Published in final edited form as:
Birth Defects Res A Clin Mol Teratol. 2010 August ; 88(8): 670–678. doi:10.1002/bdra.20675.

Periconceptional nutrient intakes and risks of neural tube defects in California [1,2]

Suzan L. Carmichael[1,*], **Wei Yang**[1], and **Gary M. Shaw**[2]

[1] California Research Division, March of Dimes Foundation, Oakland, CA, 94609, USA

[2] Department of Pediatrics, Division of Neonatal & Developmental Medicine, Stanford University School of Medicine, Palo Alto, CA, 94305-5460, USA

Abstract

Background—This study investigated the association of neural tube defects (NTDs) with maternal periconceptional intake of folic acid-containing supplements and dietary nutrients, including folate, among deliveries that occurred after folic acid fortification in selected California counties.

Methods—The population-based case-control study included fetuses and live born infants with spina bifida (189) or anencephaly (141) and 625 nonmalformed, live born controls delivered from 1999–2003. Mothers reported supplement use during telephone interviews, which included a 107-item food frequency questionnaire. For dietary nutrients, intakes <25th, 25th–<75th (reference), and ≥75th percentile were compared, based on control distributions.

Results—After adjustment for potential confounders, any versus no supplement intake resulted in ORs of 0.8 (95% CI 0.5, 1.3) for anencephaly and 0.8 (95% CI 0.6, 1.2) for spina bifida. After stratification by maternal intake of vitamin supplements, most factors in the glycemic pathway were not associated with either NTD, with the exception of low levels of fructose and glucose that were significantly associated with anencephaly. Some nutrients that contribute to one-carbon metabolism showed lowered risks (folate, riboflavin, vitamins B_6 and B_{12}); others did not (choline, methionine, zinc). Anti-oxidant nutrients tended to be associated with lowered risks (vitamins C, E, A, β-carotene, lutein).

Conclusions—Mother's intake of vitamin supplements was modestly if at all associated with a lowered risk of NTDs. Dietary intake of several nutrients contributing to one-carbon metabolism and oxidative stress were associated with reduced NTD risk.

INTRODUCTION

Many studies have demonstrated the ability of folic acid supplementation to prevent neural tube defects (NTDs) [MRC Vitamin Study Research Group, 1991; Berry et al., 1999; Milunsky et al., 1989; Shaw et al., 1995; Werler et al., 1993; Czeizel and Dudas, 1992]. Folic acid fortification of the food supply was implemented in the U.S. in early 1998, with the goal of reducing NTD prevalence. Indeed, NTD prevalence has declined in the U.S. subsequent to fortification [Honein et al., 2001; Canfield et al., 2005; Williams et al., 2002]. A recent case-control study of births from multiple U.S. states, which included deliveries

[*]Corresponding author: Suzan L. Carmichael, California Research Division, March of Dimes Foundation, 5700 Martin Luther King Jr. Way, Suite 2902, Oakland, CA 94609-1673, USA. SCarmichael@marchofdimes.com, 510-597-7056/Office, 510-450-5812/Fax.

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that occurred after fortification was in place, did not observe that folic acid-containing supplements or dietary folate were associated with reduced NTD risk [Mosley et al., 2009]. One potential explanation for the lack of association was that folic acid fortification has been a success, i.e., folic acid-responsive NTDs have been prevented [Mosley et al., 2009; Mills and Carter, 2009].

The current study investigated the association of intake of supplemental folic acid and dietary folate with risk of NTDs in a post-fortification population of births in California. We also investigated other nutrients to provide a more comprehensive examination of dietary intake. In particular, we were interested in examining other nutrients that, like folic acid, contribute to the pathway of one-carbon metabolism and nutrients that contribute to glycemic control and oxidative stress, which also may contribute to NTD etiology [Shaw et al., 2003; Groenen et al., 2004; Martin et al., 2004; Smithells et al., 1976; Loeken, 2004].

METHODS

This case-control study included live born, stillborn (fetal deaths at ≥20 wk gestation), and prenatally diagnosed, electively terminated case fetuses that occurred to mothers residing in Los Angeles, San Francisco and Santa Clara counties. The study included data on deliveries that had estimated due dates from July 1999 to June 2003. Case information was abstracted from multiple hospital reports and medical records, which were routinely reviewed by a clinical geneticist. Infants diagnosed with single gene disorders or aneusomies (based on information gathered from chart reviews) were ineligible. Spina bifida included cases of lipomeningocele, meningomyelocele, and myelocystocele. Non-malformed, live born controls were selected randomly from birth hospitals to represent the population from which cases were derived. Specifically, controls were randomly selected from area hospitals in numbers proportional to the hospital's contribution of births to the total population of live born infants in the same area.

Birth mothers were eligible for interview if they were not incarcerated and if their primary language was English or Spanish. Maternal interviews were conducted using a standardized, computer-based questionnaire, primarily by telephone, in English or Spanish, no earlier than 6 wk after the infant's estimated date of delivery. A variety of exposures were assessed, focusing on the periconceptional time period, which was defined as 2 months before through 2 months after conception. Body mass index (BMI) was estimated for each woman based on reported pre-pregnancy weight and height (kg/m^2).

To assess usual dietary intake during the periconceptional period, women answered a 107-item, modified version of the Health Habits and History Questionnaire, a well-known, semi-quantitative food frequency questionnaire (FFQ) with demonstrated reliability and validity [Block et al., 1986; Block et al., 1990]. Participants reported their usual frequency and serving size for each food item consumed, and they answered several questions about food preparation techniques (e.g., type of fat usually used in preparing foods). The FFQ also included an open-ended question which gave women the opportunity to report any additional foods they consumed at least once per week that were not included in the main body of the FFQ. The FFQ was modified to include ethnic foods appropriate to the diverse study population, especially Hispanics; a version with similar modifications demonstrated good validity and reliability, particularly among Hispanics [Mayer-Davis et al., 1999]. Analytic software developed for the survey instrument (Dietsys) was used to compute average daily dietary intake of single nutrients. The nutrient database accounts for changes in folic acid content of foods subsequent to fortification. The approach for adding glycemic index values to the database has been described previously [Shaw et al., 2003].

In total, 73% of eligible mothers of anencephaly cases (146/200), 79% of spina bifida mothers (191/241), and 80% of control mothers (626/786) were interviewed. Ten percent of eligible case mothers and 10% of control mothers declined interview, and the remainder of non-participants were not locatable. Median time between estimated date of delivery and interview completion was 10 months for cases and 8 months for controls. Cases (n=7) and controls (n=1) with mothers who had type I or II diabetes were excluded from analyses, given potential etiologic differences, leaving 141 anencephaly cases, 189 spina bifida cases and 625 controls for analyses.

Maximum likelihood estimates of odds ratios (ORs) and their corresponding 95 percent confidence intervals (CI) were calculated from logistic regression models to estimate relative risks associated with periconceptional intake of folic acid-containing vitamin supplements. Associations with vitamin supplements were also examined stratified by quartile of dietary folate intake. For analyses of dietary nutrients, we categorized intakes as <25th percentile, 25th–<75th percentile (reference), and ≥75th percentile based on their distributions among controls. In addition to comparing ORs across these categories, we conducted a linear test for trend for each nutrient, by specifying each nutrient as a continuous variable. Analyses involving maternal glycemic index and intake of fructose, glucose, galactose, sucrose, methionine, choline, betaine, lutein, and lycopene were conducted among all subjects together. Analyses of maternal intakes of folate (dietary folate equivalents), riboflavin, thiamin, vitamin B_6, vitamin B_{12}, zinc, vitamin C, vitamin E, vitamin A, β-carotene, and iron were restricted to the stratum of women who did not use vitamin supplements in the periconceptional period, because these nutrients are likely contained in multivitamin supplements, especially prenatal formulations. We also analyzed choline and lutein restricted to this sub-group, given that they were more likely to be included in newer formulations of the supplements during the study period. *A priori*, we decided to also conduct analyses of the glycemic index stratified by maternal obesity, because previous findings suggested a stronger association of NTDs and the glycemic index among obese women [Shaw et al., 2003]. Analyses were performed separately for anencephaly and spina bifida.

Analyses were adjusted for maternal race/ethnicity (foreign-born Hispanic, U.S.-born Hispanic, non-Hispanic white, other), age (<25; 25–29; 30–34; and >34 years), education (<high school, high school graduate, some college, college graduate or more), gravidity (0,1,2, and >2), periconceptional cigarette smoking (yes or no), periconceptional alcohol use (yes or no), energy intake (kilocalories), and pre-pregnancy body mass index (kg/m^2). The covariates were selected *a priori* based on their associations with nutrient intake or NTDs [Wasserman et al., 1998; Rasmussen et al., 2008; Grewal et al., 2008].

RESULTS

Compared to control mothers, case mothers were somewhat more likely to be foreign-born Hispanic; less likely to be U.S.-born Hispanic, to have attended college, and to smoke; and they had a higher mean body mass index (Table 1).

Mother's intake of vitamin supplements during the periconceptional period resulted in ORs of 0.7 (95% CI 0.5, 1.0) for anencephaly and 0.8 (95% CI 0.6, 1.1) for spina bifida (Table 2). After adjustment for covariates, the ORs were almost identical, but the CIs increased somewhat, showing a small decrease in precision. Further division of subjects based on whether they started taking supplements before or during the first or second month of pregnancy did not substantially change the level of association (data not shown). We also examined the effects of supplement intake stratified by maternal dietary folate intake (Table 2). For anencephaly, ORs for supplement intake were similar regardless of dietary folate

intake, while for spina bifida, ORs were slightly more protective among women with lower dietary folate intake, but all confidence intervals included one.

For dietary intake of nutrients that were not typically contained in multivitamin supplements, we observed substantially elevated or reduced risks (i.e., odds ratios ≥1.7 or ≤0.6) for the following groups (Table 3). For anencephaly, we observed reduced risk with high glycemic index and increased risk with low intake of fructose and glucose. The confidence intervals for these noted risk estimates excluded one or had a lower limit of one.

For dietary intake of nutrients that were typically contained in supplements, which were analyzed among women who did not take supplements periconceptionally, many of the ORs were substantially elevated or reduced (Table 4). For anencephaly, we observed increased risk with low intake of riboflavin, vitamin B_{12}, and vitamin C; reduced risk with high intake of folate, vitamin C, vitamin E, vitamin A, and β-carotene; and increased risk with high intake of thiamin, zinc, and iron. For spina bifida, we observed increased risk with low intake of folate, riboflavin, thiamin, vitamin B_6, vitamin C, and vitamin A; and increased risk with high intake of thiamin, zinc, and iron. The confidence intervals excluded one only for a few of these noted risks.

The linear test for trend was significant ($p<0.05$) for the following associations: reduced risk of anencephaly was associated with increasing intake of glucose ($p=0.037$), vitamin C ($p=0.007$) and β-carotene ($p=0.038$); and reduced risk of spina bifida was associated with increasing intake of vitamin A ($p=0.021$) and β-carotene ($p=0.026$) (data not shown).

For dietary folate intake, we also examined more extreme intake cut-offs, relative to intake from the 25^{th}–$<75^{th}$ percentile. ORs for intake $<10^{th}$ percentile were 1.8 (95% CI 0.5, 7.2) for anencephaly and 2.3 (0.6, 8.3) for spina bifida. ORs for intake $\geq 90^{th}$ percentile were 0.6 (0.1, 2.7) for anencephaly and 0.6 (0.2, 2.0) for spina bifida.

For dietary choline and lutein, we also examined intake among women who did not take supplements (data not shown). The ORs for choline intake $<25^{th}$ percentile and $\geq 75^{th}$ percentile, relative to intake from the 25^{th}–$<75^{th}$ percentiles, were 1.3 (95% CI 0.5, 3.5) and 1.1 (0.4, 3.0), respectively, for anencephaly, and 1.3 (0.5, 3.1) and 0.5 (0.2, 1.3) for spina bifida. The respective ORs for lutein were 1.3 (0.6, 2.8) and 0.3 (0.1, 0.9) for anencephaly and 1.6 (0.8, 3.3) and 0.8 (0.3, 1.7) for spina bifida. The linear test for trend was significant ($p<0.05$) for lutein and anencephaly ($p=0.026$).

Results for glycemic index were further examined among non-obese and obese women (Table 5). Among non-obese women, increasing glycemic index was protective. Among obese women, low and high intakes were associated with increased risk of anencephaly, whereas increasing intake was associated with increased risk of spina bifida, but only one of the associations was significant.

DISCUSSION

This study of California births, conceived after folic acid fortification of the food supply, found that periconceptional intake of folic acid-containing supplements was only modestly if at all protective against NTDs. After adjustment for potential confounders, ORs for any versus no intake were 0.8 for anencephaly and for spina bifida, and confidence intervals included one. Our findings for supplements are in contrast with many previous studies, which have established a stronger protective effect of folic acid against NTDs [MRC Vitamin Study Research Group, 1991; Berry et al., 1999; Milunsky et al., 1989; Shaw et al., 1995; Werler et al., 1993; Czeizel and Dudas, 1992]. The previous studies were conducted before fortification. However, in another recent study of post-fortification births, which used

data from the National Birth Defects Prevention Study (a case-control study being conducted in ten states in the U.S.), Mosley et al. did not observe a protective association of supplement intake around the time of conception against NTDs [Mosley et al., 2009]. In fact, that study actually suggested that periconceptional supplement intake may be associated with increased risks of anencephaly and spina bifida. As for dietary folate intake, as expected, we found that lower intake was associated with increased risk of anencephaly and spina bifida among women who did not take supplements, although the result for anencephaly was not significant. The Mosley et al. study also found that low dietary folate intake (i.e., below the median) tended to be associated with increased anencephaly risk, regardless of supplement intake [Mosley et al., 2009]. Their results were less clear for spina bifida. The difference in results could stem at least in part from their use of a less detailed food frequency questionnaire (a shortened version of the Willett instrument).

One explanation offered for the lack of a protective effect of supplemental folic acid in the Mosley et al. study was a “ceiling effect” [Mosley et al., 2009; Mills and Carter, 2009]. That is, the increase in folate intake subsequent to fortification may have resulted in the prevention of most folate-preventable NTDs, and the etiology of the remaining NTDs is therefore different. This interpretation may also explain a reduced or no effect, as observed in our data. Most women who take supplements periconceptionally take prenatal formulations, which in addition to folic acid contain a variety of other nutrients that have been suggested to be protective against NTDs, possibly independently of folic acid [Velie et al., 1999; Ray et al., 2007; Ray and Blom, 2003; Smithells et al., 1976; Schorah et al., 1983]. The extent to which NTD prevalence has declined – if at all – in our study population subsequent to fortification is unknown. In the Central Valley of California, we did not observe a decline in the prevalence of NTDs subsequent to fortification [Chen et al., 2008]. The current study was conducted in a different region of California, and it is unknown whether a parallel trend occurred there.

Results for nutrients from the glycemic control pathway were largely not in the expected direction. We observed that a high glycemic index was associated with a reduced risk of anencephaly, low intake of fructose and glucose were associated with increased risk of anencephaly, and these nutritional factors were not associated with spina bifida. Results limited to obese women suggested that high glycemic index may be associated with increased NTD risk, but ORs were imprecise. Results for the other nutrients from this pathway were similar among obese and non-obese women (data not shown). In a previous study of California births that occurred from 1989–1991 in counties throughout California, we observed that high glycemic index was associated with increased risk among all women, with particularly strong results among obese women [Shaw et al., 2003]. A subsequent analysis that used more recent data from the National Birth Defects Prevention Study but a more abbreviated food frequency questionnaire did not confirm those findings [Shaw et al., 2008], whereas another recent study did observe an association {Yazdy, 2010 2523/id}. The biologic plausibility for an association of glycemic index with NTDs is strong, given that NTDs in human offspring have been associated with various indicators of potentially aberrant glucose control [Aberg et al., 2001; Anderson et al., 2005; Hendricks et al., 2001; Groenen et al., 2003; Shaw et al., 1996; Shaw et al., 2000; Werler et al., 1996], and intakes of foods with higher glycemic index values are predictive of elevated serum glucose concentrations [Salmeron et al., 1997]. As for mono- and disaccharides, our finding was opposite the expected direction and previous findings {Groenen, 2004 1963/id}. The explanation for the variability in findings is uncertain. Again, one distinguishing factor is that our earlier study of California births was conducted among pre-fortification births, and the subsequent studies have been conducted among post-fortification births. Measurement error is also a potential explanatory factor, although the current study used a similar food frequency instrument as our earlier California study. Other factors that vary across the noted

studies and could contribute to differences in findings include the level of detail regarding dietary intake, time period of recall, and approach to control selection.

We also examined nutrients that contribute to one-carbon metabolism. Results tended to be in the expected direction for several of these nutrients – folate, riboflavin, and vitamins B_6 and B_{12}. However, our previous results suggesting protective effects of choline, methionine and zinc were not replicated [Shaw et al., 1997; Shaw et al., 2004; Velie et al., 1999]. Further examination of choline among women who did not take supplements, however, did suggest reduced risks for spina bifida.

Preventive effects of anti-oxidant nutrients such as vitamins C and E against NTDs were first observed many years ago [Verma and Wei, 1967; Smithells et al., 1976; Schorah et al., 1983], and more recent experimental studies support a protective role of anti-oxidant nutrients against NTDs [Wentzel et al., 2005; Li et al., 2005; Loeken, 2004]. Folic acid is also an anti-oxidant [Nakano et al., 2001; Rosenquist et al., 1996; Joshi et al., 2001; Ho et al., 2003]. Given these observations, it has been recommended that this pathway should be studied in detail in human studies [Loeken, 2004]. In the current study, anti-oxidant nutrients tended to be protective against NTDs, among women who did not take supplements. In particular, the association of lutein was modest overall, but stronger when restricted to women who did not take supplements. The current study also suggested that high dietary intake of iron, which is a pro-oxidant, was associated with increased NTD risk. Our previous study of California births suggested that lutein, but not other nutrients from this pathway, was associated with NTD risk [Carmichael et al., 2009].

Strengths of the current study include its comprehensive case ascertainment, thorough phenotypic review, population-based control selection, and detailed food frequency questionnaire. However, the study also had limitations, and alternative explanations for our results for folic acid as well as for other nutrients must therefore be considered. The current study relied on self-reported data; the extent of erroneous reporting, and whether the errors varied among mothers of cases and controls, is unknown. We expect that such errors or bias may also apply – albeit to varying degrees – to most previous studies as well. Dietary nutrient intakes in this study, as well as in previous studies of NTDs and diet, were based on non-calibrated food frequency questionnaire data, which allows ranking of intake but does not reveal actual intake levels. Therefore, although we expect that dietary folate intake increased at the population level after fortification, we cannot compare quantitative cut-offs across various studies. In addition, we do not know actual serum levels of the studied nutrients, which would be a very useful although difficult to obtain adjunct to studies of dietary intake. Although we were unable to conduct a validation study of our exact dietary questionnaire, the parent instrument has demonstrated good reliability and validity [Mayer-Davis et al., 1999], and we expect that our addition of ethnic-specific foods would further enhance rather than detract from its performance. Given the retrospective study design, we were unable to measure serum correlates of nutritional status during organogenesis. The number of cases in the current study limited our ability to rule out chance as an alternative explanation especially for weak to moderate associations and to explore effect modification of the observed associations with nutritional factors, for example by race-ethnicity and education. The study was restricted to selected California counties, and the majority of study subjects were Hispanic; as such, the generalizability of our results is uncertain. Previous studies in California and Texas suggested that supplemental folic acid and dietary folate may be less protective or not protective among Hispanic women [Shaw et al., 1995; Suarez et al., 2000], but the recent study by Mosley et al. showed similar results (of no association) for supplement intake among Hispanic and non-Hispanic white women [Mosley et al., 2009]. Small numbers in the current study limited our ability to investigate differences within these

groups of women. Some of our results appeared to be different for anencephaly versus spina bifida, while others were similar; the explanation for this variability is unknown.

The current study adds to knowledge about the association of periconceptional nutritional status with NTDs among offspring, during a time period of folic acid fortification when the epidemiology of NTDs may be changing. It is possible, albeit speculative, that increased folate intake subsequent to fortification may have resulted in the prevention of most folate-preventable NTDs, and the etiologies of those NTDs occurring in the population are different. Folic acid food fortification may have as yet undetermined effects on the association of intake of folic acid and other nutrients with NTD risk. However, given the complexity of NTD etiology and nutritional status, and various limitations of the current literature, further studies are needed to determine the current contribution of folic acid and other nutrients to NTD prevention, using varied approaches to nutritional assessment.

Acknowledgments

This research was supported by NIH R01 NS050249 and by the Centers for Disease Control and Prevention, Center of Excellence Award 1U01DD000489.

We thank the California Department of Public Health Maternal Child and Adolescent Health Division for providing data for these analyses.

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Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2011 August 1.

Table 1

Characteristics of mothers of 141 cases of anencephaly, 189 cases of spina bifida, and 625 non-malformed controls, California 1999–2003.

	Anencephaly, n=141		Spina Bifida, n=189		Controls, n=625
Maternal characteristics	**n (Percent)[1]**	**p-value[2]**	**n (Percent)[1]**	**p-value[2]**	**n (Percent)[1]**
Race/Ethnicity					
Non-Hispanic white	29 (21)		44 (23)		133 (21)
US-born Hispanic	26 (18)		30 (16)		134 (21)
Foreign-born Hispanic	65 (46)		96 (51)		235 (38)
Other	21 (15)	0.87	17 (9)	0.35	115 (18)
Age (years)					
<25	46 (33)		53 (28)		187 (30)
25–29	32 (23)		49 (26)		141 (23)
30–34	38 (27)		50 (26)		180 (29)
>34	25 (18)	0.57	37 (20)	0.82	114 (18)
Education					
<High School	59 (42)		77 (41)		177 (28)
High School	30 (21)		33 (17)		149 (24)
Some College	25 (18)		40 (21)		135 (22)
4-Year College Degree or more	27 (19)	0.21	37 (20)	0.64	153 (24)
Gravidity					
0	37 (26)		42 (22)		183 (29)
1	39 (28)		44 (23)		171 (27)
2	17 (12)	0.14	52 (28)	0.02	121 (19)
>2	48 (34)		51 (27)		149 (24)
Cigarette Use					
No	138 (98)		180 (95)		570 (91)
Yes	3 (2)	0.01	8 (4)	0.10	49 (8)
Alcohol Use					
No	114 (81)		150 (79)		511 (82)
Yes	26 (18)	0.12	38 (20)	0.39	107 (17)
Body Mass Index (kg/m^2)					

	Anencephaly, n=141		Spina Bifida, n=189		Controls, n=625
Maternal characteristics	**n (Percent)[1]**	**p-value[2]**	**n (Percent)[1]**	**p-value[2]**	**n (Percent)[1]**
<19.8	16 (11)		12 (6)		96 (15)
19.8–26.0	64 (45)		97 (51)		316 (51)
26.1–29.0	18 (13)		24 (13)		71 (11)
>29.0	29 (21)	0.07	34 (18)	0.03	101 (16)
Family history of NTD in a first degree relative					
Yes	2 (1)		2 (1)		1 (0.1)
No	139 (99)	0.08	187 (99)	0.14	624 (99.9)
			Mean (SD)		
Daily Energy Intake (Kcal)	2655 (947)	0.69	2685 (1019)	0.94	2692 (956)

[1] Percentages may not equal 100 owing to missing data or rounding.

[2] The p-values are from comparisons of distributions or means among spina bifida or anencephaly cases versus the controls.

Table 2

Association of anencephaly and spina bifida with maternal intake of folic acid-containing vitamin supplements during the periconceptional period, overall and stratified by dietary intake of folate.[1]

	Controls	Cases	Unadjusted Odds Ratio (95%CI)	Controls	Cases	Adjusted Odds Ratio (95%CI)[2]
Anencephaly						
No supplements	238	68	Reference	214	56	Reference
Any supplements	385	73	0.7 (0.5–1.0)	354	71	0.8 (0.5–1.3)
Dietary folate <25th percentile						
No supplements	54	19	Reference	51	15	Reference
Any supplements	91	17	0.5 (0.3–1.1)	85	17	0.8 (0.3–2.0)
Dietary folate 25th–<75th percentile						
No supplements	111	30	Reference	101	23	Reference
Any supplements	180	35	0.7 (0.4–1.2)	167	33	1.0 (0.5–2.0)
Dietary folate ≥75th percentile						
No supplements	57	14	Reference	48	13	Reference
Any supplements	89	19	0.9 (0.4–1.9)	82	19	0.9 (0.4–2.4)
Spina Bifida						
No supplements	238	82	Reference	214	71	Reference
Any supplements	385	107	0.8 (0.6–1.1)	354	95	0.8 (0.6–1.2)
Dietary folate <25th percentile						
No supplements	54	25	Reference	51	21	Reference
Any supplements	91	23	0.5 (0.3–1.1)	85	20	0.6 (0.2–1.3)
Dietary folate 25th–<75th percentile						
No supplements	111	36	Reference	101	30	Reference
Any supplements	180	52	0.9 (0.5–1.4)	167	46	0.8 (0.4–1.5)
Dietary folate ≥75th percentile						
No supplements	57	17	Reference	48	16	Reference
Any supplements	89	28	1.1 (0.5–2.1)	82	26	1.2 (0.5–2.8)

[1] Periconceptional period was defined as the two months before through the two months after conception.

[2] Odds ratios were adjusted for race/ethnicity, age, education, body mass index, gravidity, smoking, and alcohol use.

Table 3

Association of anencephaly and spina bifida with maternal dietary intake of compounds in the glycemic pathway and in the one-carbon metabolism pathway, California, 1999–2003.

		Anencephaly			Spina Bifida		
Nutrient Categories[1]	**Controls**	**Cases**	**Odds Ratio[2]**	**95% CI**	**Cases**	**Odds Ratio[2]**	**95% CI**
Glycemic control pathway							
Glycemic Index							
< 49.4	130	31	0.9	0.5–1.4	37	0.8	0.5–1.3
49.4–53.1	264	69	Reference		83	Reference	
≥ 53.2	140	20	0.6	0.3–1.0	39	0.9	0.6–1.4
Fructose (g)							
< 22.6	138	45	2.2	1.3–3.7	46	1.4	0.9–2.3
22.6–48.1	272	49	Reference		73	Reference	
≥ 48.2	124	26	1.0	0.6–1.8	40	1.2	0.7–2.0
Glucose (g)							
< 19.2	137	46	2.2	1.3–3.6	40	1.1	0.7–1.8
19.2–40.6	272	52	Reference		80	Reference	
≥ 40.7	125	22	0.7	0.4–1.4	39	1.1	0.7–1.9
Galactose (g)							
< 0.3	130	31	1.0	0.6–1.7	33	0.8	0.5–1.3
0.3–0.9	274	55	Reference		84	Reference	
≥ 1.0	130	34	1.3	0.8–2.2	42	1.0	0.7–1.7
Sucrose (g)							
< 26.9	136	34	1.2	0.7–2.1	41	1.2	0.7–1.9
26.9–51.6	271	57	Reference		75	Reference	
≥ 51.7	127	29	1.1	0.6–1.9	43	1.2	0.7–1.9
One-carbon metabolism pathway							
Methionine (mg)							
< 1534.1	135	29	0.8	0.4–1.5	37	0.9	0.5–1.5
1534.1–2690.5	268	62	Reference		78	Reference	
≥ 2690.6	131	29	1.0	0.5–2.0	44	1.4	0.8–2.6
Choline (mg)							

		Anencephaly			Spina Bifida		
Nutrient Categories[1]	**Controls**	**Cases**	**Odds Ratio[2]**	**95% CI**	**Cases**	**Odds Ratio[2]**	**95% CI**
< 293.0	133	32	1.1	0.6–2.0	40	1.2	0.7–2.1
293.0–505.7	269	58	Reference		84	Reference	
≥ 505.8	132	30	1.1	0.6–2.0	35	0.7	0.4–1.3
Betaine (mg)							
< 88.7	128	27	0.7	0.4–1.2	36	0.8	0.5–1.4
88.7–193.5	270	63	Reference		89	Reference	
≥ 193.6	136	30	1.0	0.6–1.8	34	0.7	0.4–1.2
Oxidative stress pathway							
Lutein (μg)							
< 1231.3	127	35	1.1	0.7–1.9	47	1.2	0.8–1.9
1231.3–5211.2	269	62	Reference		80	Reference	
≥ 5211.3	138	23	0.8	0.4–1.3	32	0.8	0.5–1.2
Lycopene (μg)							
< 2398.6	135	26	0.7	0.4–1.2	40	1.2	0.7–1.9
2398.6–7064.4	267	68	Reference		79	Reference	
≥ 7064.5	132	26	0.8	0.4–1.3	40	1.0	0.6–1.6

[1] Nutrient categories reflect <25th percentile, 25th–<75th percentile (reference), and ≥75th percentile, determined from intake levels among control mothers.

[2] Odds ratios were adjusted for maternal periconceptional intake of folic acid-containing supplements, energy intake, race/ethnicity, age, education, body mass index, gravidity, smoking, and alcohol use.

Table 4

Association of anencephaly and spina bifida with intake of selected dietary nutrients in the one-carbon metabolism pathwy and the oxidative stress pathwy, among women who did not use vitamin supplements, California 1999–2003.

		Anencephaly			Spina Bifida		
Nutrient Categories[1]	**Controls**	**Cases**	**Odds Ratio[2]**	**95% CI**	**Cases**	**Odds Ratio[2]**	**95% CI**
One-carbon metabolism pathway							
Folate (μg)							
< 293.8	51	15	1.5	0.6–3.8	21	2.4	1.1–5.4
293.8–546.3	101	23	Reference		30	Reference	
≥ 546.4	48	13	0.6	0.2–1.7	16	0.8	0.3–1.8
Riboflavin (mg)							
< 1.8	45	15	2.0	0.7–5.3	23	3.8	1.6–9.1
1.8–3.2	102	21	Reference		26	Reference	
≥ 3.3	53	15	1.2	0.5–3.2	18	0.8	0.3–1.9
Thiamin (mg)							
< 1.4	53	15	1.1	0.4–3.0	21	1.8	0.7–4.1
1.4–2.3	105	22	Reference		27	Reference	
≥ 2.4	42	14	2.0	0.7–5.7	19	1.9	0.7–4.9
Vitamin B_6 (mg)							
< 1.8	47	15	1.6	0.6–4.3	18	1.7	0.7–4.0
1.8–3.1	105	22	Reference		30	Reference	
≥ 3.2	48	14	1.2	0.5–3.2	19	1.3	0.5–3.0
Vitamin B_{12} (μg)							
< 3.6	39	15	1.7	0.7–4.5	14	1.4	0.6–3.2
3.6–7.0	110	23	Reference		37	Reference	
≥ 7.1	51	13	1.0	0.4–2.5	16	0.8	0.3–1.8
Zinc (mg)							
< 10.3	44	12	0.9	0.3–2.6	16	1.3	0.6–3.2
10.3–18.5	109	20	Reference		31	Reference	
≥ 18.6	47	19	4.0	1.5–10.8	20	1.7	0.7–4.0
Oxidative stress pathway							

		Anencephaly			Spina Bifida		
Nutrient Categories[1]	**Controls**	**Cases**	**Odds Ratio[2]**	**95% CI**	**Cases**	**Odds Ratio[2]**	**95% CI**
Vitamin C (mg)							
< 138.9	47	19	3.2	1.3–7.7	18	1.7	0.8–3.7
138.9–289.8	101	23	Reference		31	Reference	
≥ 289.9	52	9	0.6	0.2–1.5	18	1.0	0.5–2.0
Vitamin E (mg)							
< 11.7	39	11	1.4	0.5–3.7	15	1.6	0.7–3.5
11.7–20.9	107	29	Reference		32	Reference	
≥ 21.0	54	11	0.5	0.2–1.2	20	1.1	0.5–2.6
Vitamin A (RE)							
< 1040.1	48	13	1.3	0.5–3.2	21	2.2	1.0–4.9
1040.1–2134.2	105	28	Reference		32	Reference	
≥ 2134.3	47	10	0.5	0.2–1.3	14	0.7	0.3–1.6
β-Carotene (μg)							
< 2844.5	50	13	0.7	0.3–1.7	21	1.5	0.7–3.0
2844.5–7345.2	102	33	Reference		34	Reference	
7345.3	48	5	0.2	0.1–0.8	12	0.7	0.3–1.6
Iron (mg)							
< 13.0	51	14	1.0	0.3–2.8	18	1.1	0.5–2.7
13.0–22.5	108	22	Reference		29	Reference	
≥ 22.6	41	15	2.5	0.8–7.6	20	2.4	0.9–6.1

[1] Nutrient categories reflect <25th percentile, 25th–<75th percentile (reference), and ≥75th percentile, determined from intake levels among control mothers.

[2] Odds ratios were adjusted for maternal energy intake, race/ethnicity, age, education, body mass index, gravidity, smoking, and alcohol use.

Table 5

Association of anencephaly and spina bifida with glycemic index, among non-obese and obese women and among women who did not take vitamin supplements, California 1999–2003.[1]

		Anencephaly		Spina Bifida	
Glycemic Index Categories[2]	**Controls**	**Cases**	**Odds Ratio (95% CI)[3]**	**Cases**	**Odds Ratio (95% CI)[3]**
Non-Obese					
< 49.4	106	24	0.8 (0.5–1.5)	34	0.9 (0.5–1.4)
49.4–53.1	218	56	Reference	71	Reference
≥ 53.2	118	13	0.5 (0.2–0.9)	25	0.7 (0.4–1.1)
Obese					
< 49.4	24	7	1.6 (0.4–6.6)	3	0.6 (0.1–2.9)
49.4–53.1	46	13	Reference	12	Reference
≥ 53.2	22	7	2.0 (0.5–7.4)	14	2.6 (0.8–8.1)

[1] Obesity was defined as BMI > 29 kg/m^2.

[2] Nutrient categories reflect <25th percentile, 25th–<75th percentile (reference), and ≥75th percentile, determined from intake levels among control mothers.

[3] Odds ratios were adjusted for maternal periconceptional intake of folic acid-containing supplements, energy intake, race/ethnicity, age, education, gravidity, smoking, and alcohol use.