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Original Contribution

Neural Tube Defects and Maternal Folate Intake Among Pregnancies Conceived After Folic Acid Fortification in the United States

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Rates of neural tube defects have decreased since folic acid fortification of the food supply in the United States. The authors' objective was to evaluate the associations between neural tube defects and maternal folic acid intake among pregnancies conceived after fortification. This is a multicenter, case-control study that uses data from the National Birth Defects Prevention Study, 1998–2003. Logistic regression was used to compute crude and adjusted odds ratios between cases and controls assessing maternal periconceptional use of folic acid and intake of dietary folic acid. Among 180 anencephalic cases, 385 spina bifida cases, and 3, 963 controls, 21.1%, 25.2%, and 26.1%, respectively, reported periconceptional use of folic acid supplements. Periconceptional supplement use did not reduce the risk of having a pregnancy affected by a neural tube defect. Maternal intake of dietary folate was not significantly associated with neural tube defects. In this study conducted among pregnancies conceived after mandatory folic acid fortification, the authors found little evidence of an association between neural tube defects and maternal folic acid intake. A possible explanation is that folic acid fortification reduced the occurrence of folic acid-sensitive neural tube defects. Further investigation is warranted to possibly identify women who remain at increased risk of preventable neural tube defects.

folic acid; neural tube defects

Abbreviations: B3, 3 months before pregnancy; CI, confidence interval; DFE, dietary folate equivalent; OR, odds ratio; P1, first month of pregnancy.

Editor's note: *An invited commentary on this article appears on page 18, and the authors' response is published on page 22.*

After 3 decades of epidemiologic research reporting an association between neural tube defects and maternal use of folic acid (1–10), public health organizations developed recommendations and supported interventions to increase folic acid intake among women of reproductive age. In 1992, the US Public Health Service recommended that all women of childbearing age who are capable of becoming pregnant should consume 400 μg of folic acid daily (11).

In 1999, the March of Dimes, Centers for Disease Control and Prevention, and National Council on Folic Acid launched the National Folic Acid Educational Campaign. The US Food and Drug Administration had mandated that all enriched cereals and grains contain 140 μg of folic acid per 100 g of grain by January 1998 (12). In 2005, after the National Campaign and mandatory fortification, approximately 33% of women reported taking a daily supplement of folic acid (13), only a modest increase from the 25% reported in 1995 (14). However, median blood folate levels among women of childbearing age increased from 4.8 to 13.0 ng/mL between 1994 and 2000 (15), with a more recent study (16) reporting median blood folate levels at least 2 times the levels prior to fortification.

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To evaluate the impact of this public health intervention, 4 study groups have conducted time trend analyses among the US population, and all have reported a decline of neural tube defects after the introduction of mandatory folic acid fortification (17–20). Specifically, these studies reported an 11%–20% reduction in occurrence of anencephaly and a 21%–34% reduction in occurrence of spina bifida when comparing pre- versus postfortification rates. Similarly, the occurrence of anencephaly and spina bifida was observed to reduce 38% and 53%, respectively, in Canada (21) and 46% and 51%, respectively, in Chile (22) following folic acid fortification.

The objective of this study was to use data from the National Birth Defects Prevention Study to evaluate the relation between neural tube defects and maternal folic acid consumption among US women conceiving pregnancies after folic acid fortification was in place.

MATERIALS AND METHODS

The National Birth Defects Prevention Study is the largest, ongoing, birth defects case-control study in the United States. This study began in 1997 and ascertains participants from 10 population-based birth defects surveillance systems (23). With a relatively large data pool currently available on participants diagnosed with a neural tube defect and controls unaffected by a birth defect, the National Birth Defects Prevention Study offers a unique opportunity to further investigate the effects of folate following mandatory food fortification.

Study population

Case and control women were participants in the National Birth Defects Prevention Study from 1 of 10 center sites (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, or Utah). A detailed description of study methods has been published elsewhere (23). Case women had a pregnancy affected by anencephaly or spina bifida that did not result from a single gene or chromosomal abnormality. Diagnoses abstracted from medical records of fetuses or infants were confirmed by clinical dysmorphology after review of clinical descriptions and surgical/autopsy reports. Controls were a random sample of women from each center site who delivered a live-born infant without a structural birth defect. All pregnancies included in this analysis were conceived on or after July 1, 1998, 6 months following the January 1998 implementation of mandatory folic acid fortification, and included births occurring through December 2003. Cases and controls completed a structured maternal telephone interview in English or Spanish. Maternal interviews were conducted from 6 weeks to 24 months after each participant's expected date of delivery. Participation rates for maternal interviews have been reported at 62%, 76%, and 71% for anencephaly, spina bifida, and controls, respectively (National Birth Defects Prevention Study, unpublished report, 2007).

Women who had preexisting type 1 or type 2 diabetes (10 cases, 18 controls) or who reported periconceptional use of any folate antagonist medication (4 cases, 7 controls), including dilantin, valproic acid, sodium valproate, carbamazepine, methotrexate, and trimethoprim (both the hydrochloride and sulfate types), were excluded. Participants whose pregnancies resulted in multiple births (39 cases, 126 controls) and who had incomplete food frequency questionnaires (4 cases, 27 controls) or supplement use information (22 cases, 84 controls) were also excluded from the analysis. Of the 643 cases and 3,952 controls with postfortification conceptions and who participated in the study, 565 cases and 3,691 controls met all these criteria.

Folic acid supplement use and dietary intakes

Use of a multivitamin, prenatal vitamin, or single-component vitamin was reported by participants and measured in monthly units from 3 months before pregnancy through the last month of pregnancy. National Birth Defects Prevention Study investigators classified whether the specific supplement reported by each participant contained folic acid or not. Some women reported that they began to take multivitamins or prenatal vitamins during their first month of pregnancy. To better distinguish those who were exposed to folic acid supplements during the development and closure of the neural tube (within 28 days postconception), we classified supplement use into 3 categories. Women who reported consistently taking any supplement containing folic acid from 3 months before pregnancy (B3) through the first month of pregnancy (P1) were defined as ''supplement users, B3–P1''; those who reported initiation of folic acid supplements during the first month of pregnancy were ''P1 supplement initiators''; those who reported no supplement use from 3 months before pregnancy through the first month of pregnancy were defined as ''nonusers of supplements, B3–P1.'' Consistent use was defined as taking supplements at least half the number of days ($\geq$60 days) within the B3–P1 exposure period. Nonusers of supplements were the referent group for assessing the effect of supplement use on neural tube defect risk.

Maternal dietary intake during the year before pregnancy was based on completion of a modified Willett Food Frequency Questionnaire (58 food items) administered during the interview (24). Intake of breakfast cereals 12 weeks before conception was determined by additional questions. Version 19 of the USDA [US Department of Agriculture] National Nutrient Database was used to compute daily intakes of micronutrient values (25). Nutritional intake from food supplements, for example, nutritional drinks and power bars, was excluded from these analyses. Dietary folate includes naturally occurring folates (natural folate found in foods) and dietary folic acid (synthetic folic acid fortified in foods). The bioavailability of ingested natural folate, found primarily in vegetables and dried legumes, has been estimated at 50% of the folate amount consumed, while that of synthetic folic acid fortified in cereal and grains is 85% of that consumed (26). Total folate intake in the diet is expressed as dietary folate equivalents (DFEs), which account for the varying bioavailability of folates by multiplying the amount of dietary folic acid in fortified foods by 1.7 and then adding the amount of natural folate in foods (27).

The intake of dietary folates was evaluated by using measures for folic acid, natural folate, and total folate expressed as the DFE with primary focus on dietary folic acid as used in fortification. The 10th, 30th, and 50th percentile cutpoints for each of these measures were established from the distribution among controls. To determine the most appropriate cutpoints, we evaluated these data by using spline analysis to determine if any alternative cutpoint would better identify intake differences between cases and controls (28). No such cutpoints were identified; thus, we proceeded with the above percentile levels. Each participant was classified into 1 of 4 progressive categories of dietary folate intake for each of the 3 folate measures. Those consuming an amount greater than the 50th percentile were considered the referent group in our assessment of dietary folate intake and neural tube defect risk.

Statistical analysis

Bivariate comparisons of maternal demographic and behavioral characteristics among neural tube defect cases and controls were performed by using chi-square tests. For dietary folate variables, comparisons of log-transformed means between cases and controls were conducted by using Student *t* tests. Unconditional logistic regression was used to compute crude and adjusted odds ratios and 95% confidence intervals for the assessment of the association between neural tube defects and supplement use and dietary folate intake.

Potential covariates considered in the multivariable models included maternal race, age, education, household income, body mass index, periconceptional smoking, alcohol use, pregnancy intention, time to interview, and center site. Covariates remained in the final adjusted model when a 10% or greater effect was observed in the results. Interaction was assessed by using the likelihood ratio test. Results were reported stratified by specific phenotype, anencephaly and spina bifida. All data were analyzed by using SAS, version 9.1, software (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Characteristics of participants

Among the 565 infants or fetuses delivered by case women, 385 had spina bifida, 177 had anencephaly, and 3 had both defects. These 3 cases with both defects were analyzed within the anencephaly defect group. Pregnancy outcomes among anencephaly cases were 49 (27%) livebirths, 84 (47%) terminations, and 47 (26%) fetal deaths. Pregnancy outcomes among spina bifida cases were 337 (88%) livebirths, 39 (10%) terminations, and 9 (2%) fetal deaths. All 3,691 controls were delivered as liveborn infants. Maternal interviews were completed within 1 year of the expected date of delivery for 68% of anencephaly cases, 69% of spina bifida cases, and 79% of controls. All other interviews were completed within the second year of the expected date of delivery.

The frequencies of demographic and behavioral characteristics among case women and control women are shown in Table 1. Distributions of maternal race/ethnicity, education, and household income were significantly different between both anencephaly and spina bifida cases and controls ($P < 0.05$). Case women were more likely than controls to be Hispanic (35.6% vs. 23.4%, respectively), less likely to report educational levels beyond high school (49.2% vs. 57.3%), and less likely to report household incomes at or above $50,000 (24.1% vs. 33.1%). Anencephaly cases compared with controls were less likely to report smoking (12.8% vs. 18.7%) or alcohol drinking (21.7% vs. 29.7%) during the month before pregnancy. Women whose pregnancies were affected by spina bifida reported a higher proportion of body mass index in the obese range of $\geq$30 (22.1% vs. 15.3%) and were less likely to report intended pregnancies (51.2% vs. 59.3%) than control women. The distribution of maternal age was not different between cases and controls.

Folic acid supplement use

Almost half of the women in this study reported no use of a supplement containing folic acid from 3 months before pregnancy through the first month of pregnancy. Nonusers of supplements during B3–P1 composed 45.0% of anencephaly cases, 48.8% of spina bifida cases, and 48.2% of controls ($P = 0.68$). Conversely, 21.1%, 25.2%, and 26.1%, respectively, of these groups of women reported consistent use of folic acid supplement during the 3 months before pregnancy through the first month of pregnancy (B3–P1). Daily use of folic acid supplement throughout the B3–P1 period was reported by 16.7% of anencephaly cases, 22.6% of spina bifida cases, and 23.4% of controls. The remaining women in each group (33.9% of anencephaly cases, 26.0% of spina bifida cases, and 25.7% of controls) reported initiation of supplement use during the first month of pregnancy.

The overall crude odds ratio was 0.9 (95% confidence interval (CI): 0.7, 1.2) assessing use of folic acid supplements relative to no use during the B3–P1 time period. Results assessing the relation between folic acid supplement use and neural tube defect phenotype are reported in Table 2. Case women reported similar use of folic acid supplement during B3–P1 as control women. In a comparison of results from nonusers of supplements, the crude odds ratio estimating the association between supplement use and anencephaly was 0.9 (95% CI: 0.6, 1.3). A crude odds ratio of 1.0 (95% CI: 0.7, 1.2) was observed for the association between supplement use and spina bifida. After adjustment for potential confounders, odds ratios inclusive of 1.0 within the confidence intervals continued to be observed for anencephaly (adjusted odds ratio (OR) = 1.2, 95% CI: 0.8, 1.9) and spina bifida (adjusted OR = 1.4, 95% CI: 1.0, 1.8).

Among obese women (body mass index, $\geq$30), the association between supplement use in B3–P1 (vs. no use) and anencephaly was 0.5 (95% CI: 0.1, 1.7), and among nonobese women, it was 1.3 (95% CI: 0.8, 2.1). Adjusted odds ratios assessing B3–P1 supplement use and spina bifida were similar among obese (adjusted OR = 1.3, 95% CI: 0.7, 2.4) and nonobese (adjusted OR = 1.4, 95% CI: 1.0, 1.9) women.

When comparing those who initiated supplement use during the first month of pregnancy with nonusers of supplements, we found higher odds ratios for anencephaly, but not spina bifida, after adjustment for covariates (adjusted OR = 1.7, 95% CI: 1.2, 2.4).

Table 1. Maternal Demographic and Behavioral Characteristics of Neural Tube Defect Cases and Controls, National Birth Defects Prevention Study, 1998–2003

	All Neural Tube Defect Cases		Anencephaly Cases		Spina Bifida Cases		Controls	
	No.	%	No.	%	No.	%	No.	%
Total	565		180		385		3,691	
Maternal race/ethnicity	—[a]		—		—			
White, non-Hispanic	274	48.5	83	46.1	191	49.6	2,173	58.9
African American, non-Hispanic	60	10.6	18	10.0	42	10.9	431	11.7
Hispanic	201	35.6	67	37.2	134	34.8	865	23.4
Other races	29	5.1	12	6.7	17	4.4	213	5.8
Missing data	1	0.2	0		1	0.3	9	0.2
Maternal age at conception								
Median age, years	26		26		26		27	
<20 years	84	14.9	34	18.9	50	13.0	518	14.0
20–25 years	176	31.1	50	27.8	126	32.7	1,080	29.3
26–35 years	262	46.4	88	48.9	174	45.2	1,806	48.9
≥36 years	43	7.6	8	4.4	35	9.1	287	7.8
Maternal education	—		—		—			
0–11 years	117	20.7	40	22.2	77	20.0	643	17.4
High school diploma/GED	169	29.9	57	31.7	112	29.1	923	25.0
Some college/technical	155	27.4	39	21.7	116	30.1	948	25.7
Bachelor degree	100	17.7	34	18.9	66	17.1	829	22.5
Graduate degree	23	4.1	9	5.0	14	3.6	337	9.1
Missing data	1	0.2	1	0.5	0		11	0.3
Household income	—		—		—			
<$20,000	217	38.4	69	38.3	148	38.4	1,098	29.7
$20,000–49,999	169	29.9	49	27.2	120	31.2	1,151	31.2
≥$50,000	136	24.1	48	26.7	88	22.9	1,220	33.1
Missing data/refused	43	7.6	14	7.8	29	7.5	222	6.0
Maternal smoking, B1			—					
Yes, smoked	89	15.8	23	12.8	66	17.1	692	18.7
No	476	84.2	157	87.2	319	82.9	2,999	81.3
Maternal alcohol drinking, B1			—					
Yes, drank alcohol	146	25.8	39	21.7	107	27.8	1,096	29.7
No	416	73.6	141	78.3	275	71.4	2,580	69.9
Missing data	3	0.5	0		3	0.8	15	0.4
Maternal body mass index, kg/m^2	—				—			
Median values	24.4		23.3		24.8		23.5	
<18.5	23	4.1	10	5.6	13	3.4	219	5.9
18.5–<25	276	48.8	97	53.9	179	46.5	1,962	53.2
25–<30	119	21.1	36	20.0	83	21.5	788	21.3
≥30	111	19.7	26	14.4	85	22.1	563	15.3
Missing data/out of range	36	6.4	11	6.1	25	6.5	159	4.3
Intended pregnancy	—				—			
Yes, stopped contraception/ wanted to be pregnant then	300	53.1	103	57.2	197	51.2	2,189	59.3
No, all others	265	46.9	77	42.8	188	48.8	1,502	40.7

Abbreviations: B1, month before pregnancy; GED, general equivalency diploma.

[a] —, statistically different distribution from controls ($P < 0.05$). All participants with missing values were excluded from statistical comparisons.

Table 2. Association of Maternal Folic Acid Supplement Use and Neural Tube Defects, Phenotypes, Anencephaly, and Spina Bifida, National Birth Defects Prevention Study, 1998–2003

	No. of Cases	No. of Controls	Crude		Adjusted[a,b]	
			Odds Ratio	95% Confidence Interval	Odds Ratio	95% Confidence Interval
Anencephaly						
Nonusers, B3–P1	81	1,778	Referent	Referent	Referent	Referent
Users, B3–P1	38	965	0.9	0.6, 1.3	1.2	0.8, 1.9
P1 initiator	61	948	1.4	1.0, 2.0	1.7	1.2, 2.4
Spina bifida						
Nonusers, B3–P1	188	1,778	Referent	Referent	Referent	Referent
Users, B3–P1	97	965	1.0	0.7, 1.2	1.4	1.0, 1.8
P1 initiator	100	948	1.0	0.8, 1.3	1.1	0.9, 1.5

Abbreviations: B3, 3 months before pregnancy; P1, first month of pregnancy.
[a] The adjusted anencephaly model includes the covariates maternal race and education.
[b] The adjusted spina bifida model includes the covariates maternal race, body mass index, and pregnancy intent.

Race- and ethnicity-specific patterns of folic acid supplement use are reported in Table 3. Among controls, 35.6% of non-Hispanic white women compared with 63.3% of non-Hispanic black women ($P < 0.05$) and 71.3% of Hispanic women ($P < 0.05$) reported no use of supplement from 3 months before pregnancy through the first month of pregnancy. Only 7.2% of Hispanic controls and 14.9% of non-Hispanic black controls reported use of a supplement from B3 through P1, compared with 36.3% for non-Hispanic white controls.

For non-Hispanic white women, crude odds ratios for B3–P1 supplement use were 1.2 (95% CI: 0.7, 2.1) when comparing anencephaly cases and 1.3 (95% CI: 0.9, 1.9) when comparing spina bifida cases with controls. Non-Hispanic blacks had odds ratios for B3–P1 supplement use at 2.8 (95% CI: 0.8, 10.4) for anencephaly and 1.2 (95% CI: 0.5, 2.8) for spina bifida, while odds ratios among Hispanics were 0.7 (95% CI: 0.2, 2.2) for anencephaly and 0.4 (95% CI: 0.2, 1.2) for spina bifida. Supplement use–race interactions were not significant for anencephaly ($P = 0.57$) or spina bifida ($P = 0.08$). Counts among non-Hispanic black and Hispanic populations were relatively small; thus, interpretation of these data is limited.

Dietary folic acid intake

The 10th, 30th, 50th, and 90th percentile values for dietary folic acid, natural folate, and folate DFE among

Table 3. Association of Maternal Folic Acid Supplement Use and Specific Neural Tube Defects Stratified by Race/Ethnicity, National Birth Defects Prevention Study, 1998–2003

	Controls		Anencephaly				Spina Bifida			
	No.	%	No.	%	Crude Odds Ratio	95% Confidence Interval	No.	%	Crude Odds Ratio	95% Confidence Interval
White, non-Hispanic										
Nonusers, B3–P1	773	35.6	24	28.9	Referent	Referent	59	30.9	Referent	Referent
Users, B3–P1	789	36.3	30	36.1	1.2	0.7, 2.1	79	41.3	1.3	0.9, 1.9
P1 initiator	611	28.1	29	34.9	1.5	0.9, 2.6	53	27.8	1.1	0.8, 1.7
Black, non-Hispanic										
Nonusers, B3–P1	273	63.3	6	33.3	Referent	Referent	28	66.7	Referent	Referent
Users, B3–P1	64	14.9	4	22.2	2.8	0.8, 10.4	8	19.0	1.2	0.5, 2.8
P1 initiator	94	21.8	8	44.4	3.9	1.3, 11.5	6	14.3	0.6	0.3, 1.6
Hispanic										
Nonusers, B3–P1	617	71.3	45	67.2	Referent	Referent	93	69.4	Referent	Referent
Users, B3–P1	62	7.2	3	4.5	0.7	0.2, 2.2	4	3.0	0.4	0.2, 1.2
P1 initiator	186	21.5	19	28.4	1.4	0.8, 2.5	37	27.6	1.3	0.9, 2.0

Abbreviations: B3, 3 months before pregnancy; P1, first month of pregnancy.

Table 4. Descriptive Percentile Values for Dietary Folate DFE, Folic Acid, and Natural Folate Levels Among Neural Tube Defect Cases and Controls, National Birth Defects Prevention Study, 1998–2003

	Neural Tube Defect Cases	Anencephaly Cases	Spina Bifida Cases	Controls
Dietary folic acid, μg		—[a]		
10th percentile	39.3	33.3	41.3	38.1
30th percentile	75.5	67.2	77.4	87.0
50th percentile	125.3	108.7	138.7	136.1
90th percentile	392.2	335.0	404.1	368.7
Dietary folate DFE, μg DFE		—		
10th percentile	218.2	207.9	219.6	229.3
30th percentile	342.4	320.1	359.1	349.3
50th percentile	462.1	413.7	478	468.6
90th percentile	964.1	891.3	1,013.2	977.9
Dietary natural folate, μg				
10th percentile	106.9	108.5	105.0	108.3
30th percentile	164.8	160.7	166.8	165.0
50th percentile	218.3	204.8	228.2	213.0
90th percentile	454.2	432.7	466.5	416.5

Abbreviation: DFE, dietary folate equivalent.

[a] —, *t* tests comparing log-transformed means of dietary folate DFE and dietary folic acid between anencephaly cases and controls statistically significant ($P < 0.05$). All other *t* test comparisons between cases and controls were not statistically significant.

anencephaly cases, spina bifida cases, and controls are reported in Table 4. Comparison of log-transformed means of these 3 dietary intake measures among each case population and controls found statistically significant differences only among women whose pregnancies were affected by anencephaly. Specifically, women who had pregnancies affected by anencephaly reported a lower mean intake of dietary folic acid and total folate DFE than did women whose pregnancies were unaffected by a birth defect ($P < 0.05$).

Log-transformed means for dietary folic acid intake were statistically higher among non-Hispanic black and Hispanic controls compared with non-Hispanic white controls ($P < 0.05$). Median values for dietary folic acid were 129.2 μg for non-Hispanic white controls, 145.5 μg for non-Hispanic black controls, and 152.8 μg for Hispanic controls. Statistically significant differences in dietary folic acid were not observed among the racial/ethnicity groups among either anencephaly or spina bifida cases.

The odds ratios measuring the association between dietary folic acid and anencephaly and spina bifida stratified by supplement use are presented in Table 5. Among those who did not use supplements, women who had pregnancies affected by anencephaly or spina bifida were no more likely to report consuming dietary folic acid levels in the lowest 50th percentile of intake than were controls. Odds ratios measuring the association between either anencephaly or spina bifida and the 10th, 11th–30th, or 31st–50th percentile groups of dietary folic acid intake, using those in the >50th percentile as the referent group, did not present a consistent pattern providing evidence of either a positive or a negative association. Thus, women not using supplements with affected pregnancies were no more likely to report being in the lower 50th percentile of dietary folic acid intake than controls who were not taking supplements. A similar finding was observed for women who used supplements consistently between the third month prior to pregnancy and the first month of pregnancy and for those who reported initiating supplement use in the first month of pregnancy.

For women who reported initiating supplement use in the first month of pregnancy, an adjusted odds ratio of 2.5 (95% CI: 1.3, 4.6) was observed when anencephalic case women were compared with control women for the 11th–30th percentile of dietary folic acid intake relative to the >50th percentile intake level. However, for those below the 11th percentile of dietary folic acid intake, an adjusted odds ratio of 1.7 (95% CI: 0.7, 4.2) was observed. When spina bifida cases were compared with controls for initiation of supplement use in P1, all odds ratios were less than 1.0, and confidence intervals were inclusive of 1.0.

Results similar to those presented in Table 5 using dietary folic acid were observed when natural folate and total folate expressed as DFE were used to assess the risk of neural tube defects (data not shown). Given concerns that pregnancy outcomes among cases may impact our findings, we repeated the analyses including participants from only the 5 surveillance programs (Arkansas, California, Iowa, Georgia, and Texas) that have consistently included electively terminated fetuses and stillbirths throughout the study

Table 5. Association Between Anencephaly or Spina Bifida and Dietary Folic Acid Intake Stratified by Supplement Use,[a] National Birth Defects Prevention Study, 1998–2003

	No. of Cases	No. of Controls	Crude		Adjusted[b,c]	
			Odds Ratio	95% Confidence Interval	Odds Ratio	95% Confidence Interval
Anencephaly						
No supplement use B3–P1						
≤10th folic acid intake	9	193	1.2	0.6, 2.6	1.2	0.6, 2.6
11th–30th folic acid intake	19	332	1.5	0.9, 2.7	1.7	0.9, 3.0
31st–50th folic acid intake	19	342	1.5	0.8, 2.6	1.5	0.8, 2.7
>50th folic acid intake	34	911	Referent	Referent	Referent	Referent
Supplement users B3–P1						
≤10th folic acid intake	5	85	1.4	0.5, 4.0	1.5	0.5, 4.0
11th–30th folic acid intake	6	208	0.7	0.3, 1.8	0.7	0.3, 1.8
31st–50th folic acid intake	8	204	1.0	0.4, 2.2	1.0	0.4, 2.3
>50th folic acid intake	19	468	Referent	Referent	Referent	Referent
Supplement users, P1 initiators						
≤10th folic acid intake	7	92	1.7	0.7, 4.1	1.7	0.7, 4.2
11th–30th folic acid intake	22	198	2.5	1.3, 4.6	2.5	1.4, 4.8
31st–50th folic acid intake	11	193	1.3	0.6, 2.7	1.3	0.6, 2.8
>50th folic acid intake	21	465	Referent	Referent	Referent	Referent
Spina bifida						
No supplement use B3–P1						
≤10th folic acid intake	12	193	0.6	0.3, 1.1	0.5	0.3, 1.1
11th–30th folic acid intake	53	332	1.5	1.0, 2.1	1.5	1.0, 2.2
31st–50th folic acid intake	26	342	0.7	0.5, 1.1	0.7	0.4, 1.1
>50th folic acid intake	97	911	Referent	Referent	Referent	Referent
Supplement users B3–B1						
≤10th folic acid intake	13	85	1.8	0.9, 3.6	1.8	0.9, 3.5
11th–30th folic acid intake	29	208	1.7	1.0, 2.8	1.7	1.0, 2.8
31st–50th folic acid intake	16	204	0.9	0.5, 1.7	0.9	0.5, 1.7
>50th folic acid intake	39	468	Referent	Referent	Referent	Referent
Supplement users, P1 initiators						
≤10th folic acid intake	7	92	0.6	0.3, 1.4	0.7	0.3, 1.5
11th–30th folic acid intake	16	198	0.6	0.4, 1.2	0.7	0.4, 1.2
31st–50th folic acid intake	19	193	0.8	0.5, 1.4	0.8	0.4, 1.4
>50th folic acid intake	58	465	Referent	Referent	Referent	Referent

Abbreviations: B3, 3 months before pregnancy; P1, first month of pregnancy.
[a] Dietary folic acid supplement use stratifications by percentile.
[b] Adjusted anencephaly models include maternal race and education.
[c] Adjusted spina bifida models include maternal race, maternal body mass index, and pregnancy intent.

period. Results were similar to those reported here (data not shown).

DISCUSSION

Among US women who were enrolled in the National Birth Defects Prevention Study and conceived after folic acid fortification, we found insufficient evidence for an association between maternal folic acid supplement use or dietary folate intake and neural tube defect occurrence. Reported folic acid supplement use was similar among women who had neural tube defect-affected pregnancies and women who had pregnancies not affected by birth defects. Furthermore, after stratification for supplement use, there was no consistent evidence to support an association between dietary folic acid intake and occurrence of anencephaly or spina bifida. These findings, therefore, are not consistent with the larger body of evidence reported from

periods prior to fortification reporting a protective effect of supplement use on neural tube defect risk, including 2 different randomized controlled trials (5, 6), 3 nonrandomized trials (1, 2, 10), and 5 observational studies (3, 4, 7–9). In 1989, Mills et al. (29) reported no association between neural tube defect occurrence and folic acid supplement use, and Shaw et al. (8) reported in 1995 no association among higher educated women.

We postulate several hypotheses that may explain our findings. First, it is possible that we failed to find an association because of a ''ceiling effect.'' Folate intake among the US childbearing population may have reached levels where nearly all folate-sensitive neural tube defects have been prevented. Two reports from the National Health and Nutrition Examination Survey (NHANES) indicated that, following mandatory folic acid fortification, serum levels of folate in US women of reproductive age were 2–4 times higher than levels prior to fortification (15, 16). Another study from the National Health and Nutrition Examination Survey used 24-hour recall data and reported that US women of reproductive age had increased their median total folate intake by at least 100 μg/day since fortification, although the magnitude of these increases varied by race/ethnicity (30). Ecologic studies have reported declining occurrence of neural tube defects in the United States and other countries since folic acid fortification (17–22). We speculate that most women in the National Birth Defects Prevention Study may have had sufficient folic acid intake to protect their fetuses from having folate-responsive neural tube defects.

Second, our findings may be explained by potential bias. Our results relied on maternal recall of supplement use and dietary intake. By classifying the exposure period for supplement use as consistent use versus no use, we hoped to minimize this potential bias. Nevertheless, errors in reporting are possible and may be differential with respect to neural tube defect-affected offspring. Various versions of the food frequency tool used in this study had been validated in other studies (24, 31, 32), but we acknowledge the inherent limitation of this tool to measure intakes in our population. Other dietary assessment methods, such as 24-hour recalls or food diaries, were not feasible with this study design. To further evaluate potential selection bias, we analyzed data from the 5 sites where all pregnancy outcomes have been consistently monitored and found results (data not shown) similar to those reported for all sites.

Third, although the National Birth Defects Prevention Study is the largest case-control study of birth defects to be conducted in the United States, the number of affected cases included in our analyses may be insufficient to detect a true difference, particularly if the magnitude of the true difference is small. Small sample sizes prevented us from further exploring subgroup analyses, particularly potential racial/ethnic differences. In light of the evidence showing a smaller decline in the occurrence of neural tube defects since fortification among Hispanic compared with non-Hispanic white pregnancies (19), race and ethnic differences should be further evaluated.

Finally, our study estimated risks for the dietary intake of folic acid but not tissue-dose levels. Effects of genetic variation and the interactive relation with other micronutrients and neural tube defects should be considered for more comprehensive etiologic assessments. The National Birth Defects Prevention Study is an ongoing study and will provide an opportunity for continued evaluation of the association between neural tube defects and maternal micronutrient intake.

Despite these limitations, collaborative efforts of the National Birth Defects Prevention Study resulted in the accrual of a relatively large population of study participants. We recommend further studies evaluating the effects of supplement use in the postfortification era. Research is warranted to identify additional independent risk and protective factors for neural tube defects, with efforts made to identify women who may require higher levels of folic acid or alteration of other modifiable factors.

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