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# Neural Tube Defects and Maternal Intake of Micronutrients Related to One-Carbon Metabolism or Antioxidant Activity

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## Abstract

**Background—**Maternal nutritional status has been evaluated to clarify its role in development of neural tube defects (NTDs). Maternal folate intake during pregnancy has been closely evaluated for its association with NTDs.

**Objective—**The study objective was to examine associations between NTDs and other dietary periconceptional micronutrient intake, particularly nutrients involved in one-carbon metabolism or antioxidant activity.

**Design—**Using data from the National Birth Defects Prevention Study, 1997–2005, logistic regression models were used to estimate the relative risk of NTDs based on maternal micronutrient intake.

**Results—**Results were stratified according to folic acid supplement use, race/ethnicity, and maternal body mass index. Analyses included 954 cases (300 with anencephaly, 654 with spina bifida) and 6268 controls. Higher intakes of folate, thiamin, betaine, iron, and vitamin A were associated with decreased risk of anencephaly among some ethnic and clinical groups. In some groups, higher intakes of thiamin, riboflavin, vitamin $B_6$, vitamin C, vitamin E, niacin, and retinol were associated with decreased risk of spina bifida.

**Conclusion—**In addition to folic acid, other micronutrients, including thiamin, betaine, riboflavin, vitamin $B_6$, vitamin C, vitamin E, niacin, iron, retinol, and vitamin A, may decrease the risk of NTD occurrence.

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**Keywords**

Dietary periconceptional micronutrients; maternal nutrition; National Birth Defects Prevention Study; neural tube defects; one-carbon metabolism

## INTRODUCTION

The neural tube is closed by 28 days post-conception. Incomplete closure of the neural tube, most commonly results in anencephaly or spina bifida (Sadler, 2005). Maternal nutritional status has been evaluated to clarify its role in development of neural tube defects (NTDs). It is well-established that increased periconceptional folic acid intake reduces a woman's risk of a NTD-affected pregnancy (1991; Berry and others, 1999; Boulet and others, 2008; Canfield and others, 2005; Czeizel and Dudas, 1992; Williams and others, 2005). Studies have also investigated other nutrients that may be involved in the etiologies of NTDs, though the evidence for other nutrients is substantially less (Mills and others, 1992; Ray and others, 2007; Shaw and others, 2004; Shaw and others, 1997; Suarez and others, 2003; Velie and others, 1999; Wang and others, 2010).

Folate is vital to convert homocysteine to methionine and facilitate deoxyribonucleic acid methylation and synthesis (Stover, 2009). Riboflavin, vitamin $B_6$, vitamin $B_{12}$, choline, betaine and zinc are additional enzyme cofactors or methyl-donors in folate one-carbon metabolism (Benevenga, 2007; Mason, 2003; Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1998). Lack of these cofactors may result in increased levels of homocysteine, and maternal hyperhomocysteinemia has been associated with elevated NTD risk (Mills and others, 1995; Steegers-Theunissen and others, 1994; Zhao and others, 2006). Several studies demonstrate that increased maternal intake of methionine (Graham and others, 2010; Shaw and others, 1997), choline (Shaw and others, 2004), betaine (Benevenga, 2007; Shaw and others, 2004; Zhu and others, 2005), vitamin $B_{12}$ (Mills and others, 1992; Ray and others, 2007; Suarez and others, 2003; Wang and others, 2010) and zinc (Velie and others, 1999) may decrease the risk of NTDs.

Additionally, oxidative stress has been implicated in increasing NTD risk (Chang and others, 2003; Zhao and others, 2006). Maternal diabetes is a known state of oxidative stress and has long been associated with increased birth defect risks, particularly NTDs and congenital heart disease (Correa and others, 2008). Micronutrients with antioxidant capacity such as vitamins C and E may reduce NTD risk (Loeken, 2004). Lower vitamin C serum levels have been reported in women with histories of NTD-affected pregnancies (Smithells and others, 1976).

Other nutrients such as vitamin A, retinol, iron, and zinc occasionally are included in the oxidative pathway but have complex involvement in many biological systems. Similarly, niacin is often studied with other B vitamins but is not directly related to one-carbon metabolism.

The previously published report by Mosley et al. (Mosley and others, 2009) for the National Birth Defects Prevention Study (NBDPS) evaluated the association between maternal folate intake and NTDs. The current analysis is the largest case-control study examining potential associations between NTDs and dietary periconceptional intake of micronutrients involved in one-carbon metabolism and antioxidant activity.

# SUBJECTS AND METHODS

## Study population

The NBDPS, established 1997, is an ongoing, case-control study of birth defects being conducted in the US. Participants were recruited from one of 10 population-based birth defects surveillance systems. Study methods and descriptions of these programs have been published (Yoon and others, 2001). Institutional Review Boards for participating Centers approved the study along with the Centers for Disease Control and Prevention. All subjects gave written informed consent. Participation rates for NBDPS approximate 62% for anencephaly, 76% for spina bifida, and 71% for controls.

Data from case and control women with pregnancies from 1997 to 2005 are included in this analysis. Case women had a pregnancy complicated by anencephaly or spina bifida, not associated with a single gene disorder or chromosomal abnormality. NTD diagnoses were verified by clinical geneticists based on information obtained from medical records. Control women were randomly selected and had a pregnancy resulting in a live-born with no major birth defect. Cases and controls spoke English or Spanish. Women who were incarcerated or placed their infants into foster care or with adoptive parents were ineligible.

## Inclusions and exclusions

A total of 1,082 cases and 6,807 controls were evaluated for eligibility. Mothers with pregnancies resulting in multiple births were excluded (49 cases, 168 controls). Women with preexisting type 1 or 2 diabetes mellitus were excluded (15 cases, 42 controls). Women who reported periconceptional use of folate antagonist medication, including dilantin, valproic acid, sodium valproate, carbamazepine, methotrexate, trimethoprim, trimethoprim hydrochloride, trimethoprim sulfate, trimethoprim-sulfadiazine, and trimethoprim-sulfamethoxazole were excluded (4 cases, 9 controls). Furthermore, participants who had incomplete food frequency questionnaires (57 cases, 244 controls) or reported average daily energy intake <500 or >5,000 kilocalories, suggesting their intakes were implausible, (21 cases, 159 controls) were also excluded. The current data analysis included 954 (88%) cases and 6,268 (92%) controls.

## Micronutrient intake

Telephone interviews were conducted between 6 weeks to 24 months after the estimated date of delivery. Dietary intake for the year prior to pregnancy was recorded using a modified Willett Food Frequency Questionnaire (Willett and others, 1987), which consists of 58 food items. Additional questions were included in interviews regarding consumption of breakfast cereals. Micronutrient intake values were obtained from Release 20 of the US Department of Agriculture (USDA) National Nutrient Database for Standard Reference (US Department of Agriculture and Agricultural Research Service, 2008b). For choline, nutrient values were computed from the USDA Choline Database Release 2 (US Department of Agriculture and Agricultural Research Service, 2008a). We limited analysis to the following micronutrients: folate, methionine, cysteine, total choline, betaine, thiamin, riboflavin, vitamin $B_6$, vitamin $B_{12}$, vitamin C, vitamin E, alanine, niacin, iron, zinc, retinol, and vitamin A (RAE).

Periconceptional use of a multivitamin, prenatal vitamin or folic acid-only supplement was documented in monthly intervals from 3 months before pregnancy until final month of pregnancy. Supplement use was divided into 2 categories: women with any folic acid supplement intake from 3 months before pregnancy through the first month of pregnancy were “supplement users” and women with no folic acid supplement use during this time were “non-users of supplements.”

Dietary folate is a vitamin $B_9$ form naturally occurring in food sources. Dietary folic acid is the synthetic vitamin form used in food fortification. Because synthetic folic acid is more readily bioavailable than naturally occurring dietary folate, the term dietary folate equivalents (DFEs) refers to total dietary folate intake that accounts for the difference in bioavailability of the two forms by multiplying the amount of dietary folic acid in fortified foods by 1.7 and adding the amount of natural folate contained in foods (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1998).

Intakes of micronutrients from foods were categorized by quartile based on distribution of each micronutrient among controls. Women with micronutrient intakes less than 25th percentile were used as the referent group. Among micronutrients when odds ratio (OR) for 3rd quartile compared to 1st quartile were statistically significant whereas OR for 4th quartile compared to 1st quartile did not reach statistical significance, results were presented in the table but not discussed in the text. These results were most likely due to sample size limitations or random fluctuation.

### Statistical analysis

Adjusted odds ratios with 95% confidence intervals (CI) were calculated using multiple logistic regression to estimate risk associations between dietary micronutrient intake and NTDs. Models included adjustments based on center and energy intake (quartiles). Results were stratified by phenotype (anencephaly or spina bifida) and supplement use, race/ethnicity, and maternal body mass index (BMI). Groupings for self-reported race/ethnicity consisted of white, non-Hispanic; Hispanic; and black, non-Hispanic; mothers in other or unknown racial/ethnic categories were not analyzed. Pre-pregnancy BMI levels (normal: 18.5 to <25, overweight: 25 to <30, obese: ≥30) were categorized according to the National Institutes of Health (National Heart and National Institutes of Health, 1998); underweight mothers prior to pregnancy were not analyzed due to small sample size. Data were analyzed using Statistical Analysis System software, version 9.2 (SAS Institute, Inc., Cary, North Carolina).

Multiple statistical tests investigating the association between micronutrients and phenotype stratified by effect modifiers including ethnicity and BMI were computed. The OR and 95% confidence intervals are presented for each test. P-values were not adjusted for multiple comparisons.

## RESULTS

### Participants

Characteristics of case and control women are found in Table 1. Of the 954 case women, 300 had pregnancies affected by anencephaly and 654 by spina bifida. Pregnancy outcomes among anencephaly cases were 85 (28%) live births, 142 (47%) terminations, 72 (24%) fetal deaths, and 1 (<0.01%) unknown outcome. Pregnancy outcomes among spina bifida cases were 581 (89%) live births, 61 (9%) terminations, and 12 (2%) fetal deaths. Case women were more likely to be Hispanic (33.4% vs. 22.4%, respectively), less likely to report education levels above high school degree (50.5% vs. 58.2%), and less likely to be primigravida (25.1% vs. 29.1%) than control women. Spina bifida cases were more likely than controls to have maternal age ≤ 30 years at conception (75.2% vs. 70.7%) and more likely to report BMI in obese range (23.2% vs. 15.4%). Nearly half of participants were non-users of supplements (46.1%); no difference was observed among case and control women (47.6% vs. 45.9%). Use of folic acid supplementation did not vary between anencephaly or spina bifida cases.

NTD risks were assessed with and without adjustment for potential confounders (socioeconomic status, maternal smoking, alcohol consumption, age and race/ethnicity) with little or no effect on adjusted odds ratio. Thus, reported analytical models are those adjusted for center and energy intake. Using a Bonferroni correction to adjust for multiple comparisons, the p-value is $< 0.05 \times 10^{-3}$. None of the computed OR had a p-value $<0.05 \times 10^{-3}$. Being concerned that many of the findings could be due to random variation, we present findings of all OR in Tables 2 through 4, but only discuss the most significant findings in the text.

### Folic acid supplement use

Results for the association between dietary micronutrient intake and NTD stratified by folic acid supplement use are presented in Table 2. Among non-users of supplements, statistically significant results included thiamin intake ($4^{th}$ quartile) with an odds ratio of 0.47 (95% CI: 0.25, 0.91) for anencephaly. Among supplement users, iron ($4^{th}$ quartile) had an odds ratio of 0.51 (95% CI: 0.28, 0.96). OR for other micronutrients did not show a consistent protective effect.

### Maternal race/ethnicity

In Table 3, adjusted odds ratios are listed for each micronutrient and specific NTD with stratification by maternal race/ethnicity. Among Hispanic women, odds ratios for anencephaly for the $3^{rd}$ and $4^{th}$ quartiles of folate DFE were 0.28 (95% CI: 0.13, 0.58) and 0.46 (95% CI: 0.23, 0.95), respectively, while odds ratios for the same quartiles of betaine intake were 0.53 (95% CI: 0.30, 0.97) and 0.42 (95% CI: 0.21, 0.83), respectively. Similarly, Hispanic women with the highest thiamin intake had an odds ratio of 0.40 (95% CI: 0.18, 0.88) for anencephaly.

No statistically significant odds ratios for micronutrient intake and spina bifida risk were found for white, non-Hispanic women. Also for Hispanic women, we observed odds ratio of 0.53 (95% CI: 0.31, 0.92), 0.52 (95% CI: 0.31, 0.90), and 0.49 ((95% CI: 0.26, 0.91) for spina bifida for $2^{nd}$, $3^{rd}$, and $4^{th}$ quartile vitamin $B_6$ intake. Similarly, we observed in Hispanic women decreased spina bifida odds ratio of 0.40 (95% CI: 0.23, 0.70), 0.47 (95% CI: 0.28, 0.79), and 0.53 (95% CI: 0.31, 0.93) for $2^{nd}$, $3^{rd}$, and $4^{th}$ quartile vitamin E intake. Hispanic women had spina bifida odds ratio of 0.57 (95% CI: 0.34, 0.95) for vitamin C and 0.54 (95% CI: 0.30, 0.96) for niacin, each at the highest quartiles. Among Hispanics, retinol intake at the $2^{nd}$ and $4^{th}$ quartiles had odds ratio of 0.46 (95% CI: 0.29, 0.74) and 0.62 (95% CI: 0.40, 0.96), respectively. Black, non-Hispanic women had an odds ratio of 0.33 (95% CI: 0.11, 0.98) for 4th quartile riboflavin intake.

### Maternal body mass index

Due to the growing evidence that maternal obesity is associated with increased risk of having offspring affected by congenital anomalies (Stothard and others, 2009), we examined associations between dietary micronutrient intake and NTDs when stratified by pre-pregnancy BMI (Table 4). For anencephaly, women with normal weights had increased odds ratio of 2.24 (95% CI: 1.26, 3.99) and 2.39 (95% CI: 1.16, 4.91) for $3^{rd}$ and $4^{th}$ quartiles of alanine intake. Normal-weighted women with the highest intake of vitamin A had a reduced odds ratio of 0.57 (95% CI: 0.33, 0.99) for anencephaly. Overweight women had an odds ratio of 0.29 (95% CI: 0.10, 0.86) for $4^{th}$ quartile thiamin intake. Significant associations for anencephaly in obese women were not found.

No statistically significant odds ratio for spina bifida in women with normal BMIs were observed for micronutrients. In overweight women, odds ratios for spina bifida for $3^{rd}$ and $4^{th}$ quartiles of thiamin were 0.42 (95% CI: 0.24, 0.74) and 0.45 (95% CI: 0.23, 0.89).

Additionally, overweight women with riboflavin intake in the 2nd, 3rd, and 4th quartiles had odds ratios for spina bifida of 0.45 (95% CI: 0.27, 0.75), 0.51 (95% CI: 0.30, 0.87), and 0.39 (95% CI: 0.20, 0.76), respectively. Similarly, vitamin E intake in 2nd, 3rd, and 4th quartiles in overweight women had decreased odds ratios of 0.46 (95% CI: 0.28, 0.76), 0.56 (95% CI: 0.33, 0.95), and 0.51 (95% CI: 0.28, 0.95). For obese women, vitamin E at the 3rd and 4th quartiles had odds ratios of 0.51 (95% CI: 0.29, 0.89) and 0.52 (95% CI: 0.28, 0.95) for spina bifida.

## DISCUSSION

We observed several significant associations between NTDs and maternal dietary micronutrient intake. Our results suggest maternal diets higher in folate, betaine, thiamin, iron, and vitamin A may contribute to lowering anencephaly risk among some women. For spina bifida, diets higher in thiamin, riboflavin, vitamin $B_6$, vitamin C, vitamin E, niacin, and retinol appear to decrease risk in certain women. Thiamin had associations for both anencephaly and spina bifida. Interestingly, analyses revealed inverse associations for higher intakes of alanine related to anencephaly-affected pregnancies in normal-weight women.

Similarities in folic acid supplement use among cases and controls were reported in previous NBDPS data on folate and NTDs (Mosley and others, 2009). In a larger sample including two additional years of enrollment, we did not observe any difference in folic acid supplement use between cases and controls. Current analysis reveals an association between increased dietary folate intake and decreased risk of anencephaly among Hispanic women, (Shaw and others, 1995) and perhaps among supplement users and overweight women. The previous NBDPS report (Mosley and others, 2009) shows similar reduced occurrence of anencephaly with higher dietary folate. Specifically, for highest quartile of dietary folate intake, Hispanic women were 54% less likely to have offspring with anencephaly compared to Hispanic women with lowest dietary folate intake. Similarly, overweight women with high folate intake were 62% less likely to have offspring with anencephaly when compared to overweight women with lowest dietary folate intake. In the post-fortification era, these results suggest additional periconceptional folate intake may be necessary to further prevent anencephaly. Folate intake did not appear to significantly influence spina bifida risk whether stratified for folic acid supplementation, race/ethnicity, or BMI.

Recently, researchers are focusing on folate-related micronutrients involved in one-carbon metabolism including methionine, choline, betaine, thiamin, riboflavin, vitamin $B_6$, and vitamin $B_{12}$. Shaw et al. (Shaw and others, 1999) reported decreased NTD risk among women with increased thiamin intake who did not take periconceptional vitamin supplements. Our study supports an association between increased thiamin intake and decreased anencephaly risk among non-users of folic acid supplements, as well as Hispanic women. Additionally, overweight women with the highest intakes of thiamin were 71% less likely to have a pregnancy affected by anencephaly and 55% less likely to have a pregnancy affected by spina bifida. Higher levels of choline (Shaw and others, 2009) and vitamin $B_{12}$ (Suarez and others, 2003) in maternal serum have been suggested to be protective against NTDs. Diets high in choline, betaine, methionine, and vitamin $B_{12}$ have also been reported to decrease NTD risk (Shaw and others, 2004; Shaw and others, 1997; Suarez and others, 2003). This study did not observe an association between higher choline, methionine, or vitamin $B_{12}$ intake and NTD risk. We observed that increased betaine intake was associated with decreased risk of anencephaly among Hispanics. Black, non-Hispanic women and overweight women with the highest riboflavin intakes were approximately 67% and 61%, less likely to have a spina bifida affected pregnancy than women with lowest intakes of these micronutrients. Factors related to genetics or metabolic activity could explain differences among subpopulations or this could be due to chance.

Despite an overall decrease in NTD prevalence in the US, NTD prevalence among Hispanic women continues to remain higher than other racial/ethnic groups (2009; Canfield and others, 2005; Canfield and others, 2006; Williams and others, 2005). Participant demographics in the current study support this finding. Our results suggest increased intake of several micronutrients related to one-carbon metabolism, including folate, betaine, thiamin, riboflavin, and vitamin $B_6$ may be associated with decreased occurrence of NTDs among Hispanics. Additionally, higher intakes of antioxidants vitamin C and E appear to be associated with decreased likelihood of having a pregnancy affected by spina bifida. In fact, higher intakes of many micronutrients within this study were associated with decreased risk of anencephaly or spina bifida among Hispanic women. While the answer may simply be inadequate nutrition among Hispanic populations due to lower socioeconomic status, additional explanations include potential existence of an underlying genetic component affecting the folate-related metabolic pathway or the propensity towards a state of increased oxidative stress.

Other studies found associations between maternal obesity and occurrence of birth defects (Gilboa and others, 2010; Rasmussen and others, 2008; Waller and others, 2007; Watkins and others, 2003). The 2007 NBDPS report on pre-pregnancy obesity noted obese women were twice as likely to have offspring affected by spina bifida (Waller and others, 2007). Our data indicate obese women are more likely to have offspring affected with spina bifida. Higher vitamin E intake among overweight and obese women was associated with decreased risk of spina bifida by almost half compared to the lowest intake of vitamin E. A 2008 review suggests obesity and obesity-related metabolic alterations result in a state of oxidative stress similar to diabetes, a known condition leading to increased risk of birth defects (Reece, 2008). Thus, increased dietary intake of antioxidant-rich foods may decrease the risk of NTDs particularly among overweight and obese women.

When taking a broader approach than restricting discussion of odds ratio to those excluding 1, i.e. using an *a priori* threshold of 30% reduction or increase in risk, even more associations are possible between maternal dietary intake of micronutrients and NTD-risk. This view supports the need for additional research into these micronutrients contributing towards reduced NTD occurrence.

Primary limitations include potential for recall bias and small numbers of cases in certain subgroups. The validity of food questionnaires has been published (Willett and others, 1987), although we cannot discount limitations of this type of dietary assessment. Food items which are either over- or under-reported could result in false presence or absence of association of micronutrients with NTDs. Available data on subgroups based on race/ethnicity or BMI may be too limited to observe potential factors affecting NTD risk within these populations. The number of black, non-Hispanic case women was only 76, representing 8% of all NTD cases, while obese women comprised 21.1% of all NTD cases. Furthermore, small sample sizes limited our ability to stratify analyses by or adjust for multivitamin supplement use when examining the association between NTD risk and race/ethnicity or BMI. Lack of daily micronutrient intake from supplements prevented including these data in calculating the quartiles of intake. Increased numbers in these subgroups could help unearth important differences among subpopulations. We made 816 comparisons testing associations between NTDs and maternal micronutrient intake and some may insist that adjustment is made for multiple comparisons. However, adjustment of *P*-values for multiple comparisons remains controversial (Rothman and others, 2008). We did not correct for multiple comparisons; if we had, the significance level would have been affected. With 816 comparisons, many of the observed results were consistent with random variation. This trade-off is acceptable in this analysis to identify other micronutrients involved in one-carbon metabolism or antioxidant activity that might influence NTD risk for future research.

Additionally, for many statistically significant odds ratios where a lower intake of a micronutrient (2nd quartile and/or 3rd quartile) appeared protective but not higher levels (3rd and/or 4th quartiles), speculations regarding biological plausibility are challenging. Our findings consistent with other studies provide greater confidence in results. Many of the associations between specific micronutrients, phenotypes, and covariates have been evaluated for the first time and will require replication within independent populations.

Even with such limitations, the NBDPS has provided the most extensive study base to date to explore important questions between nutrients and risk of NTDs. Strengths of this study are large number of cases and amount of detailed dietary information and potential cofactors examined. This allowed for epidemiologic study of NTDs and dietary intake evaluating these relationships for potential confounders and by population subgroups. We observed that, in addition to folate, several micronutrients involved in either one-carbon metabolism or antioxidant activity may influence NTD risk and these effects might be different in certain subpopulations. Further investigation should be directed towards determining which factors are important for certain cohorts of women, particularly based upon their racial/ethnic background and pre-pregnancy body habitus.

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## Table 1

Maternal Characteristics of Neural Tube Defect Cases and Controls, National Birth Defects Prevention Study, 1997–2005

| | All NTD Cases | Anencephaly Cases | Spina Bifida Cases | Controls |
|---|---|---|---|---|
| | N (%) | N (%) | N (%) | N (%) |
| Total | 954 | 300 | 654 | 6268 |
| Maternal race/ethnicity | | | | |
| White, non-Hispanic | 494 (51.8%) | 143 (47.7%) | 351 (53.7%) | 3748 (59.8%) |
| Hispanic | 319 (33.4%) | 108 (36.0%) | 211 (32.3%) | 1404 (22.4%) |
| Black, non-Hispanic | 76 (8.0%) | 23 (7.7%) | 53 (8.1%) | 700 (11.2%) |
| Other races | 63 (6.6%) | 25 (8.3%) | 38 (5.8%) | 390 (6.2%) |
| Maternal age at conception | | | | |
| < 20 years | 137 (14.4%) | 48 (16.0%) | 89 (13.6%) | 866 (13.8%) |
| 20–25 years | 265 (27.8%) | 68 (22.7%) | 197 (30.1%) | 1794 (28.6%) |
| 26–30 years | 301 (31.6%) | 95 (31.7%) | 206 (31.5%) | 1774 (28.3%) |
| 31–35 years | 167 (17.5%) | 63 (21.0%) | 104 (15.9%) | 1322 (21.1%) |
| 36+ years | 84 (8.8%) | 26 (8.7%) | 58 (8.9%) | 512 (8.2%) |
| Maternal education | | | | |
| 0–11 years | 200 (21.0%) | 69 (23.0%) | 131 (20.0%) | 1046 (16.7%) |
| High school diploma/GED | 270 (28.3%) | 86 (28.7%) | 184 (28.1%) | 1542 (24.6%) |
| Some college/Technical | 274 (28.7%) | 73 (24.3%) | 201 (30.7%) | 1692 (27.0%) |
| Bachelor degree | 163 (17.1%) | 54 (18.0%) | 109 (16.7%) | 1411 (22.5%) |
| Graduate degree | 45 (4.7%) | 17 (5.7%) | 28 (4.3%) | 546 (8.7%) |
| Maternal body mass index, $kg/m^2$ | | | | |
| < 18.5 | 42 (4.4%) | 18 (6.0%) | 24 (3.7%) | 336 (5.4%) |
| 18.5 to < 25 | 442 (46.3%) | 151 (50.3%) | 291 (44.5%) | 3373 (53.8%) |
| 25 to < 30 | 214 (22.4%) | 64 (21.3%) | 150 (22.9%) | 1347 (21.5%) |
| 30+ | 201 (21.1%) | 49 (16.3%) | 152 (23.2%) | 966 (15.4%) |
| Previous Pregnancies | | | | |
| 0 | 239 (25.1%) | 68 (22.7%) | 171 (26.2%) | 1824 (29.1%) |
| 1 | 276 (28.9%) | 93 (31.0%) | 183 (28.0%) | 1847 (29.5%) |
| 2 | 190 (19.9%) | 53 (17.7%) | 137 (21.0%) | 1280 (20.4%) |
| 3+ | 249 (26.1%) | 86 (28.7%) | 163 (24.9%) | 1316 (21.0%) |
| Folic acid supplement use | | | | |
| No use B3-P1 | 454 (47.6%) | 142 (47.3%) | 312 (47.7%) | 2874 (45.9%) |
| Supplement use B3-P1 | 477 (50.0%) | 151 (50.3%) | 326 (49.9%) | 3288 (52.5%) |

Abbreviations: B3, 3 months before pregnancy; GED, general equivalency diploma; NTD, neural tube defect; P1, $1^{st}$ month of pregnancy.
Proportions of sums less than 100% can be attributed to missing data.

## Table 2

Association Between Anencephaly or Spina Bifida and Maternal Dietary Micronutrient Intake Stratified by Periconceptional Supplement Use, National Birth Defects Prevention Study, 1997–2005

| | Adjusted Odds Ratios[a] (95% confidence intervals) | | | |
|---|---|---|---|---|
| | Anencephaly (n=300[b]) | | Spina Bifida (n=654[b]) | |
| **Nutrient Quartiles[c]** | **Non-users supplements (n=142)** | **Supplement users (n=151)** | **Non-users supplements (n=312)** | **Supplement users (n=326)** |
| **One-carbon metabolism** | | | | |
| Folate DFE (μg DFE) | | | | |
| <338.16 | referent | referent | referent | referent |
| 338.16–496.15 | 0.78 (0.48, 1.26) | 0.89 (0.57, 1.39) | 0.88 (0.62, 1.25) | 0.87 (0.63, 1.21) |
| 496.16–720.06 | 0.65 (0.38, 1.10) | 0.55 (0.33, 0.93) | 0.92 (0.64, 1.32) | 0.79 (0.56, 1.13) |
| >720.06 | 0.65 (0.37, 1.16) | 0.72 (0.41, 1.26) | 0.87 (0.58, 1.30) | 0.91 (0.61, 1.35) |
| Methionine (g) | | | | |
| <1.10 | referent | referent | referent | referent |
| 1.10–1.43 | 1.02 (0.60, 1.74) | 0.99 (0.60, 1.64) | 0.69 (0.47, 1.00) | 1.01 (0.72, 1.42) |
| 1.44–1.87 | 1.11 (0.62, 1.98) | 1.12 (0.64, 1.94) | 0.91 (0.62, 1.35) | 0.78 (0.52, 1.15) |
| >1.87 | 1.44 (0.74, 2.84) | 1.09 (0.54, 2.20) | 1.23 (0.78, 1.95) | 1.02 (0.63, 1.65) |
| Cysteine (g) | | | | |
| <0.66 | referent | referent | referent | referent |
| 0.66–0.86 | 0.97 (0.59, 1.61) | 1.15 (0.70, 1.88) | 0.76 (0.52, 1.10) | 0.85 (0.60, 1.19) |
| 0.87–1.15 | 0.95 (0.55, 1.65) | 1.12 (0.66, 1.91) | 1.27 (0.87, 1.83) | 0.76 (0.52, 1.09) |
| >1.15 | 0.98 (0.52, 1.85) | 0.84 (0.44, 1.60) | 1.30 (0.84, 2.02) | 0.76 (0.49, 1.17) |
| Choline, Total (mg) | | | | |
| <190.41 | referent | referent | referent | referent |
| 190.41–254.08 | 0.96 (0.54, 1.69) | 0.95 (0.59, 1.54) | 0.67 (0.45, 0.99) | 0.95 (0.68, 1.33) |
| 254.09–343.19 | 1.31 (0.73, 2.34) | 0.88 (0.50, 1.53) | 0.87 (0.58, 1.30) | 0.93 (0.63, 1.37) |
| >343.19 | 1.54 (0.77, 3.07) | 0.86 (0.42, 1.77) | 1.38 (0.87, 2.20) | 0.83 (0.50, 1.38) |
| Betaine (mg) | | | | |
| <46.60 | referent | referent | referent | referent |
| 46.60–75.28 | 0.83 (0.53, 1.30) | 1.03 (0.64, 1.64) | 0.77 (0.56, 1.07) | 1.01 (0.73, 1.40) |
| 75.29–123.55 | 0.68 (0.41, 1.11) | 1.17 (0.73, 1.88) | 0.79 (0.56, 1.11) | 0.99 (0.71. 1.39) |
| >123.55 | 0.64 (0.38, 1.09) | 0.98 (0.58, 1.66) | 0.85 (0.60, 1.21) | 0.94 (0.65, 1.35) |
| Thiamin (mg) | | | | |
| <0.89 | referent | referent | referent | referent |
| 0.89–1.21 | 0.57 (0.33, 0.95) | 0.79 (0.49, 1.25) | 0.80 (0.56, 1.14) | 0.75 (0.54, 1.05) |
| 1.22–1.67 | 0.74 (0.44, 1.25) | 0.69 (0.41, 1.16) | 0.84 (0.58, 1.23) | 0.79 (0.54, 1.14) |
| >1.67 | 0.47 (0.25, 0.91) | 0.52 (0.27, 1.02) | 0.77 (0.49, 1.21) | 0.80 (0.51, 1.26) |
| Riboflavin (mg) | | | | |
| <1.39 | referent | referent | referent | referent |
| 1.39–1.91 | 0.69 (0.42, 1.13) | 0.90 (0.57, 1.44) | 0.73 (0.51, 1.04) | 0.70 (0.50, 0.97) |

| | Adjusted Odds Ratios[a] (95% confidence intervals) | | | |
|---|---|---|---|---|
| | Anencephaly (n=300[b]) | | Spina Bifida (n=654[b]) | |
| Nutrient Quartiles[c] | Non-users supplements (n=142) | Supplement users (n=151) | Non-users supplements (n=312) | Supplement users (n=326) |
| 1.92–2.62 | 0.69 (0.41, 1.18) | 0.76 (0.45, 1.29) | 0.96 (0.67, 1.38) | 0.63 (0.44, 0.91) |
| >2.62 | 0.59 (0.32, 1.11) | 0.79 (0.43, 1.47) | 0.86 (0.55, 1.33) | 0.68 (0.45, 1.05) |
| Vitamin B6 (mg) | | | | |
| <1.39 | referent | referent | referent | referent |
| 1.39–1.91 | 1.29 (0.75, 2.23) | 0.97 (0.60, 1.7) | 0.86 (0.60, 1.23) | 0.86 (0.61, 1.21) |
| 1.92–2.67 | 1.40 (0.79, 2.49) | 0.95 (0.55, 1.8) | 0.81 (0.55, 1.20) | 0.97 (0.66, 1.42) |
| >2.67 | 1.16 (0.58, 2.33) | 0.80 (0.41, 1.7) | 0.91 (0.58, 1.44) | 1.09 (0.69, 1.73) |
| Vitamin B12 (μg) | | | | |
| < 3.49 | referent | referent | referent | referent |
| 3.49–5.09 | 1.10 (0.67, 1.83) | 0.90 (0.57, 1.42) | 0.92 (0.64, 1.31) | 0.79 (0.56, 1.09) |
| 5.10–7.40 | 0.92 (0.53, 1.59) | 0.71 (0.42, 1.20) | 1.03 (0.71, 1.49) | 0.80 (0.56, 1.14) |
| >7.40 | 0.98 (0.54, 1.78) | 0.77 (0.42, 1.40) | 1.13 (0.75, 1.69) | 0.90 (0.60, 1.36) |
| **Antioxidants** | | | | |
| Vitamin C (mg) | | | | |
| <59.67 | referent | referent | referent | referent |
| 59.67–101.90 | 0.93 (0.54, 1.59) | 1.04 (0.68, 1.59) | 0.77 (0.54, 1.08) | 0.78 (0.56, 1.07) |
| 101.91–154.90 | 1.58 (0.96, 2.61) | 0.85 (0.53, 1.37) | 0.71 (0.50, 1.02) | 1.03 (0.75, 1.42) |
| >154.90 | 1.09 (0.62, 1.91) | 0.58 (0.32, 1.05) | 0.82 (0.57, 1.19) | 0.87 (0.59, 1.28) |
| Vitamin E (mg) | | | | |
| <2.86 | referent | referent | referent | referent |
| 2.86–4.13 | 1.24 (0.72, 2.12) | 0.69 (0.44, 1.07) | 0.87 (0.62, 1.24) | 0.69 (0.50, 0.97) |
| 4.14–6.09 | 1.52 (0.88, 2.63) | 0.48 (0.28, 0.83) | 0.67 (0.45, 0.99) | 0.86 (0.61, 1.21) |
| >6.09 | 1.36 (0.73, 2.56) | 0.73 (0.42, 1.26) | 1.01 (0.66, 1.52) | 0.67 (0.44, 1.00) |
| **Others** | | | | |
| Alanine (g) | | | | |
| <2.25 | referent | referent | referent | referent |
| 2.25–2.94 | 0.82 (0.46, 1.47) | 1.19 (0.72, 1.95) | 0.60 (0.40, 0.89) | 1.03 (0.74, 1.45) |
| 2.95–3.85 | 1.49 (0.84, 2.64) | 1.19 (0.67, 2.08) | 1.05 (0.71, 1.54) | 0.77 (0.52, 1.15) |
| >3.85 | 1.79 (0.90, 3.56) | 1.15 (0.56, 2.37) | 1.32 (0.83, 2.11) | 0.96 (0.59, 1.57) |
| Niacin (mg) | | | | |
| <13.45 | referent | referent | referent | referent |
| 13.45–17.77 | 1.04 (0.62, 1.73) | 0.96 (0.59, 1.56) | 0.91 (0.64, 1.29) | 0.93 (0.66, 1.31) |
| 17.78–23.65 | 1.06 (0.61, 1.83) | 1.05 (0.61, 1.79) | 0.91 (0.62, 1.33) | 1.00 (0.68, 1.45) |
| >23.65 | 0.84 (0.43, 1.63) | 0.70 (0.36, 1.39) | 0.88 (0.56, 1.38) | 0.88 (0.55, 1.39) |
| Iron (mg) | | | | |
| <8.62 | referent | referent | referent | referent |
| 8.62–12.50 | 0.80 (0.48, 1.36) | 0.77 (0.49, 1.22) | 0.90 (0.63, 1.29) | 0.90 (0.64, 1.25) |

| | Adjusted Odds Ratios[a] (95% confidence intervals) | | | |
|---|---|---|---|---|
| | Anencephaly (n=300[b]) | | Spina Bifida (n=654[b]) | |
| Nutrient Quartiles[c] | Non-users supplements (n=142) | Supplement users (n=151) | Non-users supplements (n=312) | Supplement users (n=326) |
| 12.51–17.70 | 0.81 (0.47, 1.39) | 0.59 (0.35, 1.00) | 0.82 (0.55, 1.20) | 0.74 (0.51, 1.08) |
| >17.70 | 0.68 (0.36, 1.29) | 0.51 (0.28, 0.96) | 0.96 (0.62, 1.47) | 1.08 (0.71, 1.63) |
| Zinc (mg) | | | | |
| <7.99 | referent | referent | referent | referent |
| 7.99–10.71 | 0.82 (0.47, 1.41) | 1.64 (0.98, 2.75) | 0.66 (0.45, 0.97) | 0.98 (0.70, 1.38) |
| 10.72–14.30 | 0.96 (0.54, 1.70) | 1.51 (0.83, 2.74) | 0.82 (0.55, 1.21) | 0.88 (0.59, 1.32) |
| >14.30 | 0.77 (0.38, 1.56) | 1.57 (0.76, 3.23) | 0.96 (0.59, 1.54) | 1.05 (0.65, 1.70) |
| Retinol (μg) | | | | |
| <218.60 | referent | referent | referent | referent |
| 218.60–348.59 | 0.71 (0.44, 1.15) | 0.96 (0.61, 1.51) | 0.82 (0.58, 1.16) | 0.91 (0.66, 1.25) |
| 348.60–535.10 | 0.62 (0.37, 1.04) | 0.86 (0.53, 1.38) | 0.96 (0.68, 1.35) | 0.74 (0.52, 1.04) |
| >535.10 | 0.74 (0.44, 1.23) | 0.69 (0.40, 1.21) | 0.94 (0.65, 1.36) | 0.88 (0.61, 1.29) |
| Vitamin A, RAE (μg RAE) | | | | |
| <390.88 | referent | referent | referent | referent |
| 390.88–592.60 | 0.75 (0.46, 1.25) | 0.70 (0.44, 1.12) | 0.93 (0.66, 1.30) | 0.95 (0.69, 1.30) |
| 592.61–858.76 | 1.07 (0.65, 1.75) | 0.76 (0.47, 1.22) | 0.94 (0.66, 1.35) | 0.69 (0.48, 0.98) |
| >858.76 | 0.85 (0.49, 1.48) | 0.71 (0.40, 1.23) | 1.00 (0.68, 1.46) | 0.81 (0.55, 1.20) |

Abbreviations: DFE, dietary folate equivalent; RAE, retinol activity equivalent.

[a] Adjusted model includes center and log-transformed energy intake.

[b] Cases only. For controls: Non-users of supplements (n=2874); Supplement users (n=3288).

[c] Quartiles based on daily nutrient intake of control mothers.

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## Table 3

Association Between Anencephaly or Spina Bifida and Maternal Dietary Micronutrient Intake Stratified by Race/Ethnicity, National Birth Defects Prevention Study, 1997–2005

| | Adjusted Odds Ratios[a] (95% confidence intervals) | | | | | |
|---|---|---|---|---|---|---|
| | Anencephaly (n=300[b]) | | | Spina Bifida (n=654[b]) | | |
| **Nutrient Quartiles[c]** | **White, non-Hispanic (n=143)** | **Hispanic (n=108)** | **Black, non-Hispanic (n=23)** | **White, non-Hispanic (n=351)** | **Hispanic (n=211)** | **Black, non-Hispanic (n=53)** |
| **One-carbon metabolism** | | | | | | |
| Folate DFE (μg DFE) | | | | | | |
| <338.16 | referent | referent | referent | referent | referent | referent |
| 338.16–496.15 | 1.02 (0.66, 1.58) | 0.71 (0.39, 1.27) | 0.22 (0.05, 1.07) | 0.90 (0.67, 1.22) | 0.71 (0.44, 1.16) | 1.71 (0.73, 3.99) |
| 496.16–720.06 | 0.84 (0.51, 1.39) | 0.28 (0.13, 0.58) | 0.69 (0.22, 2.11) | 0.90 (0.65, 1.25) | 0.78 (0.47, 1.28) | 1.35 (0.54, 3.39) |
| >720.06 | 0.88 (0.49, 1.57) | 0.46 (0.23, 0.95) | 0.32 (0.08, 1.33) | 1.02 (0.70, 1.48) | 0.65 (0.37, 1.14) | 0.97 (0.35, 2.73) |
| Methionine (g) | | | | | | |
| <1.10 | referent | referent | referent | referent | referent | referent |
| 1.10–1.43 | 1.29 (0.79, 2.11) | 1.07 (0.55, 2.09) | 0.48 (0.11, 2.04) | 0.99 (0.72, 1.35) | 0.84 (0.51, 1.37) | 0.35 (0.12, 1.04) |
| 1.44–1.87 | 1.48 (0.83, 2.63) | 1.02 (0.51, 2.04) | 0.82 (0.22, 3.14) | 0.92 (0.63, 1.34) | 0.78 (0.47, 1.30) | 0.94 (0.38, 2.31) |
| >1.87 | 1.87 (0.91, 3.88) | 1.01 (0.44, 2.34) | 1.01 (0.22, 4.76) | 1.52 (0.96, 2.41) | 0.80 (0.44, 1.48) | 1.09 (0.38, 3.11) |
| Cysteine (g) | | | | | | |
| <0.66 | referent | referent | referent | referent | referent | referent |
| 0.66–0.86 | 1.19 (0.74, 1.92) | 0.93 (0.49, 1.77) | 1.29 (0.39, 4.27) | 0.91 (0.66, 1.25) | 0.63 (0.38, 1.02) | 0.67 (0.27, 1.67) |
| 0.87–1.15 | 1.11 (0.65, 1.90) | 1.04 (0.53, 2.07) | 1.61 (0.45, 5.77) | 1.09 (0.77, 1.53) | 0.84 (0.51, 1.39) | 1.14 (0.47, 2.76) |
| >1.15 | 1.14 (0.61, 2.15) | 0.90 (0.39, 2.04) | 0.77 (0.14, 4.33) | 1.09 (0.73, 1.64) | 0.77 (0.43, 1.40) | 1.42 (0.52, 3.89) |
| Choline, Total (mg) | | | | | | |
| <190.41 | referent | referent | referent | referent | referent | referent |
| 190.41–254.08 | 1.08 (0.68, 1.73) | 1.05 (0.49, 2.26) | 0.63 (0.14, 2.81) | 0.96 (0.70, 1.31) | 0.69 (0.40, 1.21) | 0.41 (0.15, 1.15) |
| 254.09–343.19 | 1.02 (0.58, 1.79) | 0.93 (0.43, 2.02) | 1.82 (0.47, 7.03) | 1.09 (0.76, 1.58) | 0.59 (0.33, 1.03) | 1.14 (0.47, 2.77) |
| >343.19 | 1.00 (0.46, 2.16) | 0.92 (0.37, 2.27) | 1.33 (0.28, 6.46) | 1.30 (0.80, 2.12) | 0.76 (0.40, 1.43) | 0.75 (0.26, 2.16) |
| Betaine (mg) | | | | | | |
| <46.60 | referent | referent | referent | referent | referent | referent |

| Nutrient Quartiles[c] | Adjusted Odds Ratios[a] (95% confidence intervals) | | | | | |
|---|---|---|---|---|---|---|
| | Anencephaly (n=300[b]) | | | Spina Bifida (n=654[b]) | | |
| | White, non-Hispanic (n=143) | Hispanic (n=108) | Black, non-Hispanic (n=23) | White, non-Hispanic (n=351) | Hispanic (n=211) | Black, non-Hispanic (n=53) |
| 46.60–75.28 | 1.29 (0.82, 2.04) | 0.84 (0.51, 1.38) | 0.55 (0.09, 3.34) | 1.03 (0.77, 1.38) | 0.81 (0.54, 1.24) | 1.17 (0.43, 3.20) |
| 75.29–123.55 | 1.56 (0.97, 2.52) | 0.53 (0.30, 0.97) | 1.68 (0.43, 6.63) | 0.96 (0.70, 1.33) | 0.95 (0.63, 1.45) | 1.13 (0.45, 2.89) |
| >123.55 | 1.30 (0.73, 2.29) | 0.42 (0.21, 0.83) | 1.70 (0.43, 6.72) | 1.02 (0.71, 1.46) | 0.88 (0.56, 1.40) | 1.09 (0.43, 2.73) |
| Thiamin (mg) | | | | | | |
| <0.89 | referent | referent | referent | referent | referent | referent |
| 0.89–1.21 | 0.81 (0.52, 1.29) | 0.68 (0.36, 1.28) | 0.52 (0.15, 1.82) | 0.75 (0.55, 1.02) | 0.86 (0.53, 1.42) | 1.81 (0.76, 4.34) |
| 1.22–1.67 | 0.84 (0.50, 1.40) | 0.62 (0.32, 1.22) | 0.64 (0.19, 2.21) | 0.86 (0.61, 1.21) | 0.77 (0.45, 1.29) | 1.34 (0.51, 3.51) |
| >1.67 | 0.62 (0.31, 1.25) | 0.40 (0.18, 0.88) | 0.30 (0.06, 1.46) | 0.88 (0.58, 1.36) | 0.59 (0.32, 1.08) | 1.17 (0.39, 3.55) |
| Riboflavin (mg) | | | | | | |
| <1.39 | referent | referent | referent | referent | referent | referent |
| 1.39–1.91 | 0.95 (0.60, 1.50) | 0.89 (0.47, 1.68) | 0.59 (0.17, 2.10) | 0.83 (0.61, 1.13) | 0.59 (0.37, 0.95) | 0.42 (0.17, 1.06) |
| 1.92–2.62 | 0.82 (0.49, 1.37) | 0.86 (0.43, 1.70) | 0.57 (0.13, 2.48) | 0.83 (0.59, 1.16) | 0.57 (0.34, 0.94) | 1.19 (0.54, 2.62) |
| >2.62 | 0.77 (0.40, 1.47) | 0.77 (0.35, 1.69) | 1.01 (0.24, 4.26) | 0.91 (0.61, 1.38) | 0.60 (0.34, 1.07) | 0.33 (0.11, 0.98) |
| Vitamin B6 (mg) | | | | | | |
| <1.39 | referent | referent | referent | referent | referent | referent |
| 1.39–1.91 | 1.14 (0.72, 1.79) | 1.59 (0.71, 3.54) | 0.46 (0.11, 1.91) | 0.97 (0.72, 1.32) | 0.53 (0.31, 0.92) | 1.12 (0.48, 2.66) |
| 1.92–2.67 | 1.02 (0.59, 1.75) | 1.26 (0.55, 2.89) | 1.13 (0.34, 3.79) | 0.95 (0.67, 1.36) | 0.52 (0.31, 0.90) | 1.09 (0.44, 2.70) |
| >2.67 | 0.75 (0.36, 1.57) | 1.12 (0.44, 2.89) | 0.44 (0.08, 2.37) | 1.07 (0.69, 1.66) | 0.49 (0.26, 0.91) | 0.97 (0.33, 2.87) |
| Vitamin B12 (μg) | | | | | | |
| < 3.49 | referent | referent | referent | referent | referent | referent |
| 3.49–5.09 | 0.97 (0.61, 1.54) | 1.35 (0.73, 2.51) | 0.11 (0.01, 0.91) | 0.91 (0.67, 1.24) | 0.70 (0.43, 1.13) | 1.48 (0.59, 3.72) |
| 5.10–7.40 | 1.01 (0.60, 1.69) | 0.63 (0.31, 1.28) | 0.70 (0.22, 2.25) | 1.13 (0.81, 1.58) | 0.55 (0.34, 0.90) | 1.58 (0.61, 4.09) |
| >7.40 | 0.94 (0.49, 1.79) | 0.85 (0.43, 1.70) | 0.54 (0.14, 2.04) | 1.16 (0.76, 1.75) | 0.73 (0.45, 1.18) | 1.79 (0.66, 4.83) |
| **Antioxidants** | | | | | | |
| Vitamin C (mg) | | | | | | |
| <59.67 | referent | referent | referent | referent | referent | referent |

| Nutrient Quartiles[c] | Adjusted Odds Ratios[a] (95% confidence intervals) | | | | | |
|---|---|---|---|---|---|---|
| | Anencephaly (n=300[b]) | | | Spina Bifida (n=654[b]) | | |
| | White, non-Hispanic (n=143) | Hispanic (n=108) | Black, non-Hispanic (n=23) | White, non-Hispanic (n=351) | Hispanic (n=211) | Black, non-Hispanic (n=53) |
| 59.67–101.90 | 0.96 (0.63, 1.47) | 1.20 (0.58, 2.50) | 0.79 (0.26, 2.39) | 0.79 (0.59, 1.05) | 0.75 (0.45, 1.27) | 0.58 (0.24, 1.38) |
| 101.91–154.90 | 1.08 (0.68, 1.72) | 1.04 (0.50, 2.16) | 0.75 (0.22, 2.51) | 0.96 (0.71, 1.31) | 0.63 (0.38, 1.06) | 1.01 (0.46, 2.23) |
| >154.90 | 0.62 (0.31, 1.28) | 0.76 (0.36, 1.60) | 0.46 (0.11, 1.84) | 0.89 (0.60, 1.33) | 0.57 (0.34, 0.95) | 0.67 (0.28, 1.60) |
| Vitamin E (mg) | | | | | | |
| <2.86 | referent | referent | referent | referent | referent | referent |
| 2.86–4.13 | 0.90 (0.59, 1.37) | 0.93 (0.43, 1.99) | 0.58 (0.17, 2.03) | 0.81 (0.61, 1.09) | 0.40 (0.23, 0.70) | 1.37 (0.62, 3.03) |
| 4.14–6.09 | 0.56 (0.32, 0.97) | 1.15 (0.56, 2.39) | 0.59 (0.16, 2.22) | 0.83 (0.59, 1.16) | 0.47 (0.28, 0.79) | 1.07 (0.44, 2.57) |
| >6.09 | 0.74 (0.40, 1.35) | 0.78 (0.35, 1.77) | 1.00 (0.25, 4.08) | 0.74 (0.50, 1.11) | 0.53 (0.31, 0.93) | 0.76 (0.26, 2.22) |
| **Others** | | | | | | |
| Alanine (g) | | | | | | |
| <2.25 | referent | referent | referent | referent | referent | referent |
| 2.25–2.94 | 1.21 (0.75, 1.96) | 0.83 (0.39, 1.75) | 1.59 (0.45, 5.61) | 0.92 (0.67, 1.25) | 0.61 (0.36, 1.03) | 0.68 (0.25, 1.80) |
| 2.95–3.85 | 1.35 (0.77, 2.39) | 1.28 (0.63, 2.61) | 1.22 (0.30, 4.95) | 0.93 (0.64, 1.34) | 0.71 (0.42, 1.19) | 1.20 (0.48, 2.96) |
| >3.85 | 1.57 (0.75, 3.25) | 1.08 (0.45, 2.59) | 1.24 (0.24, 6.46) | 1.24 (0.78, 1.98) | 0.71 (0.39, 1.32) | 1.40 (0.49, 4.03) |
| Niacin (mg) | | | | | | |
| <13.45 | referent | referent | referent | referent | referent | referent |
| 13.45–17.77 | 1.23 (0.76, 1.98) | 1.06 (0.57, 1.98) | 0.27 (0.05, 1.34) | 0.99 (0.73, 1.36) | 0.82 (0.51, 1.32) | 1.18 (0.48, 2.87) |
| 17.78–23.65 | 1.42 (0.83, 2.43) | 0.76 (0.39, 1.49) | 0.78 (0.22, 2.79) | 1.13 (0.79, 1.60) | 0.70 (0.43, 1.15) | 0.95 (0.35, 2.55) |
| >23.65 | 0.85 (0.41, 1.75) | 0.71 (0.33, 1.53) | 0.58 (0.13, 2.66) | 1.17 (0.76, 2.19) | 0.54 (0.30, 0.96) | 1.20 (0.41, 3.50) |
| Iron (mg) | | | | | | |
| <8.62 | referent | referent | referent | referent | referent | referent |
| 8.62–12.50 | 0.86 (0.55, 1.34) | 0.91 (0.47, 1.76) | 0.40 (0.11, 1.44) | 1.02 (0.75, 1.38) | 0.86 (0.52, 1.42) | 0.54 (0.22, 1.32) |
| 12.51–17.70 | 0.66 (0.39, 1.11) | 0.63 (0.31, 1.28) | 0.57 (0.16, 2.01) | 0.91 (0.65, 1.28) | 0.59 (0.34, 1.02) | 0.85 (0.36, 2.02) |
| >17.70 | 0.71 (0.38, 1.32) | 0.45 (0.20, 1.01) | 0.30 (0.06, 1.48) | 1.18 (0.80, 1.76) | 0.76 (0.42, 1.35) | 0.57 (0.20, 1.59) |
| Zinc (mg) | | | | | | |
| <7.99 | referent | referent | referent | referent | referent | referent |

| Nutrient Quartiles[c] | Adjusted Odds Ratios[a] (95% confidence intervals) | | | | | |
|---|---|---|---|---|---|---|
| | Anencephaly (n=300[b]) | | | Spina Bifida (n=654[b]) | | |
| | White, non-Hispanic (n=143) | Hispanic (n=108) | Black, non-Hispanic (n=23) | White, non-Hispanic (n=351) | Hispanic (n=211) | Black, non-Hispanic (n=53) |
| 7.99–10.71 | 1.54 (0.93, 2.55) | 0.99 (0.51, 1.95) | 1.41 (0.42, 4.70) | 0.98 (0.71, 1.34) | 0.57 (0.33, 0.97) | 0.71 (0.31, 1.60) |
| 10.72–14.30 | 1.79 (1.00, 3.21) | 0.70 (0.34, 1.47) | 0.63 (0.13, 3.13) | 0.90 (0.62, 1.31) | 0.73 (0.43, 1.24) | 0.51 (0.20, 1.33) |
| >14.30 | 1.53 (0.73, 3.20) | 0.67 (0.28, 1.61) | 0.87 (0.15, 5.01) | 1.22 (0.78, 1.91) | 0.75 (0.40, 1.40) | 0.34 (0.11, 1.11) |
| Retinol (μg) | | | | | | |
| <218.60 | referent | referent | referent | referent | referent | referent |
| 218.60–348.59 | 1.33 (0.84, 2.11) | 0.69 (0.38, 1.24) | 0.97 (0.30, 3.22) | 1.05 (0.77, 1.42) | 0.46 (0.29, 0.74) | 1.69 (0.70, 4.10) |
| 348.60–535.10 | 1.08 (0.65, 1.80) | 0.70 (0.38, 1.30) | 0.74 (0.20, 2.73) | 0.97 (0.70, 1.34) | 0.79 (0.52, 1.22) | 1.59 (0.64, 3.92) |
| >535.10 | 1.24 (0.69, 2.23) | 0.73 (0.40, 1.31) | 0.88 (0.25, 3.06) | 1.19 (0.82, 1.74) | 0.62 (0.40, 0.96) | 1.60 (0.65, 3.96) |
| Vitamin A, RAE (μg RAE) | | | | | | |
| <390.88 | referent | referent | referent | referent | referent | referent |
| 390.88–592.60 | 0.90 (0.57, 1.42) | 0.61 (0.32, 1.15) | 1.11 (0.32, 3.82) | 1.00 (0.75, 1.35) | 0.74 (0.47, 1.16) | 1.76 (0.72, 4.28) |
| 592.61–858.76 | 1.12 (0.70, 1.81) | 0.89 (0.49, 1.62) | 0.78 (0.18, 3.40) | 0.98 (0.71, 1.36) | 0.62 (0.38, 1.00) | 1.54 (0.60, 3.98) |
| >858.76 | 0.76 (0.41, 1.44) | 0.72 (0.39, 1.33) | 2.38 (0.66, 8.57) | 0.94 (0.63, 1.40) | 0.69 (0.43, 1.08) | 2.46 (0.97, 6.21) |

Abbreviations: DFE, dietary folate equivalent; RAE, retinol activity equivalent.

[a] Adjusted model includes center and log-transformed energy intake.

[b] Cases only. For controls: White, non-Hispanic (n=3748); Hispanic (n=1404); Black, non-Hispanic (n=700).

[c] Quartiles based on daily nutrient intake of control mothers.

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## Table 4

Association Between Anencephaly or Spina Bifida and Maternal Dietary Micronutrient Intake Stratified by Maternal Pre-pregnancy Body Mass Index, National Birth Defects Prevention Study, 1997–2005

| | Adjusted Odds Ratios[a] (95% confidence intervals) | | | | | |
|---|---|---|---|---|---|---|
| | Anencephaly (n=300[b]) | | | Spina Bifida (n=654[b]) | | |
| **Nutrient Quartiles[c]** | **Normal BMI 18.5 to <25 (n=151)** | **Overweight BMI 25 to <30 (n=64)** | **Obese BMI 30+ (n=49)** | **Normal BMI 18.5 to <25 (n=291)** | **Overweight BMI 25 to <30 (n=150)** | **Obese BMI 30+ (n=152)** |
| **One-carbon metabolism** | | | | | | |
| Folate DFE (μg DFE) | | | | | | |
| <338.16 | referent | referent | referent | referent | referent | referent |
| 338.16–496.15 | 0.86 (0.54, 1.37) | 0.80 (0.42, 1.53) | 0.85 (0.39, 1.86) | 1.03 (0.72, 1.46) | 0.75 (0.47, 1.22) | 0.85 (0.53, 1.38) |
| 496.16–720.06 | 0.73 (0.44, 1.19) | 0.38 (0.16, 0.89) | 0.63 (0.25, 1.59) | 0.95 (0.66, 1.38) | 0.65 (0.38, 1.12) | 1.07 (0.63, 1.81) |
| >720.06 | 0.71 (0.41, 1.26) | 0.40 (0.16, 1.00) | 0.75 (0.28, 1.96) | 1.04 (0.69, 1.58) | 0.67 (0.37, 1.20) | 1.23 (0.69, 2.18) |
| Methionine (g) | | | | | | |
| <1.10 | referent | referent | referent | referent | referent | referent |
| 1.10–1.43 | 1.00 (0.58, 1.72) | 1.66 (0.78, 3.55) | 0.93 (0.41, 2.11) | 1.03 (0.72, 1.46) | 0.85 (0.50, 1.42) | 0.68 (0.41, 1.16) |
| 1.44–1.87 | 1.70 (0.99, 2.94) | 1.27 (0.50, 3.25) | 0.36 (0.13, 1.03) | 0.84 (0.56, 1.27) | 0.92 (0.51, 1.64) | 0.81 (0.46, 1.43) |
| >1.87 | 1.73 (0.88, 3.41) | 2.32 (0.77, 6.97) | 0.52 (0.17, 1.56) | 1.09 (0.67, 1.77) | 1.29 (0.64, 2.60) | 1.00 (0.50, 2.01) |
| Cysteine (g) | | | | | | |
| <0.66 | referent | referent | referent | referent | referent | referent |
| 0.66–0.86 | 1.20 (0.73, 1.97) | 1.35 (0.66, 2.75) | 0.96 (0.42, 2.22) | 0.99 (0.69, 1.42) | 0.98 (0.58, 1.63) | 0.53 (0.31, 0.91) |
| 0.87–1.15 | 1.30 (0.77, 2.19) | 1.00 (0.43, 2.32) | 0.71 (0.27, 1.86) | 1.01 (0.69, 1.49) | 1.11 (0.64, 1.92) | 0.99 (0.58, 1.67) |
| >1.15 | 1.01 (0.54, 1.90) | 1.11 (0.42, 2.98) | 0.77 (0.27, 2.16) | 1.09 (0.70, 1.71) | 1.11 (0.58, 2.12) | 0.94 (0.50, 1.75) |
| Choline, Total (mg) | | | | | | |
| <190.41 | referent | referent | referent | referent | referent | referent |
| 190.41–254.08 | 1.32 (0.78, 2.22) | 0.66 (0.30, 1.44) | 0.93 (0.38, 2.25) | 0.91 (0.63, 1.31) | 0.72 (0.43, 1.22) | 0.72 (0.43, 1.21) |
| 254.09–343.19 | 1.63 (0.93, 2.86) | 1.00 (0.43, 2.33) | 0.49 (0.16, 1.45) | 0.97 (0.64, 1.45) | 0.98 (0.56, 1.73) | 0.79 (0.45, 1.40) |
| >343.19 | 1.51 (0.73, 3.13) | 1.11 (0.39, 3.16) | 1.08 (0.34, 3.42) | 1.23 (0.74, 2.05) | 0.80 (0.39, 1.65) | 0.77 (0.37, 1.58) |
| Betaine (mg) | | | | | | |
| <46.60 | referent | referent | referent | referent | referent | referent |

| Nutrient Quartiles[c] | Adjusted Odds Ratios[a] (95% confidence intervals) | | | | | |
|---|---|---|---|---|---|---|
| | Anencephaly (n=300[b]) | | | Spina Bifida (n=654[b]) | | |
| | Normal BMI 18.5 to <25 (n=151) | Overweight BMI 25 to <30 (n=64) | Obese BMI 30+ (n=49) | Normal BMI 18.5 to <25 (n=291) | Overweight BMI 25 to <30 (n=150) | Obese BMI 30+ (n=152) |
| 46.60–75.28 | 1.07 (0.68, 1.68) | 0.57 (0.27, 1.19) | 0.92 (0.44, 1.93) | 1.06 (0.74, 1.50) | 0.59 (0.37, 0.95) | 1.04 (0.66, 1.64) |
| 75.29–123.55 | 1.00 (0.62, 1.61) | 0.93 (0.46, 1.89) | 0.77 (0.34, 1.75) | 1.13 (0.79, 1.61) | 0.56 (0.34, 0.92) | 1.00 (0.62, 1.64) |
| >123.55 | 0.91 (0.55, 1.53) | 1.01 (0.48, 2.13) | 0.53 (0.20, 1.40) | 1.23 (0.85, 1.79) | 0.80 (0.48, 1.31) | 0.82 (0.47, 1.44) |
| Thiamin (mg) | | | | | | |
| <0.89 | referent | referent | referent | referent | referent | referent |
| 0.89–1.21 | 0.93 (0.58, 1.50) | 0.57 (0.28, 1.17) | 0.58 (0.25, 1.37) | 0.94 (0.65, 1.35) | 0.66 (0.41, 1.07) | 0.61 (0.37, 1.01) |
| 1.22–1.67 | 0.84 (0.50, 1.41) | 0.55 (0.25, 1.21) | 0.57 (0.22, 1.49) | 1.15 (0.79, 1.69) | 0.42 (0.24, 0.74) | 0.90 (0.52, 1.54) |
| >1.67 | 0.53 (0.27, 1.03) | 0.29 (0.10, 0.86) | 0.89 (0.31, 2.55) | 1.00 (0.62, 1.60) | 0.45 (0.23, 0.89) | 1.01 (0.52, 1.95) |
| Riboflavin (mg) | | | | | | |
| <1.39 | referent | referent | referent | referent | referent | referent |
| 1.39–1.91 | 0.69 (0.42, 1.14) | 0.83 (0.41, 1.68) | 0.77 (0.35, 1.71) | 0.91 (0.64, 1.29) | 0.45 (0.27, 0.75) | 0.74 (0.46, 1.20) |
| 1.92–2.62 | 0.80 (0.48, 1.32) | 0.63 (0.28, 1.42) | 0.48 (0.18, 1.29) | 0.87 (0.59, 1.26) | 0.51 (0.30, 0.87) | 0.95 (0.56, 1.61) |
| >2.62 | 0.76 (0.41, 1.38) | 0.51 (0.18, 1.41) | 0.77 (0.28, 2.08) | 0.88 (0.56, 1.38) | 0.39 (0.20, 0.76) | 1.05 (0.56, 1.98) |
| Vitamin B6 (mg) | | | | | | |
| <1.39 | referent | referent | referent | referent | referent | referent |
| 1.39–1.91 | 1.20 (0.73, 1.96) | 0.97 (0.45, 2.07) | 1.00 (0.42, 2.41) | 0.94 (0.65, 1.36) | 0.88 (0.54, 1.45) | 0.74 (0.45, 1.23) |
| 1.92–2.67 | 1.14 (0.66, 1.98) | 1.32 (0.59, 2.94) | 0.79 (0.30, 2.10) | 1.16 (0.78, 1.72) | 0.61 (0.34, 1.08) | 0.76 (0.44, 1.32) |
| >2.67 | 0.84 (0.42, 1.67) | 0.74 (0.25, 2.15) | 0.94 (0.30, 2.95) | 1.30 (0.81, 2.10) | 0.67 (0.34, 1.32) | 0.88 (0.44, 1.74) |
| Vitamin B12 (μg) | | | | | | |
| < 3.49 | referent | referent | referent | referent | referent | referent |
| 3.49–5.09 | 1.04 (0.64, 1.67) | 0.70 (0.34, 1.44) | 0.93 (0.41, 2.12) | 1.04 (0.73, 1.48) | 0.67 (0.40, 1.11) | 0.84 (0.51, 1.39) |
| 5.10–7.40 | 0.88 (0.52, 1.47) | 0.63 (0.29, 1.38) | 0.48 (0.17, 1.30) | 1.07 (0.74, 1.55) | 0.69 (0.41, 1.17) | 0.94 (0.54, 1.63) |
| >7.40 | 0.99 (0.56, 1.77) | 0.64 (0.25, 1.65) | 0.74 (0.27, 2.05) | 1.08 (0.71, 1.65) | 0.79 (0.42, 1.48) | 1.14 (0.62, 2.10) |
| **Antioxidants** | | | | | | |
| Vitamin C (mg) | | | | | | |
| <59.67 | referent | referent | referent | referent | referent | referent |

| Nutrient Quartiles[c] | Adjusted Odds Ratios[a] (95% confidence intervals) | | | | | |
|---|---|---|---|---|---|---|
| | Anencephaly (n=300[b]) | | | Spina Bifida (n=654[b]) | | |
| | Normal BMI 18.5 to <25 (n=151) | Overweight BMI 25 to <30 (n=64) | Obese BMI 30+ (n=49) | Normal BMI 18.5 to <25 (n=291) | Overweight BMI 25 to <30 (n=150) | Obese BMI 30+ (n=152) |
| 59.67–101.90 | 1.19 (0.74, 1.89) | 0.80 (0.40, 1.57) | 0.81 (0.35, 1.85) | 1.04 (0.74, 1.48) | 0.63 (0.39, 1.03) | 0.53 (0.33, 0.85) |
| 101.91–154.90 | 1.31 (0.82, 2.09) | 0.84 (0.40, 1.76) | 1.03 (0.44, 2.45) | 1.08 (0.76, 1.53) | 0.76 (0.46, 1.26) | 0.66 (0.41, 1.08) |
| >154.90 | 0.73 (0.40, 1.31) | 0.67 (0.29, 1.56) | 1.38 (0.55, 3.44) | 1.03 (0.69, 1.54) | 0.64 (0.37, 1.11) | 0.72 (0.41, 1.26) |
| Vitamin E (mg) | | | | | | |
| <2.86 | referent | referent | referent | referent | referent | referent |
| 2.86–4.13 | 0.90 (0.56, 1.43) | 0.61 (0.30, 1.28) | 1.57 (0.68, 3.60) | 1.14 (0.80, 1.62) | 0.46 (0.28, 0.76) | 0.69 (0.44, 1.09) |
| 4.14–6.09 | 0.78 (0.47, 1.30) | 0.78 (0.36, 1.70) | 0.70 (0.24, 2.03) | 1.09 (0.75, 1.60) | 0.56 (0.33, 0.95) | 0.51 (0.29, 0.89) |
| >6.09 | 0.83 (0.47, 1.48) | 0.81 (0.33, 2.02) | 1.22 (0.44, 3.41) | 1.30 (0.85, 1.99) | 0.51 (0.28, 0.95) | 0.52 (0.28, 0.95) |
| **Others** | | | | | | |
| Alanine (g) | | | | | | |
| <2.25 | referent | referent | referent | referent | referent | referent |
| 2.25–2.94 | 1.41 (0.82, 2.44) | 1.38 (0.64, 2.99) | 1.07 (0.45, 2.54) | 1.10 (0.77, 1.58) | 0.83 (0.49, 1.41) | 0.54 (0.32, 0.92) |
| 2.95–3.85 | 2.24 (1.26, 3.99) | 1.54 (0.63, 3.78) | 0.67 (0.25, 1.80) | 1.02 (0.67, 1.54) | 0.93 (0.52, 1.65) | 0.79 (0.46, 1.37) |
| >3.85 | 2.39 (1.16, 4.91) | 2.42 (0.81, 7.20) | 0.63 (0.20, 1.99) | 1.39 (0.85, 2.30) | 1.26 (0.62, 2.54) | 0.67 (0.34, 1.34) |
| Niacin (mg) | | | | | | |
| <13.45 | referent | referent | referent | referent | referent | referent |
| 13.45–17.77 | 1.03 (0.63, 1.68) | 1.32 (0.63, 2.78) | 0.69 (0.29, 1.63) | 1.05 (0.73, 1.50) | 1.03 (0.62, 1.71) | 0.62 (0.37, 1.04) |
| 17.78–23.65 | 1.20 (0.72, 2.03) | 1.07 (0.46, 2.53) | 0.64 (0.25, 1.64) | 0.98 (0.66, 1.46) | 0.84 (0.48, 1.48) | 0.92 (0.54, 1.58) |
| >23.65 | 0.69 (0.35, 1.33) | 1.17 (0.41, 3.28) | 0.61 (0.20, 1.85) | 1.22 (0.77, 1.93) | 0.59 (0.29, 1.20) | 0.71 (0.36, 1.41) |
| Iron (mg) | | | | | | |
| <8.62 | referent | referent | referent | referent | referent | referent |
| 8.62–12.50 | 0.88 (0.55, 1.41) | 0.62 (0.31, 1.26) | 0.75 (0.33, 1.75) | 1.15 (0.80, 1.64) | 0.64 (0.39, 1.06) | 0.90 (0.54, 1.49) |
| 12.51–17.70 | 0.70 (0.41, 1.18) | 0.48 (0.22, 1.07) | 0.65 (0.26, 1.66) | 0.97 (0.66, 1.44) | 0.48 (0.27, 0.83) | 0.95 (0.54, 1.66) |
| >17.70 | 0.65 (0.35, 1.19) | 0.38 (0.14, 1.02) | 0.51 (0.17, 1.54) | 1.17 (0.76, 1.82) | 0.57 (0.30, 1.08) | 1.68 (0.90, 3.12) |
| Zinc (mg) | | | | | | |
| <7.99 | referent | referent | referent | referent | referent | referent |

| Nutrient Quartiles[c] | Adjusted Odds Ratios[a] (95% confidence intervals) | | | | | |
|---|---|---|---|---|---|---|
| | Anencephaly (n=300[b]) | | | Spina Bifida (n=654[b]) | | |
| | Normal BMI 18.5 to <25 (n=151) | Overweight BMI 25 to <30 (n=64) | Obese BMI 30+ (n=49) | Normal BMI 18.5 to <25 (n=291) | Overweight BMI 25 to <30 (n=150) | Obese BMI 30+ (n=152) |
| 7.99–10.71 | 1.05 (0.63, 1.77) | 1.47 (0.68, 3.20) | 0.86 (0.35, 2.15) | 0.99 (0.69, 1.42) | 0.85 (0.50, 1.43) | 0.65 (0.38, 1.12) |
| 10.72–14.30 | 1.23 (0.70, 2.16) | 1.56 (0.65, 3.76) | 0.79 (0.29, 2.13) | 0.95 (0.63, 1.44) | 0.79 (0.44, 1.42) | 0.86 (0.48, 1.54) |
| >14.30 | 1.05 (0.52, 2.10) | 1.21 (0.38, 3.84) | 0.40 (0.11, 1.45) | 1.00 (0.61, 1.65) | 1.02 (0.50, 2.10) | 1.15 (0.57, 2.31) |
| Retinol (μg) | | | | | | |
| <218.60 | referent | referent | referent | referent | referent | referent |
| 218.60–348.59 | 0.92 (0.58, 1.44) | 0.85 (0.44, 1.62) | 0.89 (0.38, 2.09) | 0.94 (0.66, 1.33) | 0.87 (0.54, 1.39) | 0.80 (0.49, 1.28) |
| 348.60–535.10 | 0.65 (0.40, 1.07) | 0.39 (0.17, 0.89) | 1.14 (0.49, 2.69) | 1.08 (0.76, 1.53) | 0.65 (0.39, 1.10) | 0.74 (0.44, 1.26) |
| >535.10 | 0.78 (0.46, 1.31) | 0.62 (0.28, 1.40) | 0.90 (0.36, 2.27) | 0.95 (0.64, 1.40) | 0.76 (0.44, 1.32) | 1.03 (0.60, 1.76) |
| Vitamin A, RAE (μg RAE) | | | | | | |
| <390.88 | referent | referent | referent | referent | referent | referent |
| 390.88–592.60 | 0.82 (0.52, 1.29) | 0.46 (0.21, 1.00) | 0.71 (0.28, 1.77) | 1.11 (0.79, 1.56) | 0.80 (0.50, 1.28) | 0.86 (0.54, 1.37) |
| 592.61–858.76 | 0.83 (0.52, 1.33) | 0.78 (0.38, 1.60) | 1.18 (0.49, 2.86) | 0.92 (0.64, 1.33) | 0.60 (0.35, 1.01) | 0.89 (0.53, 1.49) |
| >858.76 | 0.57 (0.33, 0.99) | 0.81 (0.35, 1.85) | 1.69 (0.69, 4.18) | 1.01 (0.67, 1.51) | 0.62 (0.35, 1.11) | 0.89 (0.50, 1.59) |

Abbreviations: BMI, body mass index; DFE, dietary folate equivalent; RAE, retinol activity equivalent.

[a] Adjusted model includes center and log-transformed energy intake.

[b] Cases only. For controls: Normal BMI (n=3373); Overweight BMI (n=1347); Obese BMI (n=966).

[c] Quartiles based on daily nutrient intake of control mothers.