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ORIGINAL CONTRIBUTIONS

Periconceptional Dietary Intake of Choline and Betaine and Neural Tube Defects in Offspring

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Periconceptional intake of folic acid prevents some neural tube defects (NTDs). Other nutrients may also contribute to NTD etiologies; a likely candidate is choline. Similar to folic acid, choline is involved in one-carbon metabolism for methylation of homocysteine to methionine. The authors investigated whether maternal periconceptional dietary intakes of choline and its metabolite betaine influence NTD risk. Data were derived from a case-control study of fetuses and infants with NTDs among 1989–1991 California births. In-person interviews were conducted with mothers of 424 NTD cases and with mothers of 440 nonmalformed controls. A standard 100-item food frequency questionnaire was used to assess nutrient intake. Dietary intakes of choline were associated with reduced NTD risks. Controlling for intake of supplemental folic acid, dietary folate, dietary methionine, and other covariates did not substantially influence risk estimates for choline. NTD risk estimates were lowest for women whose diets were rich in choline, betaine, and methionine. That is, for women whose intake was above the 75th percentile compared with below the 25th percentile for all three nutrients, the odds ratio was 0.17 (95% confidence interval: 0.04, 0.76). Study findings for dietary components other than folic acid offer additional clues about the complex etiologies of NTDs.

abnormalities; case-control studies; folic acid; methionine; pregnancy; vitamins

Abbreviations: CI, confidence interval; NTD, neural tube defect.

Maternal nutritional factors have been implicated in the etiologies of neural tube defects (NTDs). Foremost among these factors has been the role of periconceptional intake of folic acid in reducing risks of NTD-affected pregnancies (1, 2). Other nutrients and nutrition-related factors have also been observed to influence NTD risks. For example, increased intakes of methionine (3), zinc (4), vitamin C (5), dairy products (6), and vitamin B_{12} (7) have been associated with reduced NTD risks. Furthermore, increased intakes of sweets (8), maternal diabetes (9), maternal hyperinsulinemia (10), and prepregnancy obesity (11–13) have been associated with elevated NTD risks.

In this study, we investigated the association of intakes of two additional nutrients, choline and its metabolite betaine, with risk of NTD-affected pregnancies. These nutrients, similar to folic acid, are involved in one-carbon (methyl donors) metabolism for methylation of homocysteine to methionine and are utilized for the synthesis of cell membrane phospholipids; choline is a precursor of the neurotransmitter acetylcholine (14–16). Our motivation to investigate these two nutrients was based on previous observations implicating folate and methionine in NTD risk, nutrients that, in conjunction with choline, have an interdependence in the conversion of homocysteine to methionine (16, 17). Alterations in the methylation of homocysteine to form methionine have been implicated in the risk of NTD-affected pregnancies (18). In addition, inhibition of choline uptake and metabolism in mouse embryos

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TABLE 1. Characteristics of women with neural tube defect-affected pregnancies (cases) and those without such pregnancies (controls), California, 1989–1991

Characteristic	Cases (n = 424)		Controls (n = 440)	
	No.	%*	No.	%*
Race/ethnicity				
White	181	42.7	235	53.4
US-born Latina	49	11.6	62	14.1
Foreign-born Latina	151	35.6	92	20.9
Other	43	10.1	50	11.4
Education				
<High school graduate	164	38.7	114	25.9
High school graduate	162	38.2	180	40.9
>High school graduate	97	22.9	145	33.0
Multivitamin use				
No use	162	38.2	120	27.3
Began 3 months before conception	71	16.7	77	17.5
Began in first trimester	187	44.1	235	53.4
	Mean	SD†	Mean	SD
Height (m)	1.61	0.09	1.63	0.08
Prepregnant weight (kg)	63.6	15.0	60.8	11.9
Energy intake (kcal/day)	2,240.8	896.5	2,373.2	940.6
Dietary folate intake (μg/day)	351.7	188.7	372.0	198.6
Dietary methionine intake (mg/day)	1,862.7	830.5	2,037.0	898.7
Choline intake (mg/day)	377.3	175.6	408.5	178.8
Betaine intake (mg/day)	174.9	109.4	202.4	127.7

* Percentages may not equal 100 because of missing data or rounding.
† SD, standard deviation.

results in NTDs (19). To our knowledge, choline and betaine have not been investigated previously for their potential association with human NTDs. Thus, we examined data from a large population-based case-control study to investigate whether women who had greater periconceptional dietary intakes of choline and betaine were at decreased risk of NTD-affected pregnancies.

MATERIALS AND METHODS

This study involving human subjects was approved by the California State Committee for the Protection of Human Subjects. Details of the population-based case-control study used in this analysis have been described previously (20).

Infants or fetuses with NTDs (anencephaly, spina bifida cystica, craniorachischisis, or iniencephaly) were ascertained by reviewing medical records, including ultrasonography, at all hospitals and genetics clinics for those infants/fetuses delivered in select California counties and whose mothers gave their residence as California. Eligible were singleton liveborn infants, fetal deaths, and fetuses prenatally diagnosed and electively terminated in the cohort of 708,129 births and fetal deaths occurring between June 1989 and May 1991. Ascertained were 653 singleton infants/fetuses with an eligible NTD diagnosis. Controls were randomly selected from each area hospital in proportion to the hospital's estimated contribution to the total population of infants born alive in a given month from June 1989 to May 1991. Ascertained were 644 singleton infants born without a reportable congenital anomaly.

Women who spoke only languages other than English or Spanish or who had had a previous NTD-affected pregnancy were excluded, leaving 613 cases and 611 controls. In-person interviews were completed with mothers of 538 (87.8 percent) cases and of 539 (88.2 percent) controls an average of 4.9 months for cases and 4.6 months for controls after the actual or projected date of term delivery. Information was elicited from women about medical history, reproductive history, and activities associated with various lifestyles, primarily during the periconceptional period (6-month period from 3 months before to 3 months after conception).

A standard 100-item food frequency questionnaire was used to assess nutrient intake from diet (21). This instrument has been validated for use in epidemiologic studies (22–24). Study women themselves completed the questionnaire (English or Spanish), with interviewers present to assist. Each woman was instructed to estimate her usual frequency and portion size of the food items she consumed during the 3 months before conception. Average daily intake of nutrients was computed by using the questionnaire's software (21). Of

TABLE 2. Effect estimates (odds ratios) for neural tube defect-affected pregnancies associated with maternal choline intake during the periconceptional period, California, 1989–1991

Total choline	No. of cases	No. of controls	Odds ratio*	95% CI†
All neural tube defects				
Continuous measure‡	424	440	0.90	0.78, 1.04
Quartile measure§				
≤290.41	147	110	Reference	
290.42–371.52	99	110	0.66	0.45, 0.97
371.53–498.46	98	110	0.63	0.41, 0.98
>498.46	80	110	0.49	0.27, 0.90
Spina bifida				
Continuous measure‡	242	440	0.93	0.79, 1.09
Quartile measure§				
≤290.41	80	110	Reference	
290.42–371.52	56	110	0.66	0.42, 1.04
371.53–498.46	61	110	0.68	0.41, 1.14
>498.46	45	110	0.45	0.22, 0.93
Anencephaly				
Continuous measure‡	161	440	0.83	0.67, 1.02
Quartile measure§				
≤290.41	59	110	Reference	
290.42–371.52	38	110	0.64	0.39, 1.07
371.53–498.46	33	110	0.56	0.31, 1.02
>498.46	31	110	0.52	0.24, 1.17

* Odds ratio adjusted for maternal total energy intake.
† CI, confidence interval.
‡ Odds ratio expressed as change in risk per 100 mg of change in intake.
§ Quartiles determined from intake levels for mothers of controls.

the 1,077 women who completed an interviewer-administered questionnaire, 1,007 finished a food frequency questionnaire; of these, 916 provided data considered suitable on the basis of error checks built into the analytical software (21). The analytical program examined the data for a variety of errors to produce invalid data; more than half of the questionnaires dropped were because the number of foods selected for daily consumption was considered excessive. An additional 52 women who reported the use of food supplements such as SlimFast (SlimFast Foods Company, West Palm Beach, Florida) were excluded because of inadequate information to assess the choline or betaine content of these supplements. Of the 864, 424 were mothers of NTD cases and 440 were mothers of controls. The 424 cases included 161 with anencephaly, 242 with spina bifida, and 21 with other NTD phenotypes.

The choline and betaine composition of individual foods was added to the food frequency questionnaire's nutrient database by using values published recently by Zeisel et al. (25, 26). In general, food items in the food frequency database corresponded well with food items in the published food list (25), except for the absence of values for breakfast cereals from the food list. (Breakfast cereals were assigned choline and betaine values obtained from unpublished data from S. Zeisel, December 2003.) Choline and betaine values were adopted directly from the Zeisel et al. (25) food list when there was a 1:1 correspondence.

The food list includes 145 food items and the food frequency database 207. For foods in the database that did not have a direct correspondence, we assigned choline/betaine values associated with nutritionally comparable foods. For example, for "limas, black-eyed peas," choline and betaine values for "navy beans" were assigned. Those few foods for which no nutritionally comparable food was found (e.g., olives, coconut milk) were not assigned choline/betaine values. Foods that contained no appreciable amount of choline or betaine were assigned zero values for choline and betaine (e.g., sugar).

For food items from the food frequency questionnaire composed of multiple foods, we made assignments based on the derivation of nutrient values from National Health and Nutrition Examination Survey food consumption values (G. Block (developer of the questionnaire), University of California, personal correspondence, 2003). That is, on the basis of these other data, if frequency of consumption of one of the items was considered much higher than for other items in the group, for example, oranges in the "oranges, grapefruit, tangerines" group, that item was selected to represent the group and the choline/betaine values were assigned for that item. If frequency of consumption of the items in the group

TABLE 3. Effect estimates (odds ratios) for neural tube defect-affected pregnancies associated with maternal betaine intake during the periconceptional period, California, 1989–1991

Betaine	No. of cases*	No. of controls*	Odds ratio†	95% CI‡	Adjusted odds ratio§	95% CI	Adjusted odds ratio¶	95% CI
All neural tube defects								
Continuous measure#	391	411	0.84	0.73, 0.97	0.87	0.76, 1.01	0.96	0.82, 1.12
Quartile measure**								
≤119.70	131	103	Reference		Reference		Reference	
119.71–177.39	106	103	0.84	0.57, 1.24	0.91	0.61, 1.34	1.07	0.71, 1.61
177.40–258.36	79	103	0.64	0.42, 0.97	0.71	0.46, 1.08	0.93	0.60, 1.46
>258.36	75	102	0.64	0.41, 1.02	0.74	0.46, 1.18	0.99	0.59, 1.66
Spina bifida								
Continuous measure#	224	411	0.90	0.77, 1.06	0.94	0.80, 1.11	1.01	0.85, 1.21
Quartile measure**								
≤119.70	72	103	Reference		Reference		Reference	
119.71–177.39	63	103	0.89	0.57, 1.40	0.95	0.60, 1.50	1.08	0.67, 1.73
177.40–258.36	40	103	0.57	0.35, 0.94	0.65	0.39, 1.07	0.79	0.46, 1.35
>258.36	49	102	0.72	0.42, 1.23	0.85	0.49, 1.46	1.08	0.59, 1.95
Anencephaly								
Continuous measure#	149	411	0.77	0.62, 0.96	0.79	0.64, 0.99	0.88	0.70, 1.10
Quartile measure**								
≤119.70	49	103	Reference		Reference		Reference	
119.71–177.39	38	103	0.84	0.50, 1.41	0.88	0.52, 1.50	1.09	0.62, 1.90
177.40–258.36	37	103	0.85	0.49, 1.47	0.90	0.52, 1.57	1.26	0.70, 2.27
>258.36	25	102	0.62	0.33, 1.17	0.69	0.36, 1.33	0.93	0.46, 1.87

* Numbers of cases and controls for whom no values were missing for any of the analyzed covariates described in the fifth (¶) footnote.
† Odds ratio adjusted for maternal total energy intake.
‡ CI, confidence interval.
§ Odds ratio adjusted for methionine intake, vitamin use, total energy intake, and dietary folate intake.
¶ Odds ratio adjusted for methionine intake, vitamin use, total energy intake, dietary folate intake, race/ethnicity, education, weight, and height.
# Odds ratio expressed as change in risk per 100 mg of change in intake.
** Quartiles determined from intake levels for mothers of controls.

was considered approximately equal, choline and betaine values for the contributing items were averaged (e.g., "other snacks, crackers, chips" = one third wheat crackers, one third corn chips, one third potato chips). Assignment of choline and betaine values to mixed dishes in the food frequency database, without a comparable mixed dish on the Zeisel et al. (25) food list, was based on the proportional contribution of individual food components in the recipe. The recipe was confirmed by the originator of the food frequency questionnaire (G. Block, 2003) based on her knowledge of the original National Health and Nutrition Examination Survey food item recipe. All choline and betaine values were assigned masked to case and control status. We computed women's average daily intakes of choline and betaine by considering portion size and frequency of consumption of each food item.

NTD risk was estimated by using logistic regression models. Odds ratios and their 95 percent confidence intervals were computed to summarize the potential influence of several possible risk factors. Models were constructed to assess effects associated with continuous intake levels of choline and betaine. Models were also constructed in which intake levels were considered as quartile cutpoints. Intakes for mothers of controls were used to establish quartile categories of intake of each measure, with the lowest quartile as reference. Considered covariates in the analyses were maternal race/ethnicity (Latina, foreign born; Latina, US born; White, non-Latina; Black; other), education (<high school graduate, high school graduate, >high school graduate), height (meters), prepregnant weight (kilograms), energy intake (kilocalories/day), dietary folate intake (micrograms/day), dietary methionine intake (milligrams/day), and periconceptional multivitamin supplementation (none, use in the 3 months before conception, beginning use in the 3 months after conception). The frequencies or mean values of these data are displayed in table 1. We added daily folic acid intake from vitamin supplements in the period 3 months before conception to daily folate intake from diet also for the period 3 months before conception to estimate "total" folate intake from both sources. However, the addi-

TABLE 4. Effect estimates (odds ratios)* for neural tube defect-affected pregnancies according to quartile of average daily maternal dietary intake of choline and total folate in the 3 months before conception, California, 1989–1991

Quartile of choline (mg/day)	Quartile of folate (μg/day)			
	≤246.39	246.40–350.55	350.56–544.36	>544.36
≤290.41				
No. of cases/no. of controls	85/59	28/25	15/10	19/16
Odds ratio	Reference	0.78	1.05	0.83
95% CI†		0.41, 1.48	0.44, 2.49	0.39, 1.75
290.42–371.52				
No. of cases/no. of controls	24/28	29/37	26/21	20/24
Odds ratio	0.60	0.55	0.88	0.59
95% CI	0.31, 1.16	0.30, 1.02	0.44, 1.75	0.29, 1.18
371.53–498.46				
No. of cases/no. of controls	8/16	20/33	35/37	35/24
Odds ratio	0.35	0.43	0.68	1.05
95% CI	0.14, 0.89	0.21, 0.87	0.35, 1.30	0.52, 2.11
>498.46				
No. of cases/no. of controls	0/7	17/15	26/42	37/46
Odds ratio	N/A†	0.82	0.45	0.60
95% CI		0.34, 1.95	0.21, 0.96	0.26, 1.37

* Odds ratios adjusted for maternal total energy intake.
† CI, confidence interval; N/A, not applicable.

tion of these two sources of intake did not account for known differences in bioavailability between supplemental folic acid and dietary folate. All analyses were performed by using the Statistical Analysis System (SAS) (27).

RESULTS

Decreased risks of NTD-affected pregnancies were found for higher periconceptional intakes of choline for all NTDs as well as for spina bifida and anencephaly separately (table 2). This pattern was observed whether intakes were analyzed as continuous or discrete measures (quartiles). We adjusted for maternal prepregnant weight, height, education, race/ethnicity, periconceptional vitamin use, dietary folate intake, dietary methionine intake, and total energy intake. These adjusted risks did not differ substantially from their unadjusted counterparts. That is, for the three increasing choline intake quartiles relative to the lowest quartile, odds ratios for NTDs were 0.63 (95 percent confidence interval (CI): 0.42, 0.99), 0.65 (95 percent CI: 0.39, 1.07), and 0.51 (95 percent CI: 0.25, 1.07), respectively.

We also observed decreased risks of NTD-affected pregnancies for higher periconceptional intakes of betaine for all NTDs as well as for spina bifida and anencephaly separately (table 3), although observed risk reductions were small in magnitude and imprecise. Adjusting for covariates resulted in most of the estimated risks being much closer to unity (table 3). Analyses adjusted for maternal prepregnant weight, height, education, and race/ethnicity appeared to have the most substantial impact on estimated risks (table 3).

Compared with women categorized (referent) as having the lowest quartile intake of choline and having the lowest quartile intake of "total" folate (both supplements and diet combined), women whose intakes of choline were above the lowest quartile had lower, albeit imprecise, risks of NTD-affected pregnancies, irrespective of folate intake (table 4). Related analyses that investigated combined intakes of choline and methionine, and of choline and betaine, revealed that women whose intakes were highest of either methionine or of betaine in combination with choline were at the lowest risk of NTD-affected pregnancies. That is, odds ratios of 0.37 (95 percent CI: 0.18, 0.76) for choline and methionine (table 5) and 0.28 (95 percent CI: 0.12, 0.65) for choline and betaine (table 6) were observed. Investigating high intakes of all three nutrients (choline, methionine, and betaine) compared with low intakes of the three nutrients produced an even smaller odds ratio of 0.17 (95 percent CI: 0.04, 0.76) (data not shown). Data were sparse in the latter comparison as well as in some of the intake-level comparisons in tables 4, 5, and 6, resulting in many of the estimated risks being imprecise.

DISCUSSION

Our results indicate decreased NTD risks associated with maternal periconceptional diets containing choline and possibly betaine. Controlling for the potential effects of maternal intake of supplemental folic acid, dietary folate, dietary methionine, and other covariates did not substantially influence risk estimates for choline but did attenuate those

TABLE 5. Effect estimates (odds ratios)* for neural tube defect-affected pregnancies according to quartile of average daily maternal dietary intake of choline and methionine in the 3 months before conception, California, 1989–1991

Quartile of choline (mg/day)	Quartile of methionine (mg/day)			
	≤1,420.97	1,420.98–1,852.87	1,852.88–2,491.85	>2,491.85
≤290.41				
No. of cases/no. of controls	124/86	21/20	2/4	0/0
Odds ratio	Reference	0.70	0.33	N/A†
95% CI†		0.36, 1.38	0.06, 1.86	
290.42–371.52				
No. of cases/no. of controls	21/18	44/65	31/25	3/2
Odds ratio	0.78	0.44	0.79	0.94
95% CI	0.39, 1.56	0.27, 0.72	0.42, 1.48	0.15, 5.83
371.53–498.46				
No. of cases/no. of controls	3/4	24/22	53/61	18/23
Odds ratio	0.50	0.68	0.54	0.45
95% CI	0.11, 2.29	0.35, 1.35	0.31, 0.91	0.21, 1.00
>498.46				
No. of cases/no. of controls	0/2	2/3	20/20	58/85
Odds ratio	N/A	0.41	0.59	0.37
95% CI		0.07, 2.57	0.28, 1.28	0.18, 0.76

* Odds ratios adjusted for maternal total energy intake.
† N/A, not applicable; CI, confidence interval.

TABLE 6. Effect estimates (odds ratios)* for neural tube defect-affected pregnancies according to quartile of average daily maternal dietary intake of choline and betaine in the 3 months before conception, California, 1989–1991

Quartile of choline (mg/day)	Quartile of betaine (mg/day)			
	≤118.02	118.03–175.67	175.68–258.02	>258.02
≤290.41				
No. of cases/no. of controls	84/53	37/25	20/19	6/13
Odds ratio	Reference	0.89	0.62	0.27
95% CI†		0.48, 1.66	0.30, 1.29	0.10, 0.77
290.42–371.52				
No. of cases/no. of controls	31/26	32/39	24/29	12/16
Odds ratio	0.69	0.47	0.46	0.42
95% CI	0.37, 1.32	0.26, 0.86	0.23, 0.91	0.18, 0.99
371.53–498.46				
No. of cases/no. of controls	21/22	25/30	24/29	28/29
Odds ratio	0.53	0.45	0.44	0.49
95% CI	0.25, 1.10	0.23, 0.90	0.22, 0.90	0.23, 1.03
>498.46				
No. of cases/no. of controls	6/9	24/16	18/33	32/52
Odds ratio	0.35	0.72	0.26	0.28
95% CI	0.11, 1.09	0.30, 1.74	0.11, 0.61	0.12, 0.65

* Odds ratios adjusted for maternal total energy intake.
† CI, confidence interval.

for betaine. NTD risk estimates were lowest for women whose diets were rich in both choline and methionine as well as choline, betaine, and methionine. Extensive literature indicates an association between folates and NTDs (1, 2). However, the quantity of literature on other related nutrients (e.g., methionine (3)) and NTD risk is small, with the current study the first known to explore choline and betaine.

Our observation of an association between higher intakes of choline and NTD risk provides evidence to suggest that deficiencies in methyl donors may be associated with NTD risk; that is, a less-than-optimal methyl-donor supply and DNA methylation have been a suggested area for research regarding certain birth defects (28). It is known that methylation of DNA can be influenced by dietary contributions of methyl donors such as choline, folate, and methionine. Choline, folate, and methionine are highly interrelated in methyl-group metabolism, and an alteration in one affects the others (15). Thus, choline deficiency could impact folate and homocysteine metabolism (29). Other dietary parameters are also involved in DNA methylation, including vitamin B_{12}, vitamin B_6, and serine (30); these additional nutrients were not investigated in this study.

Choline is depleted in pregnant rats; therefore, an animal's ability to obtain sufficient choline from dietary sources may be compromised during pregnancy (29, 31). Moreover, it has been observed in experimental systems that inhibiting choline uptake and metabolism in mouse embryos results in NTDs (19) and that pregnant rabbits, given anticholinergic drugs, have reduced placental and fetal choline levels and produce higher frequencies of malformed offspring (32). However, not all experiments involving choline-deficient or folate-deficient diets have revealed increased frequencies of NTD-affected offspring (32). Furthermore, choline appears to have a role in apoptosis, one that extends beyond the repletion of methyl groups (29). Regulation of apoptosis is important to the development of the neural tube (33). Thus, choline's role in apoptosis could theoretically offer another mechanism for improper neural tube closure.

Despite this study's population-based ascertainment of cases and controls, high maternal participation rate, large sample size, and ability to control for many relevant covariates, its ability to draw firm inferences was limited by surrogate measures of tissue concentrations of choline and betaine. Choline and betaine intakes were imputed blinded to case and control status. Thus, errors that might arise from imputation of such surrogate measures would likely be the same for mothers of both cases and controls. Therefore, misclassification errors would likely produce attenuated, rather than inflated, estimates of NTD risks. The impact on estimated risks associated with using intake data to reflect tissue concentrations is unknown, however.

In interpreting our findings, we cannot exclude the possibility that observed elevated risks were attributable to recall bias. Although there is little evidence that recall bias contributes substantially to the results of studies such as these (34), it is possible, for example, that mothers of cases overreported or mothers of controls underreported intakes of foods associated with higher choline and betaine values. Foods contributing to higher choline intakes were eggs, milk, and orange juice, whereas foods contributing to high betaine intakes were breads, spinach, and biscuits. However, most women in the study (interviewed more than 10 years ago) were probably unaware of the choline and betaine values of foods they ate. It therefore seems unlikely that differential recall between mothers of cases and mothers of controls is a probable explanation for our results. We also cannot exclude the possibility that observed elevated risks were due to random variation given the sparseness of data for some comparisons.

For more than 30 years, evidence has emerged to indicate that periconceptional nutrient intakes, particularly folate, lower risks of NTD-affected pregnancies. Although fortification of the US food supply with folic acid is associated with a decreased prevalence of NTDs (35), a substantial population burden of these serious birth defects still exists. Thus, our findings identifying other dietary components may offer additional, important clues to understanding the complex etiologies of NTDs.

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