch women who had compromised B_{12} status and who had the methionine synthase reductase *MTRR* 66GG genotype. A fourth study (14) found an elevated risk of heart defects among Dutch mothers with lower B_{12} levels in the postpartum period and who had the transcobalamin II (rs1801198) genotype 776GG. A fifth study from a single clinic and a small case population found elevated homocysteine levels in amniotic fluid of women with fetuses that had various isolated congenital heart defects (16).

These observations led us to explore whether women's mid-pregnancy measurements, rather than post-partum measurements, of selected serum analytes, some indicative of the methyl-donor supply, were associated with the risk of conotruncal heart defects in offspring from a large population-base in California.

Methods

This study included data from a large and unique mid-pregnancy serum specimen bank of pregnancies in California. Specimens were collected from approximately 70% of women during the 15^{th}–18^{th} week of pregnancy. These sera were collected from women who resided in selected regions of California (Orange, San Diego, and Central Valley counties) as part of the California prenatal screening program that offers three types of screening tests

to pregnant women in order to identify individuals who are at increased risk for carrying a fetus with a specific birth defect (http://www.cdph.ca.gov/programs/pns/Pages/default.aspx). The collection and processing of specimens was as follows: 1) samples were taken at draw stations using BD™ Vacutainer 3.5 mL serum separator tubes with no anticoagulants or preservatives and centrifuged within 30 minutes; 2) samples were received by designated clinical laboratories from draw stations at room temperature, on average 3.0 days after draw; 3) AFP screening assays were run on samples usually on the day received; 4) samples were refrigerated up to 7 days if further testing was necessary; 5) samples were sent on cold packs via overnight mail to the serum storage bank; and 6) samples were aliquoted, labeled with barcodes, and frozen at −70°C within an average of 3.5 days of receipt at the serum storage bank.

Each woman's serum specimen was linked with delivery outcome information to determine whether her fetus had a conotruncal heart defect, any other structural malformation ascertained by the California Birth Defects Monitoring Program (17), or was born nonmalformed. The study included pregnancies screened in the period 2002–2007. Case information was abstracted from multiple hospital reports and medical records. Infants diagnosed with single gene disorders or chromosomal aneusomies (based on information gathered from chart reviews) were ineligible. Cases were limited to conotruncal heart defects, specifically d-transposition of the great arteries (dTGA) and tetralogy of Fallot (TOF). For each case, anatomic and physiologic features were confirmed by reviewing echocardiography, cardiac catheterization, surgery, or autopsy reports. 140 infants with conotruncal defects were identified.

We also randomly selected 280 women (based on a 2:1 frequency with case infants) who contributed mid-pregnancy specimens that were collected during the same time period and delivered nonmalformed infants (controls). Thus, this was a nested case-control study. All samples were obtained with approval from the California Health and Welfare Agency Committee for the Protection of Human Subjects.

Serum specimens for 420 cases and controls were sent on cold packs to BEVITAL AS, Bergen, Norway (www.bevital.no) for analyte measurements. Analytes measured were: total homocysteine, methylmalonic acid, folate, vitamin B_{12}, pyridoxal phosphate, pyridoxal, pyridoxic acid, pyridoxine, riboflavin, total choline, betaine, methionine, total cysteine, cystathionine, asymmetric dimethylarginine, symmetric dimethylarginine, serine, glycine, dimethylglycine, sarcosine, cystathionine, tryptophan, kynurenine, arginine, homoarginine, neopterin, 3-Hydroxykynurenine, kynurenic acid, xanthurenic acid, anthranilic acid, 3-Hydroxyanthranilic acid, quinolinic acid, nicotinic acid, nicotinamide, N1-methylnicotinamide, and creatinine.

To assess cigarette smoking exposures, the metabolite cotinine was measured. No smoke exposure was defined as values <5 nmol/L and any smoke exposure was defined as ≥5 nmol/L. Details about all laboratory assays except total choline can be found elsewhere (18,19). Total choline was measured after conversion of choline esters to free choline in the presence of phospholipase D. A serum sample of 45 μL was mixed with 18 μL solution containing phospholipase D (Sigma Chemical Company, 2.8 U/μL), CaCl2 (86 mM) and

Triton (0.44%). Then, 30 μL dithioerythritol (147mM) and 60 μL TCA containing 400 μM d7Choline were added to the incubation mixture. After centrifugation, the supernatant was analyzed by LC-MS/MS using a method optimized for the determination of free choline (20). Laboratory analyses were performed blind to case and control status.

We compared mean levels of analytes between cases and controls with t-tests. We also estimated risks using odds ratios and 95% confidence intervals (SAS 9.3). Models were constructed to assess effects associated with categories of the measured analytes. Specifically, we categorized measures as <25th percentile, 25th–74th percentile, and ≥75th percentile based on the distribution of each analyte among controls. The 25th–74th percentile was used as the reference group. We analyzed data for linear (logistic regression) and nonlinear (spline regression) effects and found no evidence for the latter. Available from intake forms associated with the screening program were the demographic factors maternal race/ethnicity (Hispanic; white, nonHispanic; Asian; Black; other) and maternal age (<25; 25–29; 30–34; and >34 years). These factors along with cigarette smoke exposure defined by cotinine levels were considered as covariates in some analyses.

Results

Table 1 shows similar racial/ethnic and age distributions between cases and controls, although case mothers were slightly more frequently nonHispanic white and older than were control mothers. Table 2 shows means and standard deviations of each measured analyte. We observed no statistically significant ($p \leq 0.05$) difference in mean levels between cases and controls for any analyte.

Given that comparisons of mean analyte values may not adequately reveal effects that could be associated with values toward the tails of the distribution, we explored lower and upper quartiles (cutpoints defined by the control distribution). As shown in Table 3, with the exception of low sarcosine (odds ratio=0.5, [0.3–0.8]), low symmetric dimethylarginine (odds ratio=1.8, [1.1–2.9]), and low folate (odds ratio=0.5, [0.3–0.9]), the many estimated effects (odds ratios) showed little evidence for associations. Analyses adjusted for maternal race/ethnicity and age are shown but crude analyses were not substantially different. Cotinine was not included in these analyses owing to the fact that most values for this analyte were zero. Analyses that excluded women with nonzero cotinine measures (cigarette smoking exposure, 15 case and 33 control women) did not produce substantially different results.

We simultaneously investigated five B-vitamin-related analytes, i.e., pyridoxal phosphate, riboflavin, betaine, vitamin B_{12}, and folate. Compared to women whose levels were not in the lowest quartile for any of these measures, women whose levels included at least one low quartile measure showed an odds ratio of 0.9 (0.6—1.6) adjusted for maternal age and race/ethnicity. Women with two or more low quartile measures showed similar effects (odds ratio=0.9 (0.5–1.4).

Discussion

In this prospective study, we examined potential associations between several gestational serum analytes, most of which are related to one-carbon metabolism B-vitamin status, and risk of conotruncal heart defects. Overall, these data did not show meaningful differences in mid-pregnancy analyte serum levels between women whose pregnancies involved conotruncal heart defects and those whose pregnancies did not involve structural malformations. Not observing an association with serum folate may be explained by the fact that women in this study were from a population whose food supply was fortified with folic acid and that most were also likely taking prenatal supplements containing folic acid as well as some of the other measured nutrients (e.g., vitamin B_{12}) at the time of serum sampling.

Previous studies by our group found that lower mid-pregnancy serum choline (a one-carbon donor) levels were associated with elevated risks of NTDs (21) and decreased risks for CLP (22). Here we did not recapitulate such an association between low or high choline levels and conotruncal heart defects. Experimental data in mice have indicated an association between dietary deficiencies in choline with elevated risks of ventricular septal defects (23).

Several previously reported observations led us to explore whether women's mid-pregnancy measurements of selected serum analytes were associated with the risk of conotruncal heart defects in offspring, particularly those associated with one-carbon metabolism. These observations included a Netherlands study (9) that found median fasting plasma homocysteine was higher and that mean B_{12} levels were lower, in mothers who delivered children with heart defects, an Arkansas study (10–12) that found post-partum plasma concentrations that were substantially higher in homocysteine, substantially lower in methionine, and lower in folate and $B_{12,}$ in mothers who had delivered babies with heart defects compared to mothers who delivered babies without birth defects, and a Dutch study (13) that found an increased risk for heart defects among women who had compromised B_{12} status. These existing studies differ with the current study in two important ways. First, our study focused on the specific phenotype of conotruncal heart defects rather than the larger more heterogeneous group of any congenital heart defects, although a follow-up report by Hobbs et al. (24) indicated that their observations were consistent across nearly all more specific heart defect phenotypes. Second, we assessed serum analytes during mid-gestation – temporally closer to embryogenesis - rather than post-partum. These differences may contribute to the contrary results observed.

A recent study by Sutton and colleagues (25) also investigated gestational serum measures of folate (red cell), homocysteine, and B_{12}. Their study included only 15 pregnancies involving conotruncal heart defects and did also not observe statistical differences in levels of the 3 studied analytes when compared to 568 control pregnancies.

Despite our study's strengths of focusing on mid-gestational serum measures and a pathogenetically specific, the current study is not without limitations. First, collection of women's serum specimens, albeit in the gestational period, was on average 8–10 weeks after fetal heart development. Bias due to such a single point-in-time measurement would tend to result in underestimation of measured effects. A related issue is that serum measures were

from a gestational time period when some women may have been using prenatal vitamin supplements. Thus, some of the measured analytes may have been directly influenced by such use. This use would have pertained primarily to folate, cobalamin pyridoxal 5-phosphate, pyridoxal, pyridoxic acid, pyridoxine, and riboflavin

A second limitation is potential degradation of analytes based on collection and storage procedures. It has been demonstrated that folate may degrade when frozen at higher temperatures (−20C) than were used for samples in this study (−80C) (25). Such degradation would likely be nondifferential to case and control status and therefore tend to lead to underestimates of measured effects. Moreover, the average length of time between collection and frozen storage was similar between cases and controls. However, we explored whether even small differences between cases and controls influenced observed effects with analytes. Analyses that incorporated length of time into models produced similar odds ratios.

Not observing a reduced risk for conotruncal heart defects associated with higher serum measures of methyl donors is counter to what we hypothesized. The reasons for a lack of association in the current study are unknown. One might speculate that the protection afforded by folic acid and other one-carbon donors might no longer be operating owing to higher dietary levels of folic acid from fortification of the US food supply during the study time period.

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Table 1

Characteristics of conotruncal heart defects and unaffected (nonmalformed controls) pregnancies, California 2002–07.

	Conotruncal Cases (n=140)		Controls (n=280)	
	No.	%*	No.	%*
Race/Ethnicity				
Hispanic	67	48.9	160	57.8
White nonHispanic	45	33.0	63	22.7
Asian	12	8.8	26	9.4
Black	8	5.8	13	4.7
Other	5	3.7	15	5.4
Age (years)				
<25	41	29.3	105	37.5
25–29	37	26.4	72	25.7
30–34	40	28.6	73	26.1
≥34	22	15.7	30	10.7

* Percentages may not equal 100 owing to missing data or rounding.

Table 2

Mean values of selected biochemical measures in mid-pregnancy serum specimens among deliveries with conotruncal heart defects and deliveries without malformations, California pregnancies 2002–07.

Serum Measurements	Case (n=140) Mean (SD)	Control (n=280) Mean (SD)	Difference of two means (95%CI)	*p*-value
Total homocysteine (μmol/L)	6.85 (3.72)	7.04 (4.19)	−0.18 (−1.00, 0.64)	0.66
Methylmalonic acid (μmol/L)	0.14 (0.05)	0.14 (0.05)	−0.00 (−0.01, 0.01)	0.45
Total cysteine (μmol/L)	220.13 (41.96)	223.64 (46.85)	−3.50 (−12.72, 5.71)	0.46
Methionine (μmol/L)	34.53 (7.52)	35.63 (9.46)	−1.10 (−2.91, 0.70)	0.23
Serine (μmol/L)	251.98 (39.26)	253.93 (49.69)	−1.95 (−11.40, 7.51)	0.69
Glycine (μmol/L)	424.33 (94.36)	428.72 (103.29)	−4.38 (−24.81, 16.05)	0.67
Sarcosine (μmol/L)	0.58 (0.19)	0.59 (0.27)	−0.02 (−0.07, 0.03)	0.52
Cystathionine (nmol/L)	0.1030 (0.0390)	0.1095 (0.0595)	−.0065 (−.0174, 0.0044)	0.24
Total choline (mmol/L)	3.19 (0.48)	3.12 (0.52)	0.06 (−0.04, 0.17)	0.23
Betaine (μmol/L)	16.97 (4.69)	16.89 (5.12)	0.08 (−0.93, 1.10)	0.87
Dimethylglycine (μmol/L)	1.85 (0.80)	1.86 (0.92)	−0.01 (−0.19, 0.17)	0.90
Creatinine (μmol/L)	51.94 (10.19)	50.21 (8.29)	1.73 (−0.09, 3.55)	0.06
Arginine (μmol/L)	145.64 (44.95)	149.10 (49.64)	−3.46 (−13.25, 6.34)	0.49
Asymmetric dimethylarginine (μmol/L)	0.47 (0.13)	0.47 (0.14)	0.00 (−0.02, 0.03)	0.77
Symmetric dimethylarginine (μmol/L)	0.51 (0.12)	0.51 (0.10)	−0.00 (−0.03, 0.02)	0.80
Homoarginine (μmol/L)	5.52 (3.06)	5.69 (2.98)	−0.17 (−0.78, 0.44)	0.59
Folate (vitamin B9) (nmol/L)	41.47 (20.44)	40.99 (24.37)	0.49 (−4.22, 5.20)	0.84
Cobalamin (vitamin B12) (pmol/L)	423.32 (341.55)	391.19 (314.44)	32.13 (−33.73, 97.99)	0.34
Pyridoxal 5-phosphate (nmol/L)	65.40 (52.36)	69.38 (72.38)	−3.97 (−17.48, 9.54)	0.57
Pyridoxal (nmol/L)	51.45 (79.23)	75.07 (208.62)	−23.62 (−59.53, 12.28)	0.20
Pyridoxic acid (nmol/L)	50.57 (87.25)	67.08 (167.10)	−16.51 (−46.12, 13.09)	0.28
Pyridoxine (nmol/L)	0.03 (0.19)	3.63 (30.76)	−3.61 (−8.72, 1.51)	0.17
Riboflavin (nmol/L)	41.91 (49.23)	45.30 (42.23)	−3.39 (−12.48, 5.70)	0.46
Neopterin (nmol/L)	7.96 (3.45)	7.77 (2.76)	0.19 (−0.43, 0.80)	0.55
Cotinine (nmol/L)	56.07 (247.71)	29.27 (167.42)	26.80 (−13.44, 67.04)	0.19
Tryptophan (μmol/L)	73.15 (9.80)	72.74 (10.96)	0.40 (−1.75, 2.56)	0.71
Kynurenine (nmol/L)	1.21 (0.23)	1.20 (0.24)	0.02 (−0.03, 0.06)	0.53
3-Hydroxykynurenine (nmol/L)	21.17 (12.47)	19.93 (10.65)	1.24 (−1.05, 3.54)	0.29
Kynurenic acid (nmol/L)	20.33 (7.30)	19.26 (8.60)	1.06 (−0.61, 2.73)	0.21
Xanthurenic acid (nmol/L)	17.18 (8.93)	15.53 (8.94)	1.65 (−0.17, 3.47)	0.08

Serum Measurements	Case (n=140) Mean (SD)	Control (n=280) Mean (SD)	Difference of two means (95%CI)	*p*-value
Anthranilic acid (nmol/L)	20.05 (18.21)	21.03 (22.21)	−0.98 (−5.24, 3.29)	0.65
3-Hydroxyanthranilic acid (nmol/L)	23.78 (21.16)	24.59 (23.12)	−0.81 (−5.39, 3.76)	0.73
Quinolinic acid (nmol/L)	326.60 (105.14)	326.76 (106.91)	−0.16 (−21.79, 21.47)	0.99
Nicotinic acid (nmol/L)	64.43 (16.22)	63.60 (15.08)	0.83 (−2.32, 3.98)	0.61
Nicotinamide (nmol/L)	463.53 (484.35)	498.95 (491.40)	−35.41 (−134.9, 64.09)	0.49
N1-methylnicotinamide (nmol/L)	198.03 (110.95)	201.09 (132.93)	−3.06 (−28.71, 22.59)	0.81

Table 3

Effect estimates (odds ratios) for pregnancies affected with conotruncal heart defects and selected biochemical measures in mid-pregnancy serum specimens.

serum_measurement	Quartile value	Case (n=137)	Control (n=277)	Adjusted OR* (95%CI)	*p*-value**
Total homocysteine (μmol/L)	<4.94	41	69	1.4 (0.9–2.3)	
	4.94–7.03	58	138	REFERENCE	
	≥7.04	38	70	1.2 (0.7–2.0)	
	Linear trend	137	277	0.92 (0.69–1.22)	0.55
Methylmalonic acid (μmol/L)	<0.11	43	69	1.5 (0.9–2.5)	
	0.11–0.15	61	137	REFERENCE	
	≥0.16	33	71	1.0 (0.6–1.7)	
	Linear trend	137	277	0.80 (0.59–1.07)	0.14
Total cysteine (μmol/L)	<197.27	43	69	1.5 (0.9–2.4)	
	197.27–232.27	61	139	REFERENCE	
	≥232.28	33	69	1.0 (0.6–1.7)	
	Linear trend	137	277	0.83 (0.62–1.11)	0.21
Methionine (μmol/L)	<29.27	33	70	0.8 (0.5–1.3)	
	29.27–39.71	75	137	REFERENCE	
	≥39.72	29	70	0.7 (0.4–1.3)	
	Linear trend	137	277	0.96 (0.71–1.30)	0.80
Serine (μmol/L)	<221.65	30	69	0.7 (0.4–1.2)	
	221.65–277.28	80	138	REFERENCE	
	≥277.29	27	70	0.6 (0.4–1.1)	
	Linear trend	137	277	0.94 (0.69–1.29)	0.72
Glycine (μmol/L)	<365.82	41	70	1.4 (0.8–2.3)	
	365.82–458.95	58	138	REFERENCE	
	≥458.96	38	69	1.2 (0.7–2.1)	
	Linear trend	137	277	0.93 (0.70–1.25)	0.64
Sarcosine (μmol/L)	<0.41	21	70	**0.5 (0.3–0.8)**	
	0.41–0.71	89	138	REFERENCE	
	≥0.72	27	69	0.6 (0.3–1.0)	

serum_measurement	Quartile value	Case (n=137)	Control (n=277)	Adjusted OR* (95%CI)	*p*-value**
	Linear trend	137	277	1.10 (0.80–1.52)	0.56
Cystathionine (nmol/L)	<0.08	31	62	0.9 (0.5–1.5)	
	0.08–0.11	80	147	REFERENCE	
	≥0.12	26	68	0.7 (0.4–1.2)	
	Linear trend	137	277	0.90 (0.66–1.24)	0.53
Total choline (mmol/L)	<2.80	31	68	0.9 (0.5–1.6)	
	2.80–3.48	71	139	REFERENCE	
	≥3.49	35	69	1.0 (0.6–1.6)	
	Linear trend	137	276	1.02 (0.75–1.38)	0.92
Betaine (μmol/L)	<13.35	33	70	0.9 (0.6–1.6)	
	13.35–19.34	69	137	REFERENCE	
	≥19.35	35	70	1.0 (0.6–1.6)	
	Linear trend	137	277	1.01 (0.75–1.35)	0.96
Dimethylglycine (μmol/L)	<1.30	30	69	0.8 (0.5–1.3)	
	1.30–2.16	74	139	REFERENCE	
	≥2.17	33	69	0.9 (0.5–1.5)	
	Linear trend	137	277	1.08 (0.79–1.46)	0.64
Arginine (μmol/L)	<127.00	38	66	1.1 (0.7–1.9)	
	127.00–178.99	69	140	REFERENCE	
	≥179.00	30	71	0.8 (0.5–1.4)	
	Linear trend	137	277	0.86 (0.64–1.16)	0.33
Asymmetric dimethylarginine (μmol/L)	<0.38	27	68	0.8 (0.5–1.3)	
	0.38–0.50	72	140	REFERENCE	
	≥0.51	38	69	1.0 (0.6–1.7)	
	Linear trend	137	277	1.15 (0.86–1.56)	0.35
Symmetric dimethylarginine (μmol/L)	<0.44	45	68	**1.8 (1.1–2.9)**	
	0.44–0.56	53	139	REFERENCE	
	≥0.57	39	70	1.4 (0.9–2.4)	
	Linear trend	137	277	0.90 (0.67–1.19)	0.46
Homoarginine (μmol/L)	<3.85	40	70	1.1 (0.6–1.8)	
	3.85–6.80	68	137	REFERENCE	

serum_measurement	Quartile value	Case (n=137)	Control (n=277)	Adjusted OR* (95%CI)	*p*-value**
	≥6.81	29	70	0.9 (0.5–1.5)	
	Linear trend	137	277	0.91 (0.67–1.25)	0.57
Folate (nmol/L)	<23.47	21	69	**0.5 (0.3–0.9)**	
	23.47–50.83	83	138	REFERENCE	
	≥50.84	33	70	0.7 (0.4–1.2)	
	Linear trend	137	277	1.17 (0.86–1.59)	0.31
Cobalamin (vitaminB12) (pmol/L)	<282.16	27	69	0.8 (0.4–1.3)	
	282.16–437.11	69	139	REFERENCE	
	≥437.12	41	69	1.2 (0.7–1.9)	
	Linear trend	137	277	1.22 (0.90–1.65)	0.19
Pyridoxal5-phosphate (nmol/L)	<31.16	26	69	0.7 (0.4–1.2)	
	31.16–80.00	78	139	REFERENCE	
	≥80.01	33	69	0.7 (0.4–1.3)	
	Linear trend	137	277	1.01 (0.74–1.39)	0.93
Pyridoxal (nmol/L)	<16.86	26	70	0.7 (0.4–1.3)	
	16.86–49.56	71	137	REFERENCE	
	≥49.57	40	70	1.0 (0.6–1.7)	
	Linear trend	137	277	1.18 (0.87–1.60)	0.28
Pyridoxicacid (nmol/L)	<14.37	22	68	0.6 (0.3–1.1)	
	14.37–47.42	77	139	REFERENCE	
	≥47.43	38	70	0.9 (0.5–1.5)	
	Linear trend	137	277	1.19 (0.86–1.63)	0.29
Riboflavin (nmol/L)	<22.70	41	71	1.2 (0.7–1.9)	
	22.70–49.32	67	136	REFERENCE	
	≥49.33	29	70	0.8 (0.5–1.4)	
	Linear trend	137	277	0.83 (0.62–1.12)	0.23
Neopterin (nmol/L)	<6.07	34	69	1.0 (0.6–1.7)	
	6.07–8.58	67	138	REFERENCE	
	≥8.59	36	70	1.0 (0.6–1.6)	
	Linear trend	137	277	0.98 (0.73–1.32)	0.90
Tryptophan (µmol/L)	<65.51	32	70	0.9 (0.6–1.6)	

serum_measurement	Quartile value	Case (n=137)	Control (n=277)	Adjusted OR* (95%CI)	*p*-value**
	65.51–79.55	69	138	REFERENCE	
	≥79.56	36	69	1.1 (0.6–1.8)	
	Linear trend	137	277	1.06 (0.79–1.43)	0.68
Kynurenine (nmol/L)	<1.04	28	68	0.7 (0.4–1.3)	
	1.04–1.30	78	140	REFERENCE	
	≥1.31	31	69	0.7 (0.4–1.2)	
	Linear trend	137	277	0.99 (0.72–1.34)	0.93
3-Hydroxykynurenine (nmol/L)	<11.83	35	68	1.2 (0.7–1.9)	
	11.83–25.99	62	139	REFERENCE	
	≥26.00	40	70	1.4 (0.8–2.3)	
	Linear trend	137	277	1.11 (0.83–1.48)	0.50
Kynurenicacid (nmol/L)	<13.48	25	69	0.7 (0.4–1.3)	
	13.48–22.83	70	139	REFERENCE	
	≥22.84	42	69	1.1 (0.7–1.9)	
	Linear trend	137	277	1.22 (0.90–1.66)	0.20
Xanthurenic acid (nmol/L)	<8.89	24	68	0.7 (0.4–1.2)	
	8.89–21.28	74	140	REFERENCE	
	≥21.29	39	69	1.0 (0.6–1.7)	
	Linear trend	137	277	1.22 (0.89–1.67)	0.21
Anthranilic acid (nmol/L)	<12.95	31	69	0.9 (0.5–1.5)	
	12.95–22.73	74	140	REFERENCE	
	≥22.74	32	68	0.9 (0.5–1.5)	
	Linear trend	137	277	1.00 (0.74–1.35)	0.99
3-Hydroxyanthranilic acid (nmol/L)	<5.65	35	69	1.0 (0.6–1.7)	
	5.65–36.63	70	140	REFERENCE	
	≥36.64	32	68	0.9 (0.5–1.6)	
	Linear trend	137	277	0.96 (0.71–1.30)	0.80
Quinolinic acid (nmol/L)	<253.67	30	68	0.8 (0.5–1.4)	
	253.67–378.61	76	139	REFERENCE	
	≥378.62	31	70	0.8 (0.5–1.3)	
	Linear trend	137	277	0.97 (0.71–1.31)	0.83

serum_measurement	Quartile value	Case (n=137)	Control (n=277)	Adjusted OR* (95%CI)	*p*-value**
Nicotinic acid (nmol/L)	<53.52	35	67	1.2 (0.7–2.0)	
	53.52–71.70	64	140	REFERENCE	
	≥71.71	38	70	1.2 (0.7–1.9)	
	Linear trend	137	277	1.01 (0.75–1.35)	0.95
Nicotinamide (nmol/L)	<218.53	38	69	1.1 (0.6–1.7)	
	218.53–527.89	71	139	REFERENCE	
	≥527.90	28	69	0.8 (0.5–1.4)	
	Linear trend	137	277	0.88 (0.65–1.18)	0.39
N1-methylnicotinamide(nmol/L)	<120.39	38	69	1.3 (0.8–2.1)	
	120.39–249.49	63	138	REFERENCE	
	≥249.50	36	70	1.2 (0.7–2.0)	
	Linear trend	137	277	0.95 (0.71–1.27)	0.72

*Adjusting for maternal race (Hispanic, white non-Hispanic, Asian, Black, and Other) and maternal age at sample collection (<25, 25–29, 30–34, and >34).

***p*-value for linear trend model where quartiles are evaluated for trend in response.