

## Dietary Methionine Is Involved in the Etiology of Neural Tube Defect-Affected Pregnancies in Humans<sup>1,2</sup>

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**ABSTRACT** Research has provided evidence of the role of multivitamin supplementation in the prevention of neural tube defects (NTD). Failure of the neural tube to close is one of the most frequent and severe human developmental defects. The etiology of NTD is complex, encompassing genetic, dietary and environmental factors. The purpose of this study was to explore the relationship between maternal dietary intake of methionine and the risk of having a NTD-affected pregnancy. We hypothesized that women with high maternal dietary methionine intake were at a decreased risk for a NTD. Combinations of methionine, folate and vitamin B-12 intakes and NTD risk were also examined. Data from a 5-y, population-based, case-control study of 170 NTD-affected pregnancies and 269 controls were provided by the South Carolina NTD Surveillance, Prevention, and Research Project. There was a 30–55% lower NTD risk among women whose average daily dietary intake of methionine was greater than the lowest quartile of intake (>1580 mg/d). The odds ratios associated with the three quartiles of methionine intake > 1580 mg/d after adjusting for energy, race and body mass index were 0.72 ( $P < 0.07$ ), 0.68 ( $P < 0.07$ ) and 0.45 ( $P < 0.06$ ), respectively. These findings indicate that a reduction in the risk of having a NTD-affected pregnancy is associated with maternal dietary intake of methionine (3 mo pre- to 3 mo postconception). This finding is consistent with the hypothesis that methionine plays a role in the etiology of NTD and suggests the need for further research in the area of maternal diet and pregnancy. *J. Nutr.* 131: 2653–2658, 2001.

**KEY WORDS:** • *methionine* • *neural tube defects* • *pregnancy* • *maternal diet* • *humans*

Research has provided evidence of the role of multivitamin supplementation in the reduction of risk for neural tube defects (NTD)<sup>4</sup> (1). Pregnancy is associated with a significant increase in cellular proliferation as the result of increased maternal erythropoiesis, maternal uterine and mammary tissue growth as well as placental and fetal development (2). Epidemiologic investigation has provided evidence to demonstrate that multivitamin supplementation with folic acid (3 mo pre- to 3 mo postconception) significantly decreases the risk of having a NTD-affected pregnancy (3,4). However, the exact mechanism by which folate decreases the risk is unknown (5). Recent research has indicated that folate may not be the only component involved in the causal

network (5). The etiology of NTD is complex, encompassing genetic, dietary and other environmental factors.

Animal and laboratory studies have demonstrated that methionine plays an important role in the normal closure of the neural tube in rodents. Coelho et al. (6) conducted a study using whole rat embryos cultured in cow serum without supplemental methionine and found an increase in neural tube closure defects in these embryos compared with methionine-supplemented embryos. Essien and Wannberg (7) conducted a study in mice and found that closure of the neural tube in mice embryos required methionine. It has been suggested that different closure initiation sites are disrupted for anencephaly and spina bifida. Also, these studies suggest that encephaloceles result from disruption of still other closure initiation sites, all through a common mechanism (8). Due to the rarity of encephaloceles as well as other subcategories of NTD, the different types of NTD are treated as a homogeneous group.

Shaw et al. (5) hypothesized that women with greater dietary intakes of methionine would be at a lower risk for an occurrent NTD-affected pregnancy. Shaw et al. (5) estimated the risk of having a NTD-affected pregnancy according to quartiles of average daily maternal dietary intake of methionine in the 3 mo before conception on the basis of the dietary intake of methionine in the control group (<1341.87 mg/d, 1341.87–1750.35 mg/d, 1750.36–2347.61 mg/d, >2347.61

<sup>1</sup> Presented at the 2000 Annual Meeting of the American Public Health Association, Boston, MA and at the 2000 Annual Meeting of the American College of Epidemiology, Atlanta, GA. [Shoob, H. D., Thompson, S. J., Sargent R. G., Drane, J. W., Best, R. G. & Tocharoen, A. (2000) Dietary Methionine in the Multifactorial Etiology of Neural Tube Defect Affected Pregnancies.] Abstract published by the American Public Health Association and the American College of Epidemiology.

<sup>2</sup> Supported by South Carolina Neural Tube Defect Surveillance, Prevention and Research Initiative Cooperative Agreement with the CDC, Cooperative Initiative Agreement #408774.

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<sup>4</sup> Abbreviations used: B-12-CH<sub>3</sub>, methionine synthetase; BMI, body mass index; CDC, Centers for Disease Control and Prevention; 5-CH<sub>3</sub>-THF, methyltetrahydrofolate; CI, confidence interval; FFQ, food-frequency questionnaire; NTD, neural tube defect; OR, odds ratio; WFFQ, Willett Food Frequency Questionnaire.

mg/d). There was a 30–40% reduction in the number of NTD-affected pregnancies among women whose average daily dietary intake of methionine was above the lowest quartile of intake (>1341.86 mg/d) after adjusting for maternal race/ethnicity and education. The adjusted odds ratios (OR) of 0.67, 0.66 and 0.50 were similar to the crude estimates. The reductions in risk were independent of maternal level of folate intake. These investigators could not determine definitively whether the observed reductions were the result of maternal methionine intake (3 mo pre- to 3 mo postconception) or due to another highly correlated nutrient, but that study did provide evidence to support the involvement of maternal diet in neural tube closure.

Methionine is one of nine essential amino acids; as such, it is not produced sufficiently by the body and must be obtained in the diet. Methionine is found in complete proteins (contain all of the essential amino acids), most specifically animal proteins. Complete proteins are found in great abundance in meats (e.g., beef and lamb), fish, poultry, dairy products and eggs. Animal products are the only important dietary sources of methionine.

Methionine is closely linked to folate metabolism, and in animal studies has been shown to contribute to the closure of the neural tube. This study investigates dietary methionine intake levels and other maternal dietary components and their association with NTD.

## SUBJECTS AND METHODS

The South Carolina Neural Tube Defect Surveillance, Prevention, and Research Project was conducted via a cooperative agreement between the Centers for Disease Control and Prevention (CDC) and the three regional genetic centers in South Carolina. The research objectives of the project were as follows: to identify all cases of NTD, to obtain in-depth dietary information on NTD-affected pregnancies and to conduct a population-based, case-control study of dietary intake and multivitamin supplement use and occurrent NTD.

The case control study included all women with NTD-affected pregnancies who agreed to participate and randomly selected controls from women who delivered live births during the study period. The CDC provided a roster of control numbers by delivery hospital, based on the number of deliveries at each hospital during the previous year. Hospital labor and delivery suites were monitored monthly to determine when the randomly selected control birth numbers occurred.

Of the 260 isolated NTD-affected pregnancies ascertained for the 5-y period, 190 (73%) agreed to participate and were enrolled in the case-control study. Excluded from the data analysis were women who were diabetic, who reported taking anticonvulsant medications while pregnant, one woman who gave birth to twins and one woman who had a NTD herself. For the purposes of this study, a case mother was identified as a resident of South Carolina who had a pregnancy in which a fetus was affected with an isolated NTD. Each NTD-affected pregnancy was verified either by direct examination of the affected fetus or infant or by review of obstetric, genetic, pediatric or pathology records of such an examination elsewhere and classified by a medical geneticist. Isolated NTD were cases having no other physical features except those secondary to the NTD. Nonisolated cases, which included all syndromic forms of NTD, were excluded from the case-control study and followed for surveillance purposes only.

For this study, 179 NTD affected cases were eligible. However, nine did not complete the Willett Food Frequency Questionnaire (WFFQ) (9). Of the 398 eligible controls, 288 (72.4%) agreed to participate, but one control taking anticonvulsant medication during pregnancy was excluded. An additional 19 controls failed to complete the WFFQ. After all exclusions, our final study population included 170 cases and 269 controls. The distribution of NTD by type was as follows: anencephaly, 65; spina bifida, 84; and encephalocele, 21. The percentage of eligible cases and controls included in this study were 65 and 68%, respectively.

Ascertainment of NTD-affected pregnancies occurring in the

state was obtained from multiple sources. Maternal serum  $\alpha$  fetoprotein programs, prenatal diagnosis programs, all fetal/neonatal pathology departments of hospitals with delivery and newborn units, medical practices providing care to pregnant women and the State Vital Records Department were monitored continuously for NTD-affected pregnancies throughout the study period. All medical record departments of hospitals with delivery and newborn units were surveyed monthly for any NTD live births, stillbirths and pregnancy terminations. The well-established network of genetic centers in the state with a history of working well with medical practitioners facilitated the identification of NTD-affected pregnancies that spontaneously aborted or were electively terminated as well as births and fetal deaths. Officers from the CDC verified completeness of case ascertainment at the end of y 1 and 4.

Each mother enrolled in the project was interviewed using The South Carolina NTD Prevention Initiative Questionnaire, which is a modification of the CDC Birth Defects Risk Factor Surveillance Questionnaire. Demographic, health, behavioral and environmental exposure information relevant to the period (3 mo pre- to 3 mo postconception) of the NTD-affected pregnancy was obtained.

Each mother also completed the WFFQ (9) and reported the frequency of individual foods and use of multivitamins for the period 3 mo before conception through the first 3 mo of pregnancy. Completed questionnaires were sent to Harvard University for computation of average daily energy intake and intakes of major nutrients, vitamins and minerals using analytic software developed specifically for the survey instrument. The WFFQ was used in this population-based study because it is relatively simple to administer, does not require prompting or food models and, most importantly, allows for seasonal variations to be taken into account.

Once the mother of an NTD-affected pregnancy was reported to the surveillance project or a control mother was identified, the physician was contacted to obtain approval to contact the subject. Patient contact was made within 30 d of ascertainment unless the patient's physician requested a delay. With approval, the patient was contacted and the NTD prevention initiative explained to her and her participation requested. If she agreed, an interview date was determined. All persons who agreed to participate in the study signed an informed consent document approved by the participating hospitals and Department of Health and Environmental Control Institutional Review Boards.

Frequencies were run to describe the initial data;  $\chi^2$  analysis was used to determine differences between the cases and controls with regard to dietary intake of selected nutrients, vitamins and minerals.

Dietary intakes of methionine (mg), animal protein (g), folate ( $\mu$ g) and vitamin B-12 ( $\mu$ g) in the control group were used to establish quartile categories of intake. The OR and their 95% confidence intervals (CI) were calculated to estimate the risk using the lowest quartile of dietary intake of methionine, animal protein, folate, and vitamin B-12 as the referent levels. The variables included for analysis were the following: intakes of energy (kJ), protein (g), carbohydrate (g), fat (g), cholesterol (mg), fiber (g), vitamin A (retinol equivalents), thiamin (mg), riboflavin (mg), niacin (mg), vitamin B-6 (mg), folate ( $\mu$ g), folic acid ( $\mu$ g), vitamin B-12 ( $\mu$ g), vitamin C (mg), vitamin D ( $\mu$ g), vitamin E (mg), calcium (mg), iron (mg) and zinc (mg), age (<20, 20–29, 30–34,  $\geq$ 35 y), gravidity (1, 2, 3,  $\geq$ 4), race/ethnicity (Caucasian, African-American and other), prenatal care (1st, 2nd, 3rd trimester or none), marital status (married, single, divorced, widowed and other), education ( $\leq$ 11, 12 y of school,  $\geq$ 13), smoking-active (no, every month, some months), smoking-passive (no, every month, some months), alcohol (beer, wine, and liquor no, yes), preconception body mass index (BMI) (<19.8, 19.8–26.0, 26.1–29.0,  $\geq$ 29.0 kg/m<sup>2</sup>), and multivitamin use (no use, 1st use in 4–6 or 7–9 mo of pregnancy, some use 3 mo before conception to 3 mo gestation, regular use 3 mo before conception to 3 mo gestation).

The OR were calculated for methionine and NTD and for each of the other covariates and NTD. Unconditional logistic regression was used to estimate the association of each major exposure and NTD while simultaneously controlling for the other variables. Energy intake (kJ) was adjusted for in all models because nutrient content is strongly affected by energy change in the diet.

Backward stepwise regression was used to test for interactions and to select a minimal set of confounders. Tests for statistical interaction were conducted by including cross-product terms of the variables methionine and folate and methionine and vitamin B-12 in the model. When the OR of the major independent variable changed appreciably from one model to the next (>5%), the variable removed from the model was treated as a confounder and retained in the final model. Several logistic models were used to explore the hypothesized relationships. The first full model included methionine and the covariates energy, carbohydrate, thiamin, riboflavin, niacin, vitamin B-6, vitamin B-12, calcium, vitamin D, iron, race, age and BMI.

It appears that in humans, a combination of low levels of methionine, folate and vitamin B-12 may lead to the occurrence of NTD. Three separate models were used to examine combinations of methionine and folate intake and methionine and vitamin B-12 intake to investigate this possibility. A new variable was created to reflect the combined levels of each of the two nutrients in each of the three combinations. Each of the OR for the newly created levels of each of the combined variables was calculated using the lowest quartile of each nutrient as the referent level.

## RESULTS

A slightly higher proportion of cases than controls were Caucasian ( $P = 0.06$ ), had completed high school ( $P = 0.08$ ), had a prepregnant BMI  $\geq 29.1$  kg/m<sup>2</sup> ( $P = 0.06$ ), reported first prenatal care in mo 7–9 or had no care ( $P = 0.07$ ), reported consuming alcohol ( $P = 0.06$ ) and being exposed to passive smoking in the home or at work in the 3 mo before to 3 mo postconception ( $P = 0.06$ ) (Table 1). Significant differences were found between the cases and controls in intakes of methionine, protein, animal protein, animal fat, cholesterol and dietary fiber with the cases reporting lower intakes than the controls (Table 2). No significant differences were found between cases and controls for energy or carbohydrate intake. A significant difference was found between the cases and controls in intakes of vitamin A, thiamin, riboflavin, niacin, vitamin B-6, folate, vitamin B-12, vitamin C and vitamin D with the cases reporting lower intakes than the controls. Calcium, iron and zinc intakes were also different for the cases and controls and again were lower for the cases than the controls (Table 2).

There was an ~55% reduction in NTD risk among women whose average daily dietary intake of methionine was > 2830 mg/d. There was an ~30% reduction in NTD risk among women whose average daily dietary intake of methionine was >1580 mg/d. The OR associated with the three quartiles of methionine intake >1580 mg/d after adjusting for energy, race, and BMI were, in increasing order, 0.72 ( $P < 0.07$ ), 0.68 ( $P < 0.07$ ) and 0.45 ( $P < 0.06$ ), respectively. The adjusted risk ratios were very similar to the crude estimates. It should be noted that no significant interactions were found (Table 3).

Similar risks were observed for anencephaly and spina bifida (plus encephalocele) separately and across maternal race/ethnicity strata. Although risk does not consistently decrease at all folate intakes, associations with animal protein were similar to those with methionine.

Separate models were used to examine combinations of methionine and folate intake and methionine and dietary vitamin B-12 intake. A new variable was created to reflect the combined levels of each of the two nutrients in the three combinations and was included in a logistic model. Each of the OR for the newly created levels of the combined variables was calculated using the lowest quartile of each nutrient as a common referent level. When the referent group was women with the lowest level of methionine and the lowest level of folate, intake of methionine above the lowest quartile was associated with lower NTD risk at all levels of folate intake. In

TABLE 1

Characteristics of the case mothers and the control mothers

Characteristic	Cases (n = 170)	Controls (n = 269)
	n (%)	
Race/Ethnicity		
Caucasian	134 (78.8)	188 (69.9)
African-American/other	36 (21.2)	81 (30.1)
Age at Conception, y		
<20	26 (15.3)	45 (16.7)
20–29	102 (60.0)	143 (53.2)
30–34	31 (18.2)	62 (23.1)
$\geq 35$	11 (6.5)	19 (7.1)
Educational Level, y		
$\leq 11$	29 (17.1)	41 (15.2)
12 (high school)	60 (35.3)	80 (29.7)
$\geq 13$	75 (44.1)	145 (53.9)
Missing	6 (3.5)	3 (1.1)
Prepregnant BMI, kg/m <sup>2</sup>		
<19.8	22 (12.9)	50 (18.6)
19.8–26.0	90 (52.9)	139 (51.7)
26.1–29.0	19 (11.2)	40 (14.8)
$\geq 29.1$	39 (22.9)	40 (14.8)
Prenatal care		
1–3 mo	152 (89.4)	237 (88.1)
4–6 mo	11 (6.5)	28 (10.4)
7–9 mo or none	7 (4.1)	4 (1.5)
Alcohol intake (–3 to 3)		
No	96 (56.5)	173 (64.3)
Yes	74 (45.4)	96 (35.7)
Cigarette smoking (–3 to 3)		
No	117 (68.8)	202 (75.1)
Every Mo	30 (17.7)	40 (14.9)
Some Mo	23 (13.5)	27 (10.0)
Passive smoking (–3 to 3)		
No	82 (48.2)	172 (63.9)
Every Mo	72 (42.4)	82 (30.5)
Some Mo	16 (9.4)	15 (5.6)
Gravidity, n		
1	39 (22.9)	69 (25.6)
2	57 (33.5)	104 (38.7)
3	43 (25.3)	45 (16.7)
$\geq 4$	31 (18.2)	51 (19.0)

addition, we observed an ~70% reduction in NTD risk (OR = 0.32,  $P < 0.05$ ) in the group with both the highest methionine and folate intakes (Table 4).

The effect of combinations of methionine and vitamin B-12 was also investigated. Three levels of methionine and three levels of vitamin B-12 were compared with the referent level (lowest quartile) of <1580 mg methionine and <4.19  $\mu$ g of vitamin B-12. NTD risk with methionine did not show a consistent decrease at all levels of vitamin B-12 intake. However, we did observe a 50% reduction in NTD risk (OR = 0.05,  $P < 0.05$ ) in the group with the greatest methionine intake and the second highest vitamin B-12 intake (Table 5).

## DISCUSSION

Failure of the neural tube to close is one of the most frequent and severe human developmental defects. The prevalence of neural tube closure defects in the United States has been estimated to be 1.3–2.0 fetuses per 1000 live births (10). Neural tube closure defects are the most frequent abnormality seen in spontaneously aborted fetuses (11). The etiology of NTD is complex, encompassing genetic, dietary and environmental factors. Established dietary relationships appear to exist between maternal folate and methionine intake and the de-

TABLE 2

Intake of selected nutrients, vitamins and minerals by the case mothers and the control mothers<sup>1,2</sup>

	Cases (n = 170)	Controls (n = 269)	P-value
Methionine, mg/d	2120.0	2540.0	0.01*
Energy, kJ/d	9892.0	11057.4	0.07
Protein, g/d	91.2	108.5	0.01*
Animal protein, g/d	66.4	80.0	0.01*
Carbohydrate, g/d	316.7	356.0	0.06
Animal fat, g/d	49.3	57.3	0.03*
Cholesterol, mg/d	312.7	388.1	<0.01*
Dietary fiber, g	20.8	24.4	0.02*
Vitamins			
Vitamin A <sup>3</sup> , RE/d	2735.0	3791.9	<0.01*
Thiamin, mg/d	1.5	1.9	<0.01*
Riboflavin, mg/d	2.0	2.5	<0.01*
Niacin, mg/d	31.8	36.7	0.03*
Vitamin B-6, mg/d	2.2	2.6	0.02*
Folate, µg/d	315.8	405.5	<0.01*
Vitamin B-12, µg/d	8.8	11.1	0.05
Vitamin C, mg/d	165.5	216.7	<0.01*
Vitamin D, µg/d	6.2	7.9	<0.01*
Vitamin E, mg/d	7.3	8.3	0.12
Minerals			
Calcium, mg/d	864.6	1067.5	<0.01*
Iron, mg/d	15.0	17.9	0.01*
Zinc, mg/d	12.9	15.2	0.01*

<sup>1</sup> Values are means.

<sup>2</sup> Chi-square test was used to determine differences; \* significantly different at  $P < 0.05$ .

<sup>3</sup> RE, retinol equivalents.

velopment of NTD. Methionine levels can be influenced by a lack of adequate amounts of other amino acids such as tyrosine and cysteine in the diet. However, due to a very high protein intake in this population, we would not assume this to be a factor. In addition, the exact mechanism by which folate and/or methionine decreases the risk is unknown (5). No other clearly established dietary factor has been identified. Our data show a reduction in risk of NTD-affected pregnancies with high maternal dietary intake of methionine in the period (3 mo pre- to 3 mo postconception).

Dietary intakes of methionine above the lowest quartile were associated with lower NTD risk regardless of level of folate intake. However, risk does not show a consistent pattern of decrease. Although these data are suggestive of a further reduction in risk at the highest level of methionine and folate in combination than for methionine alone, the data are too sparse to make this definitive conclusion.

A diet deficient in folate may result in lower levels of methyltetrahydrofolate (5-CH<sub>3</sub>-THF), the methyl donor that is needed for the production of methionine from homocysteine (5). Vitamin B-12 (in the form of methylcobalamin) aids in the removal of the methyl group (CH<sub>3</sub>) from the 5-CH<sub>3</sub>-THF to form tetrahydrofolate (THF). Methionine synthetase (B-12-CH<sub>3</sub>) is an enzyme available for use in the synthesis of methionine from homocysteine. Homocysteine is methylated by B-12-CH<sub>3</sub> in a reaction requiring vitamin B-12 as a cofactor, which yields methionine as its product. When this occurs, methionine (CH<sub>3</sub>-homocysteine) is formed (12). However, one must be cautious in assuming that methionine alone is responsible for the reduction in NTD risk because methionine may affect the status of folate, vitamin B-6 and vitamin B-12.

Few studies have investigated the developmental abnormal-

ities resulting from amino acid deficiencies. Zamenof et al. (13) found that omission of dietary tryptophan, lysine or methionine in pregnant rats resulted in offspring with reduced body and brain weights and reduced cerebral DNA and protein content. Leclerc (14) found that the addition of methionine to low protein diets fed during pregnancy increased the body weight of rat progeny. In 1973, Benedetti et al. (15) reported that the addition of methionine to the diet of rats during pregnancy increased fetal RNA, DNA and protein contents.

Our findings were similar to those of Shaw et al. (5), the only other human study to investigate the role of methionine in neural tube closure. They hypothesized that women with greater dietary intakes of methionine would be at a lower risk for an occurrent NTD-affected pregnancy. They found a 30–40% reduction in the number of NTD-affected pregnancies among women whose average daily dietary intake of methionine was above the lowest quartile (>1341.86 mg/d) after adjusting for maternal race/ethnicity and education. The adjusted OR and 95% CI were 0.67 (0.46–0.98), 0.66 (0.45–0.96) and 0.50 (0.34–0.73) and were similar to the crude estimates. The reductions in risk were independent of maternal folate intake.

An advantage of the present study was that it was a population-based, case control study in a state with a relatively small population, reporting one of the highest prevalence rates of NTD in the United States. In addition, the racial distribution of the study among both the cases and controls was 70% Caucasian and 30% African-American, which was representative of the state's population. A second advantage is all cases were thoroughly established, including elective terminations. Each potential case was carefully assessed and classified by one medical geneticist. Only isolated occurrent NTD-affected pregnancies were included in the analysis.

A possible limitation is that the data were based on self-reported dietary information, which is subject to bias. The women were interviewed after delivering a NTD-affected fetus/infant or a normal infant and asked to recall what they had eaten in the period 3 mo pre- to 3 mo postconception. Interviews were conducted ~1 mo after delivery. This could have been problematic because in some instances, women were required to recall dietary intakes up to 13 mo in the past. The WFFQ, used to assess maternal nutrient intake, was originally intended for categorizing individuals by intake of selected nutrients. The WFFQ is typically self-administered and asks respondents to report usual frequency of consumption from a list of foods for a specified time period and includes questions on portion size. It does not require prompting or food models

TABLE 3

Odds ratios (OR) of NTD-affected pregnancies by quartile of average daily intake of methionine in the 3 mo pre- to the 3 mo postconception, assessed from the WFFQ and adjusted for energy, race and BMI<sup>1</sup>

Quartile of methionine, mg/d	Cases (n = 170)	Controls (n = 269)	Adjusted OR	P-value
<1580 Referent	50	65	1.0	—
1580–2070	44	69	0.72	<0.07
2070–2830	46	68	0.68	<0.07
>2830	30	67	0.45	<0.06

<sup>1</sup> NTD, neural tube defect; WFFQ, Willett Food Frequency Questionnaire; BMI, body mass index.

TABLE 4

Unadjusted odds ratios (OR) and 95% confidence intervals (CI) for risk of NTD-affected pregnancies based on quartile of dietary methionine and folate intakes<sup>1</sup>

Quartile of methionine, mg/d	Quartile of folate, µg/d			
	<235.79 Cases/Controls OR 95% CI	235.79–322.26 Cases/Controls OR 95% CI	322.26–457.36 Cases/Controls OR 95% CI	>457.36 Cases/Controls OR 95% CI
<1580				
Cases/Controls	44/39	2/21	3/3	1/2
OR	Referent	0.08	0.89	0.44
95% CI		(0.02, 0.38)	(0.17, 4.6)	(0.04, 5.1)
1580–2070				
Cases/Controls	13/23	16/17	12/22	3/7
OR	0.50	0.83	0.48	0.38
95% CI	(0.22, 1.1)	(0.37, 1.9)	(0.21, 1.1)	(0.09, 1.6)
2070–2830				
Cases/Controls	9/5	16/22	16/27	5/14
OR	1.6	0.65	0.53	0.32
95% CI	(0.49, 5.2)	(0.30, 1.4)	(0.25, 1.1)	(0.10, 0.96)
>2830				
Cases/Controls	2/0	1/7	11/16	16/44
OR	—	0.13	0.61	0.32*
95% CI		(0.02, 1.1)	(0.25, 1.5)	(0.16, 0.66)

<sup>1</sup> NTD, neural tube defect.

\* Significant at  $P < 0.05$ .

and allows for seasonal variations to be taken into account; however, it has a limited number of foods and has been updated only three times since its inception (16). Compared with diet recalls, food-frequency questionnaires (FFQ) typically indicate higher intakes. This inherent quality of the FFQ may contribute to increased micronutrient values found in this study. We would expect errors associated with this diet instrument to lead to biased effect estimates toward the null value.

Therefore, elevated effect estimates are unlikely to be the consequence of misclassified methionine levels.

The findings of this study demonstrate that a reduction in the risk of having a NTD-affected pregnancy is associated with greater maternal dietary methionine intake (3 mo pre- to 3 mo postconception). The findings are important in that this is one of the first human studies to investigate the role of methionine and NTD. This study provides evidence of the influence of methio-

TABLE 5

Unadjusted odds ratios (OR) and 95% confidence intervals (CI) for risk of NTD-affected pregnancies for combined levels of dietary methionine and vitamin B-12

Quartile of methionine, mg/d	Vitamin B-12, µg/d			
	<4.19 Cases/Controls OR 95% CI	4.19–6.59 Cases/Controls OR 95% CI	6.59–12.55 Cases/Controls OR 95% CI	>12.55 Cases/Controls OR 95% CI
<1580				
Cases/Controls	36/49	5/8	7/6	2/2
OR	Referent	0.85	1.6	1.4
95% CI		(0.26, 2.8)	(0.49, 5.1)	(0.18, 10.1)
1580–2070				
Cases/Controls	12/16	19/30	8/10	5/13
OR	1.0	0.86	1.1	0.52
95% CI	(0.43, 2.4)	(0.42, 1.8)	(0.39, 3.0)	(0.17, 1.6)
2070–2830				
Cases/Controls	5/1	16/27	17/22	8/18
OR	6.8	0.81	1.1	0.61
95% CI	(0.76, 60.8)	(0.38, 1.7)	(0.49, 2.3)	(0.24, 1.5)
>2830				
Cases/Controls	0/0	1/3	11/30	18/34
OR	—	0.45	0.50*	0.72
95% CI		(0.05, 4.5)	(0.22, 1.1)	(0.35, 1.5)

<sup>1</sup> NTD, neural tube defect.

\* Significant at  $P < 0.05$ .

nine in the biochemical pathway leading to closure of the neural tube and demonstrates the need for further research in the area of maternal diet, nutrition, and vitamin intake and pregnancy.

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